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RESEARCH ARTICLES

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RESEARCH ARTICLE

Effects of Bromelain on Oxidative Stress and Lung Tissue Histopathology in an Experimental Rat Sepsis Model

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ABSTRACT

Sepsis is an inflammatory syndrome that targets the lung tissue. Bromelain (BRO) is an enzyme complex known for its anti-inflammatory properties. In this study, the protective effect of BRO on lung injury due to sepsis induced by cecal ligation and puncture (CLP) in *Sprague-Dawley* rats was investigated by biochemical and histopathological methods. Forty *Sprague dawley* rats were randomly divided into five groups as control, CLP, BRO, CLP+BRO 50 and CLP+BRO 100. In the study, after oral administration of 50 and 100 mg/kg BRO (total of 7 applications for 7 days) to rats, laparotomy and CLP were performed. The data showed that due to its antioxidant properties, BRO decreased CLP-induced myeloperoxidase (MPO) and lipid peroxidation (MDA) ($p < 0,001$), increased glutathione (GSH) levels, superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities ($p < 0,001$). Additionally, CLP-induced lung histological changes were reduced with BRO supplementation. The results showed that BRO treatment has an antioxidant, anti-inflammatory effect on CLP-induced lung toxicity and helps preserve lung tissue architecture owing to its regulatory effect.

Keywords: Lung, Bromelain, Inflammation, Oxidative Stress, Sepsis

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Deneysel Sıçan Sepsis Modelinde Bromelainin Oksidatif Stres ve Akciğer Doku Histopatolojisi Üzerine Etkileri

ÖZ

Sepsis, inflamatuvar bir sendrom olup başta akciğeri hedef almaktadır. Bromelainin (BRO) anti-inflamatuvar özelliği ile bilinen enzim kompleksidir. Bu çalışmada *Sprague dawley* sıçanlarında çekal ligasyon ve perforasyon (CLP) ile indüklenen sepsise bağlı akciğer hasarında BRO koruyucu etkisi biyokimyasal ve histopatolojik yöntemlerle araştırıldı. Kırk adet *Sprague dawley* sıçan, kontrol, CLP, BRO, CLP+BRO 50 ve CLP+BRO 100 olmak üzere rastgele beş gruba ayrıldı. Çalışmada sıçanlara 50 ve 100 mg/kg BRO oral olarak (7 gün boyunca toplam 7 uygulama) verildikten sonra laparotomi ve CLP uygulanmıştır. Veriler, BRO'nun antioksidan özelliklerinden dolayı CLP ile indüklenen miyeloperoksidaz (MPO) ve lipid peroksidasyonu (MDA) azalttığını ($p < 0,001$), glutatyon (GSH) seviyelerini, süperoksit dismutaz (SOD) ve glutatyon peroksidaz (GPx) aktivitelerini arttırdığını göstermiştir ($p < 0,001$). Ayrıca CLP kaynaklı akciğer histolojik değişiklikler BRO takviyesi ile azalmıştır. Sonuçlar BRO tedavisin CLP kaynaklı akciğer toksisitesi üzerinde antioksidan, anti-inflamatuvar etkiye sahip olduğunu ve düzenleyici etkisi sayesinde akciğer doku mimarisinin korunmasına yardımcı olduğunu göstermiştir.

Anahtar Kelimeler: Akciğer, Bromelain, İnflamasyon, Oksidatif Stres, Sepsis

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GİRİŞ

Sepsis, enfeksiyona bağlı çoklu organ yetmezliği ve ölüme sebep olabilen sistemik inflamatuvar bir sendromdur (Zhang ve ark. 2021). Her yıl yoğun bakım ünitelerinde 5 milyondan fazla hasta sepsise bağlı hayatını kaybetmektedir (Chen ve ark. 2018). Akciğerler, sepsisli hastada ilk fonksiyonu bozulan organlardır. Yoğun bakımda yatan hastalarda, akciğer disfonksiyonu ve akut respiratuar distres sendromu (ARDS) ölüm riskini artıran nedenler içerisinde en sık görülenlerdendir (Zeng ve ark. 2015). Sepsisin hem akciğer hem de çoklu organ disfonksiyonu patogenezinde reaktif oksijen türlerinin (ROS) artması ve antioksidan savunma sistemlerinin azalması yatmaktadır. Artış gösteren oksidatif stres ile birlikte özellikle akciğer dokularında inflamatuvar hücre infiltrasyonu, konjesyon ve ödem gibi patolojik değişiklikler meydana gelmektedir (Chen ve ark. 2018; Giustina ve ark. 2019). Mevcut tedavi seçenekleri arasında antibiyotik ve sürekli pozitif basınçlı ventilasyonla tedavi ön plandadır. Fakat bu tedavilerin etkisi hem sınırlı hem de maliyetlidir (Chen ve ark. 2021). Son yıllarda sepsisin sebep olduğu akciğer disfonksiyonunun önüne geçmek için farklı tedavi ve stratejiler üzerinde durulmaktadır. Fakat başvurulan yöntemler yeterince etki gösterememiş ve altta yakan kesin mekanizma tam olarak çözülememiştir. Oksidatif stres, çoklu organ fonksiyon bozukluğunda önemli bir rol oynadığından antioksidanlar ile tedavi umut verici bir strateji olarak kabul edilmektedir.

Doğal bileşikler özellikle birçok hastalığın tedavisinde yaygın olarak kullanılan antioksidan ve anti-inflamatuvar etkilerinden dolayı birçok araştırmacının ilgisini çekmektedir (Hu ve ark. 2020; Genin ve ark. 2016). Doğal bir ürün olan *Ananas comosus* (Ananas), *Bromeliaceae* familyasına ait önemli terapatik özelliği olan bir bileşiktir. Bromelain (BRO), ananasın saplarından elde edilen proteaz bir enzim kompleksidir (El-Demerdash ve ark. 2020; Ferah Okkay ve ark. 2023). BRO'nun kullanım alanları çok yaygınlaşmış, güvenilir ve özellikle gıdada, sağlık alanlarında ve kozmetikte yerini almıştır (El-Demerdash ve ark. 2020). Dünyanın birçok ülkesinde kullanılmakta olan BRO, özellikle Amerika ve Avrupada besin takviyesi olarak, Çin'de ise antimodülatör bir ilaç olarak kullanılmaktadır (Abo ve ark. 2021). BRO daha önceki çalışmalarda antioksidan, anti-inflamatuvar, antikanser ve anti-trombosit etkisi ortaya konmuştur (El-Demerdash ve ark. 2020; Ferah Okkay ve ark. 2023). Bu etkilerinden dolayı sepsisin neden olduğu akciğer disfonksiyonunda kullanılması alternatif bir yol olabilir.

Bu çalışmada amaç, sepsis kaynaklı akciğer toksisitesine karşı BRO'nun potansiyel koruyucu etkisini biyokimyasal ve histopatolojik yöntemler kullanarak araştırmaktır.

MATERYAL ve METOT

Deneysel Prosedür ve Gruplar

Bu çalışmada, Kafkas Üniversitesi Deneysel Araştırma ve Uygulama Merkezi bünyesindeki deneysel hayvan laboratuvarından temin edilen toplam 40 adet *Sprague dawley* (250-300gr, 3 aylık) cinsi dişi sıçanlar kullanıldı. Deney süresince, deney hayvanlarına yeteri kadar (ad libitum) su ve pellet yem verildi. Hayvanlar deney öncesi gruplar halinde standart laboratuvar koşullarında (sıcaklık: 23±2 °C) ve (nem: % 55 ± 5) barındırıldı. Bu çalışma Kafkas Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu tarafından onaylanmıştır (Tarih: 22 Eylül 2022, protokol numarası: 2022–8/145). Çalışma 5 grup olacak şekilde her bir grup rastgele 8 sıçandan oluşmaktadır.

- 1. Grup: Kontrol (Sahte operasyon)
- 2. Grup: CLP
- 3. Grup: BRO 100 mg/kg
- 4. Grup: CLP + BRO 50 mg/kg
- 5. Grup: CLP + BRO 100 mg/kg

Çalışmada uygulanan BRO dozu literatüre göre belirlenerek bir hafta boyunca oral olarak uygulandı (Ferah Okkay ve ark. 2023; Sehirli ve ark. 2021). CLP+BRO gruplarında 7. günün sonunda literatüre göre belirlenmiş CLP modeli oluşturuldu (Eraslan ve ark. 2020). Sepsis modeli oluşturmak için sıçanlara intraperitoneal yoldan 90 mg/kg ketamin ile anestezi uygulandı. CLP için 2 cm ebatında orta hatta boylamasına insizyonla batın açıldı. Ardından çekum eksplore edildi. Çıkan kolon dikkatlice sıvazlandı ve çekum gaita ile doldurulduu. Daha sonra ileoçekal valvin distalinden 3/0 ipekle bağlanıp, çekum ön yüzü 18 G iğne ile iki defa delindi. Feçes çıkışı gözlendikten sonra laparotomi 4/0 ipek sütürle kapatıldı. CLP gruplarındaki sıçanlara takiben laparotomi ve çekal ligasyon perforasyon (CLP) uygulandı. Kontrol grubuna ise sıçanlara operatif işlem yapılmış, fakat CLP yöntemiyle sepsis modeli uygulanmamış, sadece çekum eksplore edilmiştir. Sıçanlar, CLP uygulamasından 16 saat sonra anestezi (ketamin ve ksilazin) altında sakrifiye edilerek akciğer dokuları alındı. Dokuların bir kısmı biyokimyasal analiz için -80^{0}C'de, diğer kısmı ise histolojik değerlendirme yapmak için %10'luk formalin içerisinde saklandı.

Biyokimyasal Analiz

Akciğer dokuları -80^{0}C'den çıkarıldıktan sonra sıvı nitrojen içerisine bırakıldı ve porselen bir tokmak ile toz haline getirildi. Toz haline getirilen dokular %1,15 KCl ile seyreltildi, homojenize edildi ve santrifüjlendi. Elde edilen süpernatant kısımlarından Placer ve arkadaşlarının (Placer ve ark. 1966) geliştirdiği methoda göre MDA seviyesi, Sun ve ark. (Sun ve ark. 1988) metoduna göre SOD aktivitesi, Sedlak ve Lindsay (Sedlak ve ark. 1968) kullandığı methoda göre GSH seviyesi, Bradleyin (Bradley ve ark. 1982) methoduna göre MPO aktivitesi, Lawrence ve Burk (Lawrence ve ark. 1976) methoduna göre ise GPx

aktivitesi ölçüldü. Toplam protein analizinde ise Lowry ve ark. (Lowry ve ark. 1951) geliştirdiği method kullanıldı.

Histopatolojik Analiz

Sıçan akciğer doku örnekleri %10'luk formalin içerisinde 48 saat boyunca tespit edilmiştir. Ardından 4 saat süresince yıkamaya alınan dokular, devamında artan dereceli alkol serilerinde dehidratasyon, ksilende şeffaflaştırma ve parafinde infiltrasyon işlemleri yapılarak bloklar haline getirilmiştir. Bloklanan dokulardan konvansiyonel ışık mikroskobik incelemeleri için mikrotom ile 5 μm'lik kesitler elde edilerek lamlar üzerine bırakılmıştır. Alınan kesitler hematoksilen-eozin (H&E) ile prosedüre uygun şekilde boyanmıştır. Histolojik analizler için; kesitler, bilgisayar destekli mikroskop ile fotoğraflanıp ayrı ayrı 2 histolog tarafından körleme yöntemiyle incelenmiştir. Akciğer hasarı, daha önceki çalışmalarda bildirildiği gibi histolojik bir skorlama sistemi kullanılarak değerlendirildi (Liu ve ark. 2019). Değerlendirmede, dört patolojik parametre kullanıldı ve 0 ile 4 arasında bir ölçekte puanlandı: alveolar konjesyon, kanama, lökosit infiltrasyonu veya nötrofil agregasyonu ve alveolar duvar kalınlığı. Karşılaştırmalı analiz için dört parametrenin tümü için puanların toplamı kaydedildi.

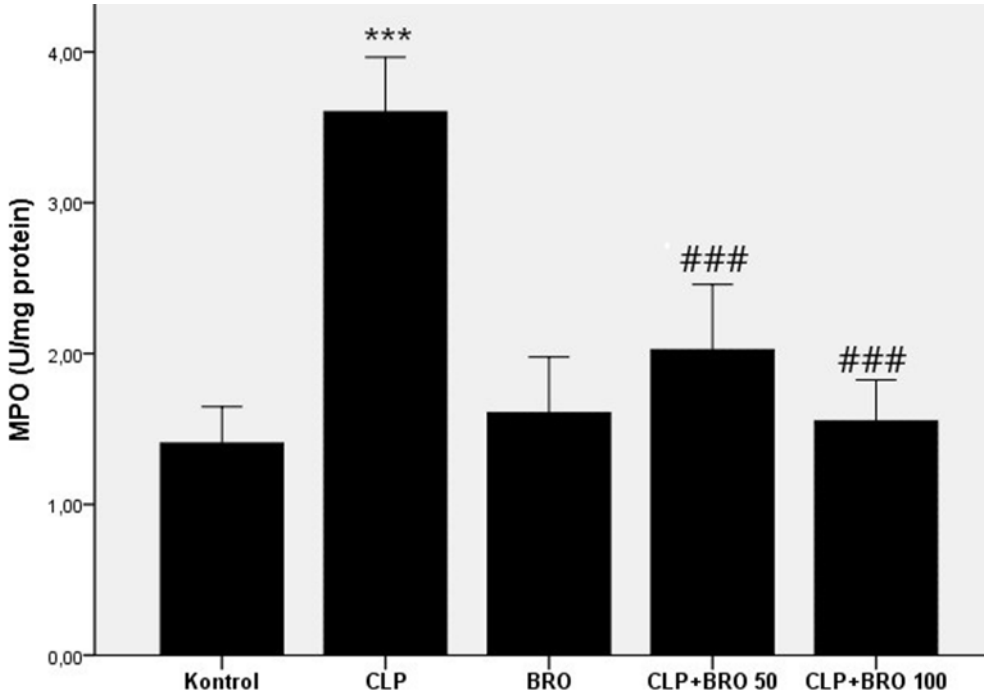
İstatistiksel Analiz

İstatistiksel analiz için SPSS versiyon 20.0 bilgisayar programı kullanıldı. Grupların biyokimyasal ve histopatolojik verilerinin analizi ve karşılaştırılmasında tek yönlü varyans analizi (ANOVA) ve Tukey karşılaştırma testi kullanıldı. İstatistiksel analiz sonuçları ortalama ± standart sapma (SD) olarak ifade edildi. $p<0,05$ istatistiksel olarak anlamlı kabul edildi.

BULGULAR

BRO'nun ve CLP'nin Akciğer Dokusu MPO Aktivitesine Etkisi

İnflamasyon belirteçlerinden MPO aktivitesi Şekil 1'de gösterilmiştir. CLP modeli sıçanlarda akciğer dokusunda MPO aktivitesini arttırarak inflamasyon düzeyini arttırmıştır. BRO'nun CLP uygulaması ile birlikte uygulandığında MPO aktivitesini, CLP grubuna kıyasla önemli ölçüde azalttığı tespit edilmiştir ($p<0,001$).



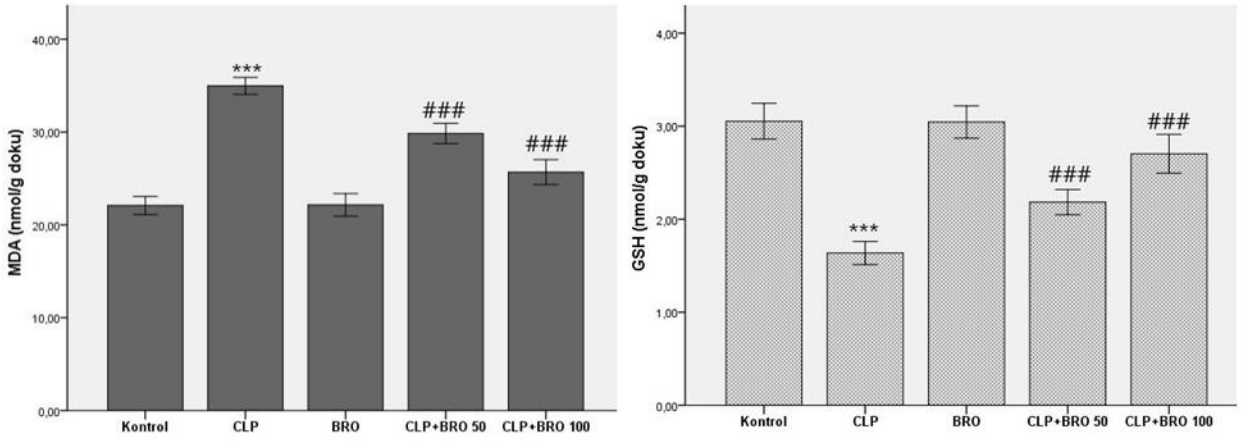
Şekil 1: Tüm deney gruplarına ait myeloperoksidaz (MPO) sonuçları. *** Kontrol grubuna karşı $p<0,001$, ### CLP grubuna karşı $p<0,001$

Figure 1: Myeloperoxidase (MPO) results of all experimental groups. *** $p<0.001$ vs. control group, ### $p<0.001$ vs. CLP group.

BRO'nun ve CLP'nin Akciğer Dokusu MDA ve GSH Düzeylerine Etkisi

Sıçan akciğer dokusunda analiz edilen MDA ve GSH düzeyleri Şekil 2'de gösterilmiştir. Akciğer dokuları MDA düzeylerinde kontrol grubuna kıyasla CLP oluşturulan grupta artış olduğu tespit edilmiştir. BRO'nun (50 ve 100 mg/kg) CLP ile birlikte uygulanması, sepsisin neden olduğu artan MDA seviyesini doza bağlı olarak önemli ölçüde hafifletmiştir ($p<0,001$). GSH seviyesi, CLP

grubunda kontrol grubuna göre akciğer dokusunda önemli ölçüde azaldığı tespit edilmiştir (p<0,001). CLP+BRO 50 ve CLP+BRO 100 grupları CLP oluşturulan grubu ile kıyaslandığında akciğer GSH düzeylerinde artış olduğu tespit edilmiştir (p<0,001).

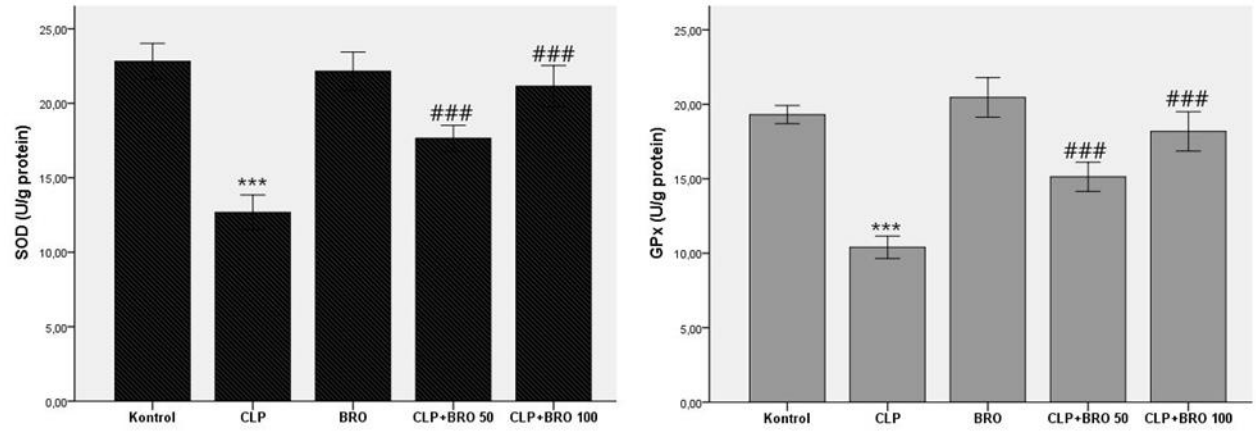


Şekil 2: Tüm deney gruplarına ait malondialdehit (MDA) ve Glutatyon (GSH) sonuçları. *** Kontrol grubuna karşı p<0,001, ### CLP grubuna karşı p<0,001

Figure 2: Malondialdehyde (MDA) and Glutathione (GSH) results of all experimental groups. *** p<0.001 vs. control group, ### p<0.001 vs. CLP group

BRO'nun ve CLP'nin Akciğer Dokusu Antioksidan Enzim Belirteçleri Üzerine Etkisi

Akciğer dokusu antioksidan enzim (SOD, GPx) aktiviteleri Şekil 3'de verilmiştir. Bulgulara göre, CLP grubunda SOD ve GPx enzim aktivitelerinin kontrol grubuna göre düştüğü tespit edilmiştir (p<0,001). CLP ile birlikte BRO uygulanması SOD ve GPx enzim aktivitelerini doza bağlı olarak artırarak iyileşme sağlamıştır (p<0,001).

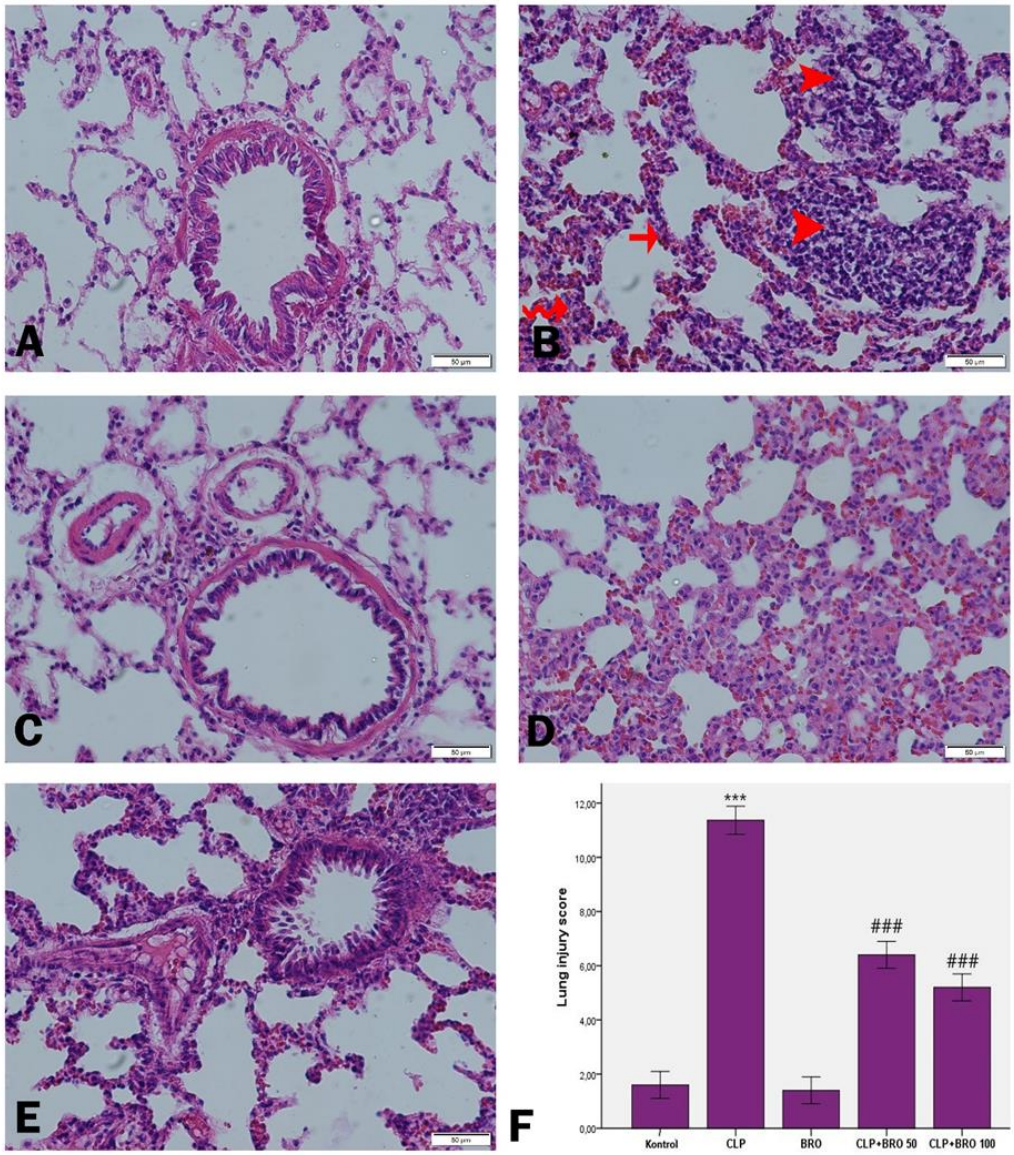


Şekil 3: Tüm deney gruplarına ait Süperoksit dismutaz (SOD) ve Glutatyon peroksidaz (GPx) sonuçları *** Kontrol grubuna karşı p<0,001, ### CLP grubuna karşı p<0,001

Figure 3: Superoxide dismutase (SOD) and Glutathione peroxidase (GPx) results for all experimental groups *** p<0.001 vs. control group, ### p<0.001 vs. CLP group

BRO'nun ve CLP'nin Akciğer Dokusu Histopatolojik Değişiklikler Üzerine Etkisi

H&E boyaması sonucu akciğer dokuları histolojik olarak incelendiğinde kontrol grubu sıçanların akciğer dokuları normal morfolojiye sahip ve belirgin bir hasar yoktu (Şekil 4.A). Özellikle alveol duvarları normal kalınlıkta ve bronşiollerin düzgün histolojik yapı gösterdiği gözlendi. Aynı şekilde BRO grubunda kontrole yakın görüntüler olduğu gözlendi (Şekil 4.C). CLP uygulanan grupta ise akciğer dokularında inflamatuvar hücre infiltrasyonunun dikkat çekici olduğu saptandı. Bu gruptaki akciğerlerin alveol duvarları kalınlaşmış, ödem, kapiller hiperemi olduğu gözlendi. Ayrıca bazı bronşiollerin ve bronşların epitel hücrelerinin deforme olduğu saptandı (Şekil 4.B). Aksine CLP ile birlikte BRO uygulanması akciğer hasarını modüle ettiği gözlendi (Şekil 4.D, 4.E). Akciğer hasar skorları değerlendirildiğinde, kontrol grubuna göre CLP grubunda akciğer hasarının arttığı gözlendi ($p<0,001$). Ancak CLP grubuna göre CLP+BRO gruplarında histolojik skorların düştüğü gözlendi (Şekil 4.F).



Şekil 4: Akciğer dokusunun ışık mikrografları. (A) Kontrol grubu (sahte operasyon), (B) CLP (sepsis) grubu; kıvrık ok: alveolar duvar kalınlığı, ok başı: inflamatuar hücre infiltrasyonu, ok: kanama, (C) BRO (Bromelain) grubu, (D) CLP+BRO 50 (sepsis+ bromelain 50) grubu, (D) CLP+BRO 100 (sepsis + bromelain 100) grubu. (F) Akciğer hasarı, yöntemlerde açıklanan akciğer skorları kullanılarak değerlendirildi. *** Kontrol grubuna karşı $p<0,001$, ### CLP grubuna karşı $p<0,001$. (H&E boyama; bar: 50µm)

Figure 4: Light micrographs of lung tissue. (A) Control group (fake operation), (B) CLP (sepsis) group; curved arrow: alveolar wall thickness, arrowhead: inflammatory cell infiltration, arrow: bleeding, (C) BRO (Bromelain) group, (D) CLP+BRO 50 (sepsis+ bromelain 50) group, (D) CLP+BRO 100 (sepsis) + bromelain 100) group. (F) Lung injury was assessed using lung scores described in the methods. *** $p<0.001$ versus control group, ### $p<0.001$ versus CLP group. (H&E staining; bar: 50µm)

TARTIŞMA

Sepsis, çoklu organ hasarına sebep olan ve mortalitesi giderek artan küresel bir sağlık sorunudur. Özellikle akciğer dokusu sepsis için birincil hedef organdır. Bu bağlamda, sepsisin zararlı etkilerini azaltmak ve tedavi seçenekleri sunmak için araştırmalar giderek artmaktadır. Özellikle son yıllarda tedavi amaçlı olarak, doğal yöntemlere ve fitokimyasallara ilgi artmaktadır. BRO güvenilir, ucuz ve toksik etkisi olmayan doğal bir etken maddedir. Daha önce yapılan çalışmalar, BRO'nun anti-inflamatuvar ve antioksidan etkilerinin olduğunu rapor etmiştir (El-Demerdash ve ark. 2020; Ferah Okkay ve ark. 2023). Fakat sepsis hasarına karşı etkisi ve mekanizması henüz tam olarak bilinmemektedir. Bu çalışmada amaç, sepsis modeli oluşturulan sıçanlarda akciğer doku hasarına karşı BRO'nun etkisini belirlemektir.

Sepsise maruz kalan dokularda ilk savunma hattı nötrofiller, monositler ve makrofajlardır. Sepsis geliştiğinde özellikle nötrofiller inflamatuvar mediyötörleri serbest bırakarak anti-mikrobiyal öldürmeye ve akciğer patolojik değişikliklere katkıda bulunurlar. Ayrıca nötrofiller, sitokinlerin, proteazların ve ROS'ların konsantrasyonlarını da artırır (Giustina ve ark. 2019; Yu ve ark. 2020). İnflamasyon ve sepsisin önemli belirteçlerinden birisi, MPO enzim aktivitesidir (Margotti ve ark. 2022). Mevcut çalışmada, sepsise bağlı olarak MPO seviyesinin arttığı ve bu artışla inflamatuvar yanıtın ortaya çıktığı tespit edilmiştir. Bulgularımıza benzer şekilde literatürdeki diğer sepsis çalışmalarında da MPO aktivitesinin arttığı gösterilmiştir (Baradaran Rahimi ve ark. 2019; Li ve ark. 2019). CLP ile birlikte BRO uygulaması ise MPO aktivitesini düşürerek inflamatuvar yanıtı azaltmıştır.

Sepsisin akciğer üzerindeki patolojik hasar mekanizmalarından birisi, oksidatif strestir (Li ve ark. 2022). Oksidatif stres, ROS'ların artması ve antioksidan kapasitenin baskılanmasına dayanan bir hasar olarak tanımlanır (Akaras ve ark. 2017; Gür ve ark. 2022; Kandemir ve ark. 2022; Semis ve ark. 2022; Şimşek ve ark. 2023a; Şimşek ve ark. 2023b). Sepsis hasarında dokularda yüksek oranda biriken inflamatuvar sitokinler, ROS oluşumunu artırmaktadır. Artan ROS özellikle akciğer hücre zarlarına, lipitlere, proteinlere ve nükleik asitlere zarar verebilmektedir. Ayrıca artan ROS lipid peroksidasyonunu aktive eder ve mitokondriyal membran hasarına sebep olmaktadır (Gür ve ark. 2023; Petronilho ve ark. 2016; Anter ve ark. 2022). Mevcut çalışmada, CLP uygulamasının akciğer dokusunda lipid peroksidasyon belirteçlerinden MDA seviyesini artırdığı ve bu artışın oksidatif strese neden olduğu görülmüştür. CLP ile birlikte BRO uygulaması ise MDA seviyesini doza bağlı olarak azaltmıştır. Yakın zamanda yapılan toksisite çalışmalarında, BRO'nun MDA seviyesini azalttığına dair kanıtlar sunulmuştur (Khazaeel ve ark. 2022; Ferah Okkay ve ark. 2023). Mevcut çalışma verileri değerlendirildiğinde, BRO'nun membran kaynaklı hasarlara karşı koruyucu olabileceği sonucuna varıldı. İnflamasyona bağlı olarak gelişen oksidatif stresin gelişmesinde önemli katkısı olan süreçlerden birisi antioksidanların baskılanması veya inhibe olmasıdır. Oksidatif stres enzimatik ve enzimatik olmayan antioksidan savunma sistemleriyle detoksifiye edilebilir (Kandemir ve ark. 2020; Gur ve ark. 2022). Antioksidanlar, oksidan moleküllerin dokular üzerindeki olumsuz etkilerini sınırlayan ve serbest radikalleri temizleyerek oksidatif hücre hasarına karşı koruyan hücresel savunma mekanizmalarının bir parçasıdır. Hücrede bol miktarda bulunan GSH enzimatik olmayan tripeptit yapıda bir antioksidandır. GSH, oksidanlar ile ksenobiyotiklerin temizlenmesinde ve ilaçların detoksifikasyonunda aktif rol alır (Turk ve ark. 2019). Mevcut çalışmada, akciğer dokularında sepsise bağlı GSH düzeyinin azaldığı tespit edilmiştir. GSH azalması, sepsis kaynaklı ROS'ların artmasına bağlı olarak artan GSH kullanımına bağlı olabilir. CLP ile birlikte BRO uygulamasının, doza bağlı olarak GSH miktarını artırdığı tespit edilmiştir. Sonuç olarak yüksek doz olan 100mg/kg dozunda BRO uygulaması, GSH seviyesini kontrol grubuna yakın bir düzeye getirmiştir. Antioksidan enzimlerinden SOD, süperoksit radikallerinin hidrojen peroksite (H_2O_2) dönüşümünü katalizlerken, GPx ise H_2O_2'yi su ve oksijene dönüştürür (Akaras ve ark. 2023). Mevcut çalışmada, CLP uygulaması sıçan akciğer dokularında, SOD ve GPx enzim aktivitelerinde azalmaya sebep olurken, BRO takviyesinin enzim miktarlarını modüle ettiği tespit edilmiştir. Önceki araştırmalar, çalışmamıza benzer şekilde sepsisin antioksidan enzimlerin aktivitesini azalttığını rapor etmiştir (Cınar ve ark. 2019, Liu ve ark. 2021). Agarwal ve ark. mevcut çalışma ile paralel olarak BRO'nun antioksidan kapasitesini artırdığını rapor etmiştir (Agarwal ve ark. 2016).

Mevcut çalışmada, CLP kaynaklı akciğer histopatolojik bulgular biyokimyasal bulgularla uyumlu sonuçlar göstermiştir. Özellikle CLP uygulaması ile akciğerde inflamatuvar hücre infiltrasyonu, hiperemi ve alveoler duvar kalınlaşması görülmüştür. Hu ve ark. çalışmalarında, sepsisin akciğerde oksidatif strese bağlı olarak konjesyona ve ödeme sebep olduğunu rapor etmiştir (Hu ve ark. 2020). Mevcut çalışmada, CLP ve BRO'nun birlikte uygulanması dejenaratif değişiklikleri iyileştirmiştir.

SONUÇ

Bütün veriler birlikte değerlendirildiğinde, CLP'nin oksidatif stresi ve inflamasyonu arttırarak akciğer dokusunda histopatolojik değişikliklere neden olduğu gösterilmiştir. Diğer taraftan, BRO akciğer dokularında ROS'u temizleme özelliğinden dolayı

oksidatif stresi, inflamasyonu, patolojik değişiklikleri azaltmış ve CLP'ye karşı koruma sağlamıştır.

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KAYNAKLAR

Agarwal, S., Chaudhary, B., & Bist, R. (2016). Bacoside A and bromelain relieve dichlorvos induced changes in oxidative responses in mice serum. *Chemico-biological interactions*, *254*, 173–178. https://doi.org/10.1016/j.cbi.2016.05.017

Akaras, N., Bal, T., Atilay, H., Selli, J., & Halici, M. B. (2017). Protective effects of agomelatine on testicular damage caused by bortezomib. *Biotechnic&histochemistry:official publication of the Biological Stain Commission*, *92*(8), 552–559. https://doi.org/10.1080/10520295.2017.1350748

Akaras, N., Gur, C., Kucukler, S., & Kandemir, F. M. (2023). Zingerone reduces sodium arsenite-induced nephrotoxicity by regulating oxidative stress, inflammation, apoptosis and histopathological changes. *Chemico-biological interactions*, *374*, 110410. https://doi.org/10.1016/j.cbi.2023.110410

Anter, A., Ahmed, A. F., Hammad, A. S. A., Almalki, W. H., Abdel Hafez, S. M. N., Kasem, A. W., El-Moselhy, M. A., Alrabia, M. W., Ibrahim, A. R. N., & El-Daly, M. (2022). The Severity of Acute Kidney and Lung Injuries Induced by Cecal Ligation and Puncture Is Attenuated by Menthol: Role of Proliferating Cell Nuclear Antigen and Apoptotic Markers. *Frontiers in medicine*, *9*, 904286. https://doi.org/10.3389/fmed.2022.904286

Baradaran Rahimi, V., Rakhshandeh, H., Raucci, F., Buono, B., Shirazinia, R., Samzadeh Kermani, A., Maione, F., Mascolo, N., & Askari, V. R. (2019). Anti-Inflammatory and Anti-Oxidant Activity of *Portulaca oleracea* Extract on LPS-Induced Rat Lung Injury. *Molecules (Basel, Switzerland)*, *24*(1), 139. https://doi.org/10.3390/molecules24010139

Bradley, P. P., Priebat, D. A., Christensen, R. D., & Rothstein, G. (1982). Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. *The Journal of investigative dermatology*, *78*(3), 206–209. https://doi.org/10.1111/1523-1747.ep12506462

Chen, G., Hou, Y., Li, X., Pan, R., & Zhao, D. (2021). Sepsis-induced acute lung injury in young rats is relieved by calycosin through inactivating the HMGB1/MyD88/NF-κB pathway and NLRP3 inflammasome. *International immunopharmacology*, *96*, 107623. https://doi.org/10.1016/j.intimp.2021.107623

Chen, X., Wang, Y., Xie, X., Chen, H., Zhu, Q., Ge, Z., Wei, H., Deng, J., Xia, Z., & Lian, Q. (2018). Heme Oxygenase-1 Reduces Sepsis-Induced Endoplasmic Reticulum Stress and Acute Lung Injury. *Mediators of inflammation*, *2018*, 9413876. https://doi.org/10.1155/2018/9413876

Cinar, I., Sirin, B., Aydin, P., Toktay, E., Cadirci, E., Halici, I., & Halici, Z. (2019). Ameliorative effect of gossypin against acute lung injury in experimental sepsis model of rats. *Life sciences*, *221*, 327–334. https://doi.org/10.1016/j.lfs.2019.02.039

El-Demerdash, F. M., Baghdadi, H. H., Ghanem, N. F., & Mhanna, A. B. A. (2020). Nephroprotective role of bromelain against oxidative injury induced by aluminium in rats. *Environmental toxicology and pharmacology*, *80*, 103509. https://doi.org/10.1016/j.etap.2020.103509

Eraslan, E., Tanyeli, A., Akdemir, F. N. E., & Güler, M. C. (2020). Reno-Protective Effects of 6-Shogaol on Kidney Tissue in Cecal Ligation and Puncture-Induced Polymicrobial Sepsis Rat Model. Atatürk Üniversitesi Veteriner Bilimleri Dergisi, 15(3), 244-250.

Ferah Okkay, I., Okkay, U., Bayram, C., Cicek, B., Sezen, S., Aydin, I. C., Mendil, A. S., & Hacimuftuoglu, A. (2023). Bromelain protects against cisplatin-induced ocular toxicity through mitigating oxidative stress and inflammation. *Drug and chemical toxicology*, *46*(1), 69–76. https://doi.org/10.1080/01480545.2021.2011308

Gerin, F., Sener, U., Erman, H., Yilmaz, A., Aydin, B., Armutcu, F., & Gurel, A. (2016). The Effects of Quercetin on Acute Lung Injury and Biomarkers of Inflammation and Oxidative Stress in the Rat Model of Sepsis. *Inflammation*, *39*(2), 700–705. https://doi.org/10.1007/s10753-015-0296-9

Giustina, A. D., Danielski, L. G., Novochadlo, M. M., Goldim, M. P. S., Joaquim, L., Metzker, K. L. L., Carli, R. J., Denicol, T., Cidreira, T., Vieira, T., & Petronilho, F. (2019). Vitamin B6 reduces oxidative stress in lungs and liver in experimental sepsis. *Anais da Academia Brasileira de Ciencias*, *91*(4), e20190434. https://doi.org/10.1590/0001-3765201920190434

Gur, C., Kandemir, F. M., Caglayan, C., & Satıcı, E. (2022). Chemopreventive effects of hesperidin against paclitaxel-induced hepatotoxicity and nephrotoxicity via amendment of Nrf2/HO-1 and caspase-3/Bax/Bcl-2 signaling pathways. *Chemico-biological interactions*, *365*, 110073. https://doi.org/10.1016/j.cbi.2022.110073

Cihan, G. Ü. R., Kandemir, Ö., & Kandemir, F. M. (2022). Ratlarda diklofenak ile indüklenen kardiyotoksisite üzerine krisinin etkilerinin oksidatif stres, endoplazmik retikulum stresi ve apoptoz belirteçleri ile değerlendirilmesi. *Kocatepe Veterinary Journal*, *15*(2), 151-160.

Gur, C., Akarsu, S. A., Akaras, N., Tuncer, S. C., & Kandemir, F. M. (2023). Carvacrol reduces abnormal and dead sperm counts by attenuating sodium arsenite-induced oxidative stress, inflammation, apoptosis, and autophagy in the testicular tissues of rats. Environmental toxicology, 38(6), 1265–1276. https://doi.org/10.1002/tox.23762

Hu, Q., Wang, Q., Han, C., & Yang, Y. (2020). Sufentanil attenuates inflammation and oxidative stress in sepsis-induced acute lung injury by downregulating KNG1 expression. *Molecular medicine reports*, *22*(5), 4298–4306. https://doi.org/10.3892/mmr.2020.11526

Kandemir, F. M., Caglayan, C., Aksu, E. H., Yildirim, S., Kucukler, S., Gur, C., & Eser, G. (2020). Protective effect of rutin on mercuric chloride-induced reproductive damage in male rats. *Andrologia*, *52*(3), e13524. https://doi.org/10.1111/and.13524

Kandemir, F. M., Ileriturk, M., & Gur, C. (2022). Rutin protects rat liver and kidney from sodium valproate-induce damage by attenuating oxidative stress, ER stress, inflammation, apoptosis and autophagy. *Molecular biology reports*, *49*(7), 6063–6074. https://doi.org/10.1007/s11033-022-07395-0

Khazaeel, K., Rad, O. R., Jamshidian, J., Tabandeh, M. R., Mohammadi, G., & Atashfaraz, A. (2022). Effect of bromelain on sperm quality, testicular oxidative stress and expression of oestrogen receptors in bisphenol-A treated male mice. *Andrologia*, *54*(11), e14584. https://doi.org/10.1111/and.14584

Lawrence, R. A., & Burk, R. F. (1976). Glutathione peroxidase activity in selenium-deficient rat liver. *Biochemical and biophysical research communications*, *71*(4), 952–958. https://doi.org/10.1016/0006-291x(76)90747-6

Li, L., Wang, H., Zhao, S., Zhao, Y., Chen, Y., Zhang, J., Wang, C., Sun, N., & Fan, H. (2022). Paeoniflorin ameliorates lipopolysaccharide-induced acute liver injury by inhibiting oxidative stress and inflammation via SIRT1/FOXO1a/SOD2 signaling in rats. *Phytotherapy research: PTR*, *36*(6), 2558–2571. https://doi.org/10.1002/ptr.7471

Li, R., Ren, T., & Zeng, J. (2019). Mitochondrial Coenzyme Q Protects Sepsis-Induced Acute Lung Injury by Activating PI3K/Akt/GSK-3*β*/mTOR Pathway in Rats. *BioMed research international*, *2019*, 5240898. https://doi.org/10.1155/2019/5240898

Liu, Y., Mu, S., Li, X., Liang, Y., Wang, L., & Ma, X. (2019). Unfractionated Heparin Alleviates Sepsis-Induced Acute Lung Injury by Protecting Tight Junctions. *The Journal of surgical research*, *238*, 175–185. https://doi.org/10.1016/j.jss.2019.01.020

Liu, Y., Zhang, Y., Feng, Q., Liu, Q., Xie, J., Li, H., Yang, F., Liu, X., Gao, W., Bai, X., Li, Z., & Wang, Y. (2021). GPA Peptide Attenuates Sepsis-Induced Acute Lung Injury in Mice via Inhibiting Oxidative Stress and Pyroptosis of Alveolar Macrophage. *Oxidative medicine and cellular longevity*, *2021*, 5589472. https://doi.org/10.1155/2021/5589472

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. The Journal of biological chemistry, *193*(1), 265–275.

Margotti, W., Goldim, M. P. S., Machado, R. S., Bagio, E., Dacoregio, C., Bernades, G., Lanzzarin, E., Stork, S., Cidreira, T., Denicol, T. L., Joaquim, L., Danielski, L. G., Metzker, K. L. L., Bonfante, S., Margotti, E., & Petronilho, F. (2022). Oxidative stress in multiple organs after sepsis in elderly rats. *Experimental gerontology*, *160*, 111705. https://doi.org/10.1016/j.exger.2022.111705

Petronilho, F., Florentino, D., Danielski, L. G., Vieira, L. C., Martins, M. M., Vieira, A., Bonfante, S., Goldim, M. P., & Vuolo, F. (2016). Alpha-Lipoic Acid Attenuates Oxidative Damage in Organs After Sepsis. *Inflammation*, *39*(1), 357–365. https://doi.org/10.1007/s10753-015-0256-4

Placer, Z. A., Cushman, L. L., & Johnson, B. C. (1966). Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. *Analytical biochemistry*, *16*(2), 359–364. https://doi.org/10.1016/0003-2697(66)90167-9

Sedlak, J., & Lindsay, R. H. (1968). Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Analytical biochemistry*, *25*(1), 192–205. https://doi.org/10.1016/0003-2697(68)90092-4

Semis, H. S., Gur, C., Ileriturk, M., Kandemir, F. M., & Kaynar, O. (2022). Evaluation of Therapeutic Effects of Quercetin Against Achilles Tendinopathy in Rats via Oxidative Stress, Inflammation, Apoptosis, Autophagy, and Metalloproteinases. *The American journal of sports medicine*, *50*(2), 486–498. https://doi.org/10.1177/03635465211059821

Simsek, H., & Akaras, N. (2023). Acacetin ameliorates acetylsalicylic acid-induced gastric ulcer in rats by interfering with oxidative stress, inflammation, and apoptosis. *International Journal of Medical Biochemistry*, *6*(2).

Sun, Y., Oberley, L. W., & Li, Y. (1988). A simple method for clinical assay of superoxide dismutase. *Clinical chemistry*, *34*(3), 497–500.

Şehirli, A. Ö., Sayiner, S., Savtekin, G., & Velioğlu-Öğünç, A. (2021). Protective effect of bromelain on corrosive burn in rats. Burns : journal of the International Society for Burn Injuries, 47(6), 1352–1358.

Şimşek, H., Akaras, N., Gür, C., Küçükler, S., & Kandemir, F. M. (2023). Beneficial effects of Chrysin on Cadmium-induced nephrotoxicity in rats: Modulating the levels of Nrf2/HO-1, RAGE/NLRP3, and Caspase-3/Bax/Bcl-2 signaling pathways. *Gene*, *875*, 147502. Advance online publication. https://doi.org/10.1016/j.gene.2023.147502

Turk, E., Kandemir, F. M., Yildirim, S., Caglayan, C., Kucukler, S., & Kuzu, M. (2019). Protective Effect of Hesperidin on Sodium Arsenite-Induced Nephrotoxicity and Hepatotoxicity in Rats. *Biological trace element research*, *189*(1), 95–108. https://doi.org/10.1007/s12011-018-1443-6

Yu, H., Liu, Y., Wang, M., Restrepo, R. J., Wang, D., Kalogeris, T. J., Neumann, W. L., Ford, D. A., & Korthuis, R. J. (2020). Myeloperoxidase instigates proinflammatory responses in a cecal ligation and puncture rat model of sepsis. *American journal of physiology. Heart and circulatory physiology*, *319*(3), H705–H721. **https://doi.org/10.1152/ajpheart.00440.2020**

Zeng, M., He, W., Li, L., Li, B., Luo, L., Huang, X., Guan, K., & Chen, W. (2015). Ghrelin attenuates sepsis-associated acute lung injury oxidative stress in rats. *Inflammation*, *38*(2), 683–690. https://doi.org/10.1007/s10753-014-9977-z

Zhang, J., Wang, C., Wang, H., Li, X., Xu, J., & Yu, K. (2021). Loganin alleviates sepsis-induced acute lung injury by regulating macrophage polarization and inhibiting NLRP3 inflammasome activation. International immunopharmacology, 95, 107529. https://doi.org/10.1016/j.intimp.2021.107529

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RESEARCH ARTICLE

Investigation of the Effects of Rutin on Valproic Acid Induced Testicular Damage in Rats

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ABSTRACT

Valproic acid (VALP) is a drug used for many psychiatric diseases such as epilepsy. However, the use of VALP has potential side effects on various tissues, including the testicles. Rutin (RUT) is a flavonoid with protective effects against oxidative stress-induced diseases and lipid peroxidation. In this study, the protective effects of RUT against testicular damage caused by VALP were investigated. For this purpose, 35 male Spraque-Dawley rats weighing 220-250 g were used in the study. The rats were randomly divided into 5 groups as Control (physiological saline), RUT (100 mg/kg/bw), VALP, (500mg/kg/bw), VALP+RUT 50 (500 mg/kg/bw VALP+50 mg/kg/bw RUT), and VALP +RUT 100 (500 mg/kg/bw VALP+100 mg/kg/bw RUT). At the end of the RUT and VALP administrations, the rats were sacrificed and testicular tissues were taken to be used for biochemical and spermatological analyzes. According to the results of this study, the MDA level in the testicular tissues of the VALP group was found to be statistically higher than the other experimental groups ($p<0.05$). Testicular tissue GSH and Nrf-2 level was the lowest in the VALP group. TNF-α, IL-1B and MAPK14 levels in testicular tissue were highest in the VALP group, while it was decreased in the RUT treatment groups. Similarly, the highest levels of Bax, Caspase-3 and MMP-9 were observed in the VALP group, while RUT treatment decreased this value. When the spermatological analyzes were examined, it was observed that the total motility value decreased significantly in the VALP group, but the total motility value increased in the RUT treatment group ($p<0.05$). A significant increase in the rate of dead and abnormal sperm was found in the VALP group, but these parameters improved in the RUT treatment group. As a result, RUT has protective effects against VALP-induced testicular damage in rats.

Keywords; Oxidative stress, rat, rutin, semen, valproic acid

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Sıçanlarda Valproik Asite Bağlı Testis Hasarına Rutin Etkisinin Araştırılması

ÖZ

Valproik asit (VALP), epilepsi gibi birçok psikiyatrik hastalık için kullanılan bir ilaçtır. Ancak VALP kullanımının testisler de dahil olmak üzere çeşitli dokularda potansiyel yan etkileri vardır. Rutin (RUT), oksidatif stres kaynaklı hastalıklara ve lipid peroksidasyonuna karşı koruyucu etkileri olan bir flavonoiddir. Bu çalışmada VALP'in neden olduğu testis hasarına karşı RUT'un koruyucu etkisi araştırıldı. Bu amaçla çalışmada ağırlıkları 220-250 g olan 35 adet erkek Sprague-Dawley cinsi rat kullanıldı. Ratlar kontrol (serum fizyolojik), RUT (100 mg/kg/va), VALP, (500mg/kg/va), VALP+RUT 50 (500 mg/kg/va VALP+50 mg/kg/va RUT) ve VALP +RUT 100 (500 mg/kg/v VALP+100 mg/kg/va RUT) olarak 5 gruba ayrıldı. Uygulamalarının sonunda ratlar sakrifiye edilerek testis dokuları biyokimyasal ve spermatolojik analizlerde kullanılmak üzere alındı. Sunulan çalışmanın sonuçlarına göre VALP grubunun testis dokularındaki MDA düzeyi diğer deney gruplarına göre istatistiksel olarak yüksek bulundu ($p<0,05$). Testis dokusu GSH ve Nrf-2 düzeyi VALP grubunda en düşüktü. Testis dokusunda TNF-α, IL-1β ve MAPK14 seviyeleri VALP grubunda en yüksek iken, RUT tedavi gruplarında azaldı. Benzer şekilde en yüksek Bax, Caspase-3 ve MMP-9 seviyeleri VALP grubunda gözlenirken, RUT tedavisi bu değeri azaltmıştır. Spermatolojik analizler incelendiğinde total motilite değerinin VALP grubunda anlamlı olarak düştüğü, ancak RUT tedavi grubunda total motilite değerinin arttığı görüldü ($p<0,05$). VALP grubunda ölü ve anormal sperm oranında önemli bir artış bulundu, ancak bu parametreler RUT tedavi grubunda düzeldi. Sonuç olarak RUT, sıçanlarda VALP'nin neden olduğu testis hasarına karşı koruyucu etkilere sahiptir.

Anahtar kelimeler; Oksidatif stres, rat, rutin, sperma, valproik asit.

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INTRODUCTION

Valproic acid (VALP, 2-propylpentanoic acid) or valproate is an eight-carbon branched chain fatty acid used as an antiepileptic drug (Tolou-Ghamari & Palizban, 2015). VALP and its salts are among non-specific histone deacetylase inhibitors (Zhou et al., 2020). VALP has been used for its anti-migraine, neuroprotective, and anti-manic properties (Safdar & Ismail, 2022). Despite its medical use, side effects are reported after VALP administration (Abdelkader et al., 2020; Pourahmad et al., 2012; Saleh et al., 2012). VALP can cause dyspepsia, obesity, hematological toxicity, teratogenicity, hepatotoxicity, nephrotoxicity and reproductive toxicity (Adewole et al., 2021; Galaly et al., 2014; Kandemir et al., 2022). VALP induces oxidative stress and inflammation, leading to toxication (Jin et al., 2014; Tong et al., 2005). Like other histone deacetylase (HDAC) inhibitors, VALP has the potential to alter the expression of protein levels (Bradbury et al., 2005). Increased levels of phospho-nuclear factor kappa beta (p-Nf-kB) and Caspase-3 were observed in testicular damage induced by VALP (Savran et al., 2020). Moreover, VALP causes a decrease in testicular weight and sperm quality in rats (Alsemeh et al., 2022).

Flavonoids are known for their anti-inflammatory and anti-apoptotic properties (Akaras, Gur, et al., 2023; Akaras, Ileriturk, et al., 2023; Ileriturk et al., 2023; Kandemir et al., 2017; Şimşek et al., 2023; Şimşek et al., 2023; Yardim et al., 2020). Rutin (RUT) is a flavone with antioxidant and anti-inflammatory effects, consisting of quercetin and the disaccharide rutinose (Aktaş et al., 2017; Kandemir, Caglayan, et al., 2020). Previous studies indicate that RUT has antidiabetic (Kamalakkannan & Prince, 2006), neuroprotective (Ola et al., 2015), and cardioprotective (Wang et al., 2015) effects. In addition, RUT also has protective effects against testicular toxicity (Abarikwu et al., 2013; Hozayen, 2012). It has also been determined that RUT has protective effects in rats with testicular ischemia perfusion (Akondi et al., 2011; Wei et al., 2011). In acrylamide administered rats, RUT reduced testicular toxicity (Salem et al., 2017). In addition, RUT has protective effects against testicular toxicity caused by doxorubicin (Hozayen, 2012), lead acetate (Ansar et al., 2015) and busulfan (Abarikwu et al., 2022) induced in rat.

In the literature review, we could not find a study examining the effects of RUT against VALP-induced testicular damage. In this study, the protective properties of RUT against VALP induced testicular toxicity were investigated.

MATERIAL AND METHODS

Animals and Ethical Approval

Presented study 35 male Sprague Dawley rats, 10-12 weeks old and weighing 220-250 g, were used. Animals were purchased from …… University Medical Experiment Application and Research Center and the study was conducted here. Animals were provided with feed and water ad libitum throughout the study. Ethical approval was obtained for the study from …… University Animal Experiments Local Ethics Committee (Protocol No: 2023-4-5). VALP (Depakin) was purchased from the pharmaceutical company Sanofi (Sanofi, France). Other chemicals were purchased from Sigma (Sigma Aldrich Company, USA) unless otherwise stated.

In the study, five different groups consisting of seven rats in each group were formed. The rats in the control group were given oral saline solution daily for 14 days. Rats in the RUT group were given oral RUT at a dose of 100 mg/kg/bw for 14 days. The rats in the VALP group were given sodium valproate orally at a dose of 500 mg/kg/bw for 14 days. Rats in the VALP+ RUT 50 group were given orally 500 mg/kg sodium valproate + RUT 50 mg/kg/bw. Rats in the VALP+ RUT 100 group were given orally 500 mg/kg/bw sodium valproate + RUT 100 mg/kg/bw. At the end of the study, the rats were sacrificed under mild sevoflurane (Abbvie, England) anesthesia and testicular tissues were taken. While testicular tissues were used for biochemical analysis, cauda epididymis parts were used for semen analysis.

Testicular tissue oxidative stress analysis

The measurement of malondialdehyde (MDA) was determined as a lipid peroxidation marker by its reaction with thiobarbituric acid. Testicular tissue MDA analyzes were determined by the method described by Placer et al.(1966) Glutathion (GSH) level was measured for antioxidant status in testicular tissue. Testicular tissue GSH analyzes were determined by the method described by Sedlak and Lindsay's(1968).

RT-PCR Analysis

After VALP and RUT treatments in testis tissues, mRNA transcript levels of tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), mitogen-activated protein kinase 14 (MAPK14), Bcl-2-associated X protein (Bax), Caspase-3, nuclear factor erythroid 2-related factor 2 (Nrf-2) and matrix metallopeptidase 9 (MMP-9) genes were analyzed by RT-PCR method. In the first step of the assays, total RNA was isolated from tissues using QIAzol Lysis Reagent (79306; Qiagen) according to the manufacturer's instructions. The concentration of total RNAs was measured in the NanoDrop (BioTek Epoch) device. In the second step, total RNAs were converted into cDNAs with the iScript cDNA Synthesis Kit (Bio-Rad). In the third and final step, a mixture of primers of the relevant genes, cDNAs, iTaq Universal SYBR Green Supermix (BIORAD) and DNase/RNase-free water was prepared

according to the manufacturer's instructions. The mixture was then reacted in the Rotor-Gene Q (Qiagen) device at the temperature cycles given by the manufacturer. After the cycles were completed, the CT values taken from the device were normalized according to β -actin with the 2-^{deltadeltaCT} method developed by Livak KJ and Schmittgen TD (2001). Primer sequences are given in Table 1.

Table 1. Sequences of primers

Gene	Sequences (5'-3')	Length (bp)	Reference No
Nrf2	F: TTTGTAGATGACCATGAGTCGC R: TCCTGCCAAACTTGCTCCAT	161	NM_031789.2
TNF-α	F: CTCGAGTGACAAGCCCGTAG R: ATCTGCTGGTACCACCAGTT	139	NM_012675.3
IL-1β	F: ATGGCAACTGTCCCTGAACT R: AGTGACACTGCCTTCCTGAA	197	NM_031512.2
MAPK14	F: GTGGCAGTGAAGAAGCTGTC R: GTCACCAGGTACACATCGTT	170	NM_031020.2
Bax	F: TTTCATCCAGGATCGAGCAG R: AATCATCCTCTGCAGCTCCA	154	NM_017059.2
Caspase-3	F: ACTGGAATGTCAGCTCGCAA R: GCAGTAGTCGCCTCTGAAGA	270	NM_012922.2
MMP9	F: AGCTGGCAGAGGATTACCTG R: ATGATGGTGCCACTTGAGGT	230	NM_031055.2
β-Actin	F: CAGCCTTCCTTCTTGGGTATG R: AGCTCAGTAACAGTCCGCCT	360	NM_031144.3

Semen Analysis

Testes taken from rats were separated from the cauda epididymis. The cauda epididymis was trimmed in 5 mL saline in a Petri dish. The resulting liquid was used in semen analysis. Sperm motility determination was determined by the method described by Aksu et al.(2021) Approximately 20 µL semen sample was dripped onto the heating plate, covered with a coverslip and examined with a light microscope (Zeiss Primo Star; Carl Zeiss, Oberkochen, Germany). The results were calculated as percentage.

For density analysis, 10 µL of semen fluid was taken and 990 µL physiological saline was added to it. It was vortexed for 15 sec at 2500 rpm and counted at 400X magnification on a Thoma slide to ensure homogeneity. The mean value of both compartments was multiplied by 5×10^6 and the result was considered as semen density (Aksu et al., 2018; Gur, Akarsu, et al., 2022). The rate of dead spermatozoa and sperm abnormalities were performed by the method described by Aksu et al. (2017). 10 µL of semen sample was taken and 10 µl of eosin-nigrosin mixture was dripped onto it, mixed with a coverslip and smear was taken. Dried smears were evaluated under a light microscope. To calculate the rate of dead spermatozoa and abnormal spermatozoa, 200 sperm cells were examined on each slide. Results were expressed as percentage.

Statistical Analysis

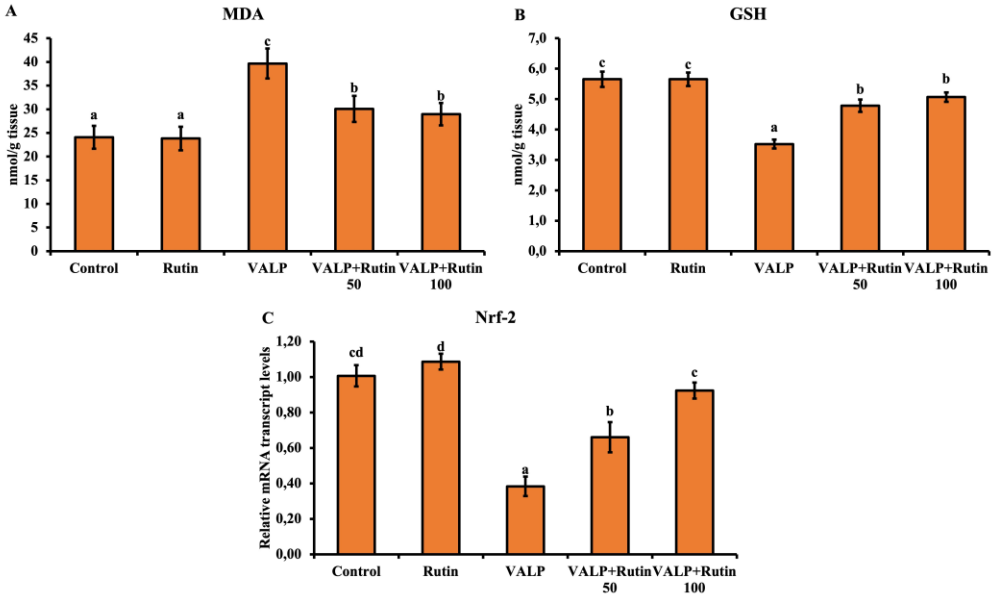
Statistical evaluation of the data obtained from the study was made in IBM SPSS (Version 26.0) program. One-way ANOVA and Tukey post hoc tests were used to determine whether there was a statistical difference between the groups. The results were given as mean ± standart deviation. $p<0,05$ was considered statistically significant.

RESULTS

Oxidative stress analysis results

MDA and GSH levels and Nrf-2 mRNA transcript levels in testicular tissue are shown in Figure 1. Accordingly, it is noteworthy that in the VALP group, the level of MDA, which is a biomarker of lipid peroxidation, increased, and the level of GSH and Nrf-2 mRNA transcripts decreased ($p<0,05$). However, it was observed that the MDA level decreased and the GSH level increased in the RUT treatment groups. Nrf-2 level increased dose-dependently with RUT treatment ($p<0.05$).

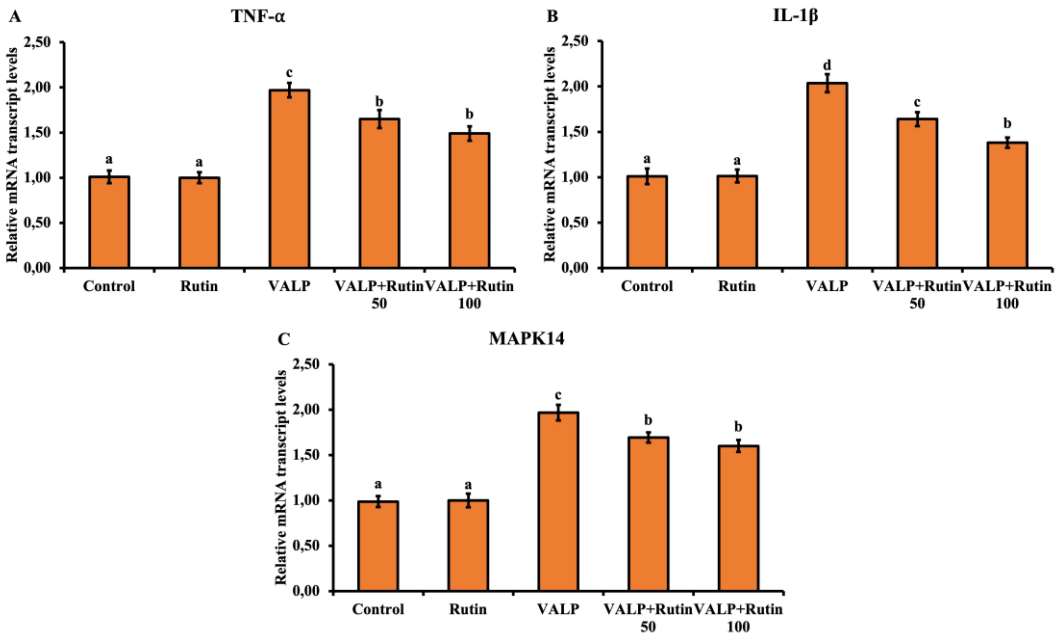
Figure 1. Testicular tissue MDA, GSH and Nrf-2 levels.



Inflammation-related gene expression level results

TNF-α, IL-1β and MAPK14 mRNA transcript levels in testicular tissue are shown in Figure 2. TNF-α and MAPK14 gene expression levels were significantly decreased in the RUT treatment groups compared to the VALP group. IL-1β mRNA transcript level decreased in a dose-dependent manner with RUT treatment ($p<0,05$).

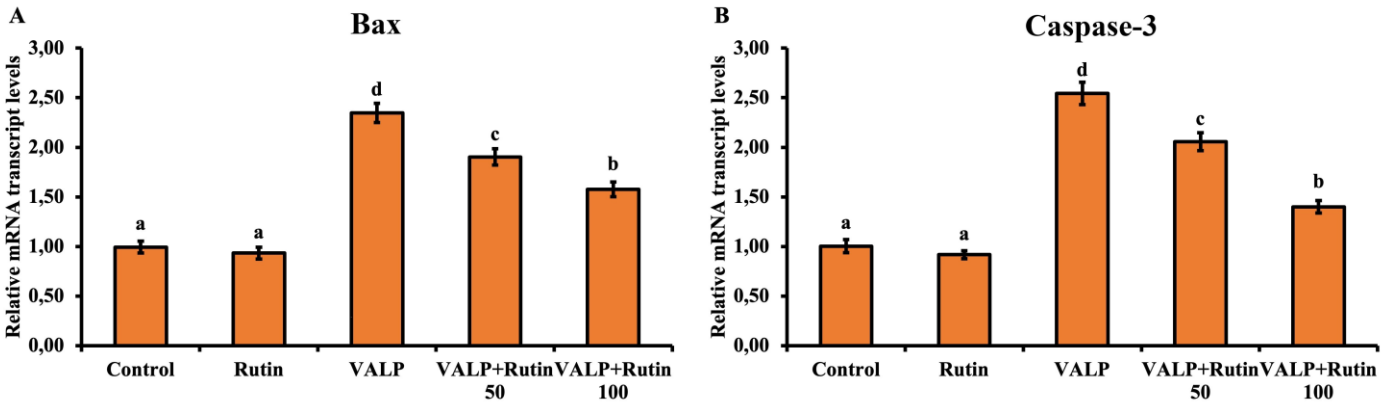
Figure 2. Testicular tissue TNF-α, IL-1β and MAPK14 mRNA transcript levels.



Bax, Caspase-3 level results in testis tissue

The mRNA transcript level results of Bax and Caspase-3, which are apoptosis-related genes in testis tissue, are shown in Figure 3. The expression levels of Bax and Caspase-3 genes were increased in the VALP group. Bax and Caspase-3 levels were significantly decreased in the RUT treatment groups compared to the VALP group in a dose-dependent manner ($p<0,05$).

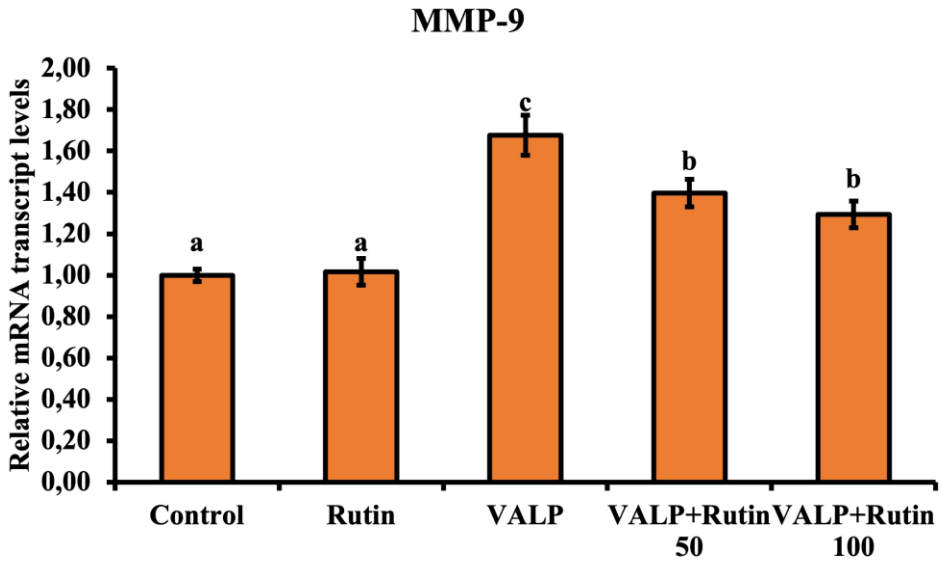
Figure 3. Testicular tissue Bax and Caspase-3 mRNA trancripts levels.



mRNA Transcript Levels of Metalloproteinases in Testis Tissue

The testicular tissue MMP9 gene expression level of rats in the whole experimental group is shown in Figure 4. Accordingly, it was determined that MMP9 expression level increased in testicular tissue after VALP application, while RUT treatment decreased these expression levels ($p<0.05$).

Figure 4. Testis tissue MMP9 levels.



Semen Analysis Results

The epididymal semen analysis results of the rats in the study groups are shown in Table 2. In the VALP group, it was observed that the total motility value decreased significantly compared to the other experimental groups. In addition, the rate of dead and abnormal sperm was significantly increased in the VALP group ($p<0,05$). Sperm quality improved in the RUT treatment group. However, there was no difference between the groups in terms of sperm density and testicular weight.

Table 2. Spermatological parameter results

	Total Motility (%)	Density x10⁶	Live-Dead Spermatozoa Rate(%)	Abnormal Spermatozoon Rate(%)	Total Testis Weight (mg)
Control	72,75±2,48[a]	73±2,36	11,06±2,04[ab]	11,01±1,67[ab]	2935,66±168,71
RUT	82,21±4,03[b]	76,5±3,55	8,83±0,98[a]	10,16±0,98[a]	2881,33±243,16
VALP	62,18±4,02[c]	74,863±2,4	16,33±2,16[c]	15,01±4,43[b]	2956,33±218,42
VALP+RUT 50	78,80±5,54[b]	72,33±3,14	12,83±1,83[b]	13,51±1,04[ab]	3089,16±385,02
VALP+RUT 100	79,95±4,22[b]	73±2,01	12,23±2,92[b]	12,33±0,81[ab]	2961,33±160,12

DISCUSSION

VALP has long been widely used as a broad-spectrum anticonvulsant drug (Nalivaeva et al., 2009). However, VALP has a toxic effect on many tissues, including the reproductive system (Bairy et al., 2010; Kandemir et al., 2022; Mandana et al., 2013). In this study, the effects of RUT against testicular toxicity induced by VALP were tried to be determined by biochemical pathways and spermatological analyzes.

Toxic compounds are sources of oxidative stress in testicles (Akaras et al., 2020; Belhan et al., 2017; Gur, Kandemir, et al., 2022). Oxidative stress is one of the main mechanisms in VALP toxicity. Measurement of MDA levels is necessary to determine lipid peroxidation, which is an indicator of oxidative stress (Kandemir et al., 2022). Previous studies show that VALP induces an increase in MDA level in various tissues (Hamza & Amin, 2007; Raza et al., 1997). In the presented study, it was determined that VALP caused an increase in lipid peroxidation and thus in MDA levels. However, it was determined that RUT administration in the treatment groups decreased the MDA level. This may be due to increased free radicals released by VALP.

GSH is a non-enzymatic antioxidant compound (Kucukler et al., 2020). Decreased levels of antioxidant substances cause oxidative damage in testis tissue (Akaras et al., 2017; Aksu et al., 2019). It has been stated in previous studies that RUT is a good antioxidant scavenger (Moshahid Khan et al., 2012). In our study, it was observed that there was a significant decrease in GSH levels in the VALP group and RUT treatment provided a significant improvement in GSH levels. Nrf2 is one of the cellular responses to oxidative damage (Ma, 2013). Nrf2 is suppressed when ROS is excessive (Gur & Kandemir, 2023). VALP leads to a decrease in Nrf2 levels in various tissues(Adewole et al., 2021).

In our study, a significant decrease in Nrf2 level occurred in the VALP group. There was a dose-dependent increase in Nrf2 levels in the RUT treatment groups. This indicates that cellular response to oxidative damage occurs in RUT groups and that RUT is a good reactive oxygen species scavenger.

Inflammation occurs in response to a chemical, physical or biological substance in the body (Ambriz-Pérez et al., 2016). TNF-α, IL-1β and MAPK14 are proinflammatory cytokines (Gur, Kandemir, et al., 2022; Temel et al., 2020) Previous studies have shown that RUT inhibits TNF-α and IL-1β levels in various tissues (Cihan et al., 2022; Kandemir, Caglayan, et al., 2020; Youssef et al., 2022). In the presented study, it was determined that there were significant increases in TNF-α, IL-1β and MAPK14 mRNA transcript levels in testicular tissue triggered by VALP application to rats. It was observed that RUT administration alleviated oxidative stress and suppressed the expression of TNF-α, IL-1β and MAPK14. Previous studies have reported that RUT reduces oxidative stress and alleviates inflammation, thus protecting target tissues from damage by toxic agents (Caglayan, Kandemir, Darendelioğlu, et al., 2019; Caglayan, Kandemir, Yildirim, et al., 2019).

In testicular toxicity, apoptosis occurs, which is programmed cell death (Gur, Kandemir, et al., 2022; Tuncer et al. 2023). It is stated that ROS produced in mitochondria in the apoptotic process have a function (Kandemir, Yıldırım, et al., 2020). Bax and Caspase 3 are used as indicators to determine the level of apoptosis (Kucukler et al., 2020). It has been stated that VALP triggers apoptosis in cells (Phillips et al., 2003). In the present study, it was determined that VALP application increased the mRNA transcript levels of Bax and Caspase-3 in testicular tissue, thus causing apoptosis. On the other hand, it

was observed that RUT treatment suppressed Bax and Caspase-3 expressions, thus protecting the testicular tissue from the destructive effect of VALP. In previous studies, it has been reported that phytochemicals suppress the apoptotic pathway induced by toxic agents (Kandemir, Caglayan, et al., 2020). In this respect, our study is compatible with previous studies.

MMPs are enzymes that have an important function in cell membrane disruption (Yıldız et al., 2022). These enzymes are inhibited when the cell Zinc (Zn) and Copper (Cu) levels are too low (De Souza et al., 2000). The increase in oxidative stress causes an increase in MMP level in parallel. In our study, there was a significant increase in MMP9 levels in the VALP group. It is thought that this situation may be related to the decrease in the concentration of Zn or Cu, which is suppressed against increasing oxidative damage.

Sperm motility provides information about the health status of rats (Aksu et al., 2017). In our study, there was a significant decrease in sperm motility in the VALP group. This may have occurred due to increased oxidant activity. Germ cells are extremely sensitive to toxic substances due to their high mitotic activity and low antioxidant capacity (Martin et al., 1999). In our study, the increase in the ratio of dead and abnormal spermatozoa in the VALP group can be explained by this mechanism. The decrease in the ratio of dead and abnormal sperm in RUT-treated groups can be explained by the fact that RUT scavenges ROS with increased antioxidant activity.

CONCLUSION

As a result, VALP administration cause to an increase in oxidative stress, inflammation and apoptosis in testicular tissue of rats, thus decreasing sperm quality. RUT treatment improved sperm quality by partially ameliorating the testicular damage induced by VALP.

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

Authors' Contributions: All authors contributed to the study. SAA, NAK, and EE contributed to the project idea, design and execution of the study. SAA, NAK and EE contributed to obtaining the data. SAA analyzed the data. NAK and EE drafted and wrote. The SAA critically reviewed the manuscript. All authors have read and approved the finalized article.

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REFERENCES

Abarikwu, S., Iserhienrhien, B., & Badejo, T. (2013). Rutin-and selenium-attenuated cadmium-induced testicular pathophysiology in rats. Human & experimental toxicology, 32(4), 395-406.

Abarikwu, S. O., Mgbudom-Okah, C. J., & Onuah, C. L. (2022). The protective effect of rutin against busulfan-induced testicular damage in adult rats. Drug and Chemical Toxicology, 45(3), 1035-1043.

Abdelkader, N. F., Elyamany, M., Gad, A. M., Assaf, N., Fawzy, H. M., & Elesawy, W. H. (2020). Ellagic acid attenuates liver toxicity induced by valproic acid in rats. Journal of Pharmacological Sciences, 143(1), 23-29.

Adewole, K. E., Attah, A. F., & Osawe, S. O. (2021). Exploring phytotherapeutic approach in the management of valproic acid-induced toxicity. Advances in Traditional Medicine, 1-21.

Akaras, N., Abuc, O. O., Koc, K., Bal, T., Geyikoglu, F., Atilay, H., Erol, H. S., Yigit, S., & Gul, M. (2020). (1→ 3)-β-d-glucan enhances the toxicity induced by Bortezomib in rat testis. Journal of food biochemistry, 44(3), e13155.

Akaras, N., Bal, T., Atilay, H., Selli, J., & Halici, M. (2017). Protective effects of agomelatine on testicular damage caused by bortezomib. Biotechnic & Histochemistry, 92(8), 552-559.

Akaras, N., Gur, C., Kucukler, S., & Kandemir, F. M. (2023). Zingerone reduces sodium arsenite-induced nephrotoxicity by regulating oxidative stress, inflammation, apoptosis and histopathological changes. Chemico-Biological Interactions, 374, 110410.

Akaras, N., Ileriturk, M., Gur, C., Kucukler, S., Oz, M., & Kandemir, F. M. (2023). The protective effects of chrysin on cadmium-induced pulmonary toxicity; a multi-biomarker approach. Environmental Science and Pollution Research, 1-16.

Akondi, B. R., Challa, S. R., & Akula, A. (2011). Protective effects of rutin and naringin in testicular ischemia-reperfusion induced oxidative stress in rats. Journal of reproduction & infertility, 12(3), 209.

Aksu, E., Kandemir, F., Özkaraca, M., Ömür, A., Küçükler, S., & Çomaklı, S. (2017). Rutin ameliorates cisplatin-induced reproductive damage via suppression of oxidative stress and apoptosis in adult male rats. Andrologia, 49(1), e12593.

Aksu, E. H., Kandemir, F. M., & Küçükler, S. (2021). Ameliorative effect of hesperidin on streptozotocin-diabetes mellitus-induced testicular DNA damage and sperm quality degradation in Sprague–Dawley rats. Journal of food biochemistry, 45(10), e13938.

Aksu, E. H., Kandemir, F. M., Küçükler, S., & Mahamadu, A. (2018). Improvement in colistin-induced reproductive damage, apoptosis, and autophagy in testes via reducing oxidative stress by chrysin. Journal of biochemical and molecular toxicology, 32(11), e22201.

Aksu, E. H., Kandemir, F. M., Yıldırım, S., Küçükler, S., Dörtbudak, M. B., Çağlayan, C., & Benzer, F. (2019). Palliative effect of curcumin on doxorubicin-induced testicular damage in male rats. Journal of biochemical and molecular toxicology, 33(10), e22384.

Aktaş, M., Kandemir, F. M., Ozkaraca, M., Hanedan, B., & Kirbaş, A. (2017). Protective effects of rutin on acute lung injury induced by oleic acid in rats. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 23(3).

Alsemeh, A. E., Ahmed, M. M., Fawzy, A., Samy, W., Tharwat, M., & Rezq, S. (2022). Vitamin E rescues valproic acid-induced testicular injury in rats: Role of autophagy. Life Sciences, 296, 120434.

Ambriz-Pérez, D. L., Leyva-López, N., Gutierrez-Grijalva, E. P., & Heredia, J. B. (2016). Phenolic compounds: Natural alternative in inflammation treatment. A Review. Cogent Food & Agriculture, 2(1), 1131412.

Ansar, S., AlGhosoon, H. T., & Hamed, S. (2015). Evaluation of protective effect of rutin on lead acetate-induced testicular toxicity in Wistar rats. Toxin Reviews, 34(4), 195-199.

Bairy, L., Paul, V., & Rao, Y. (2010). Reproductive toxicity of sodium valproate in male rats. Indian journal of pharmacology, 42(2), 90.

Belhan, S., Özkaraca, M., Kandemir, F. M., Gülyüz, F., Yildirim, S., ÖMÜR, A. D., & Yener, Z. (2017). Effectiveness of hesperidin on methotrexate-induced testicular toxicity in rats. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 23(5).

Bradbury, C. A., Khanim, F., Hayden, R., Bunce, C., White, D., Drayson, M., Craddock, C., & Turner, B. (2005). Histone deacetylases in acute myeloid leukaemia show a distinctive pattern of expression that changes selectively in response to deacetylase inhibitors. Leukemia, 19(10), 1751-1759.

Caglayan, C., Kandemir, F. M., Darendelioğlu, E., Yıldırım, S., Kucukler, S., & Dortbudak, M. B. (2019). Rutin ameliorates mercuric chloride-induced hepatotoxicity in rats via interfering with oxidative stress, inflammation and apoptosis. Journal of Trace Elements in Medicine and Biology, 56, 60-68.

Caglayan, C., Kandemir, F. M., Yildirim, S., Kucukler, S., & Eser, G. (2019). Rutin protects mercuric chloride-induced nephrotoxicity via targeting of aquaporin 1 level, oxidative stress, apoptosis and inflammation in rats. Journal of Trace Elements in Medicine and Biology, 54, 69-78.

Cihan, G., Aydın, G., & Kandemir, Ö. (2022). Ratlarda Deltametrin'in Neden Olduğu Dalak Toksisitesine Karşı Rutin'in Potansiyel Koruyucu Etkilerinin Oksidatif Stres, Apoptoz ve İnflamasyon Belirteçleri Üzerinden Araştırılması. Atatürk Üniversitesi Veteriner Bilimleri Dergisi, 17(1), 6-10.

De Souza, A., Gerlach, R., & Line, S. (2000). Inhibition of human gingival gelatinases (MMP-2 and MMP-9) by metal salts. Dental Materials, 16(2), 103-108.

Galaly, S. R., Abdella, E. M., & Mohammed, H. M. (2014). Effects of royal jelly on genotoxicity and nephrotoxicity induced by valproic acid in albino mice. Beni-Suef University journal of basic and applied sciences, 3(1), 1-15.

Gur, C., Akarsu, S. A., & Kandemir, F. M. (2022). Carvacrol reduces abnormal and dead sperm counts by attenuating sodium arsenite-induced oxidative stress, inflammation, apoptosis and autophagy in testicular tissues of rats.

Gur, C., & Kandemir, F. M. (2023). Molecular and biochemical investigation of the protective effects of rutin against liver and kidney toxicity caused by malathion administration in a rat model. Environmental Toxicology, 38(3), 555-565.

Gur, C., Kandemir, O., & Kandemir, F. M. (2022). Investigation of the effects of hesperidin administration on abamectin-induced testicular toxicity in rats through oxidative stress, endoplasmic reticulum stress, inflammation, apoptosis, autophagy, and JAK2/STAT3 pathways. Environmental Toxicology, 37(3), 401-412.

Hamza, A. A., & Amin, A. (2007). Apium graveolens modulates sodium valproate-induced reproductive toxicity in rats. Journal of Experimental Zoology Part A: Ecological Genetics and Physiology, 307(4), 199-206.

Hozayen, W. G. (2012). Effect of hesperidin and rutin on doxorubicin induced testicular toxicity in male rats. Int J Food Nutr Sci, 1(1), 31-42.

Ileriturk, M., Kandemir, O., Akaras, N., Simsek, H., Genc, A., & Kandemir, F. M. (2023). Hesperidin has a protective effect on paclitaxel-induced testicular toxicity through regulating oxidative stress, apoptosis, inflammation and endoplasmic reticulum stress. Reproductive Toxicology, 118, 108369.

Jin, J., Xiong, T., Hou, X., Sun, X., Liao, J., Huang, Z., Huang, M., & Zhao, Z. (2014). Role of Nrf2 activation and NF-κB inhibition in valproic acid induced hepatotoxicity and in diammonium glycyrrhizinate induced protection in mice. Food and chemical toxicology, 73, 95-104.

Kamalakkannan, N., & Prince, P. S. M. (2006). Rutin improves the antioxidant status in streptozotocin-induced diabetic rat tissues. Molecular and cellular biochemistry, 293, 211-219.

Kandemir, F. M., Caglayan, C., Aksu, E. H., Yildirim, S., Kucukler, S., Gur, C., & Eser, G. (2020). Protective effect of rutin on mercuric chloride-induced reproductive damage in male rats. Andrologia, 52(3), e13524.

Kandemir, F. M., Ileriturk, M., & Gur, C. (2022). Rutin protects rat liver and kidney from sodium valproate-induce damage by attenuating oxidative stress, ER stress, inflammation, apoptosis and autophagy. Molecular Biology Reports, 49(7), 6063-6074.

Kandemir, F. M., Kucukler, S., Caglayan, C., Gur, C., Batil, A. A., & Gülçin, İ. (2017). Therapeutic effects of silymarin and naringin on methotrexate-induced nephrotoxicity in rats: Biochemical evaluation of anti-inflammatory, antiapoptotic, and antiautophagic properties. Journal of food biochemistry, 41(5), e12398.

Kandemir, F. M., Yıldırım, S., Kucukler, S., Caglayan, C., Darendelioğlu, E., & Dortbudak, M. B. (2020). Protective effects of morin against acrylamide-induced hepatotoxicity and nephrotoxicity: A multi-biomarker approach. Food and chemical toxicology, 138, 111190.

Kucukler, S., Caglayan, C., Darendelioğlu, E., & Kandemir, F. M. (2020). Morin attenuates acrylamide-induced testicular toxicity in rats by regulating the NF-κB, Bax/Bcl-2 and PI3K/Akt/mTOR signaling pathways. Life Sciences, 261, 118301.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. methods, 25(4), 402-408.

Ma, Q. (2013). Role of nrf2 in oxidative stress and toxicity. Annual review of pharmacology and toxicology, 53, 401-426.

Mandana, S. N., Fatemeh, N., Hossein, N., Azam, M., & Saleh, A. (2013). Protective effect of ghrelin on sodium valproate-induced liver injury in rat. Journal of Stress Physiology & Biochemistry, 9(1), 96-105.

Martin, R. H., Ernst, S., Rademaker, A., Barclay, L., Ko, E., & Summers, N. (1999). Analysis of sperm chromosome complements before, during, and after chemotherapy. Cancer genetics and cytogenetics, 108(2), 133-136.

Moshahid Khan, M., Raza, S. S., Javed, H., Ahmad, A., Khan, A., Islam, F., Safhi, M. M., & Islam, F. (2012). Rutin protects dopaminergic neurons from oxidative stress in an animal model of Parkinson's disease. Neurotoxicity research, 22, 1-15.

Nalivaeva, N. N., Belyaev, N. D., & Turner, A. J. (2009). Sodium valproate: an old drug with new roles. Trends in pharmacological sciences, 30(10), 509-514.

Ola, M. S., Ahmed, M. M., Ahmad, R., Abuohashish, H. M., Al-Rejaie, S. S., & Alhomida, A. S. (2015). Neuroprotective effects of rutin in streptozotocin-induced diabetic rat retina. Journal of Molecular Neuroscience, 56, 440-448.

Phillips, A., Bullock, T., & Plant, N. (2003). Sodium valproate induces apoptosis in the rat hepatoma cell line, FaO. Toxicology, 192(2-3), 219-227.

Placer, Z. A., Cushman, L. L., & Johnson, B. C. (1966). Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. Analytical biochemistry, 16(2), 359-364.

Pourahmad, J., Eskandari, M. R., Kaghazi, A., Shaki, F., Shahraki, J., & Fard, J. K. (2012). A new approach on valproic acid induced hepatotoxicity: involvement of lysosomal membrane leakiness and cellular proteolysis. Toxicology in vitro, 26(4), 545-551.

Raza, M., Al-Bekairi, A., Ageel, A., & Qureshi, S. (1997). Biochemical basis of sodium valproate hepatotoxicity and renal tubular disorder: time dependence of peroxidative injury. Pharmacological research, 35(2), 153-157.

Safdar, A., & Ismail, F. (2022). A Comprehensive Review on Pharmacological Applications and Drug-Induced Toxicity of Valproic Acid. Saudi Pharmaceutical Journal.

Saleh, D., Ismail, M. A., & Ibrahim, A. M. (2012). Non alcoholic fatty liver disease, insulin resistance, dyslipidemia and atherogenic ratios in epileptic children and adolescents on long term antiepileptic drug therapy. Pakistan journal of biological sciences: PJBS, 15(2), 68-7.

Salem, E., Salem, N., & Hellstrom, W. (2017). Therapeutic effect of ozone and rutin on adriamycin-induced testicular toxicity in an experimental rat model. Andrologia, 49(1), e12603.

Savran, M., Ascı, H., Armagan, İ., Erzurumlu, Y., Azırak, S., Kaya Ozer, M., Bilgic, S., & Korkmaz, D. T. (2020). Thymoquinone could be protective against valproic acid-induced testicular toxicity by antioxidant and anti-inflammatory mechanisms. Andrologia, 52(7), e13623.

Sedlak, J., & Lindsay, R. H. (1968). Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Analytical biochemistry, 25, 192-205.

Şimşek, H., Akaras, N., Cihan, G., Küçükler, S., & Kandemir, F. M. (2023). Beneficial effects of Chrysin on Cadmium-induced nephrotoxicity in rats: Modulating the levels of Nrf2/HO-1, RAGE/NLRP3, and Caspase-3/Bax/Bcl-2 signaling pathways. Gene, 147502.

Şimşek, H., Küçükler, S., Gür, C., İleritürk, M., Aygörmez, S., & Kandemir, F. M. (2023). Protective effects of zingerone against sodium arsenite-induced lung toxicity: A multi-biomarker approach. Iranian Journal of Basic Medical Sciences, 26(9), 1098-1106. https://doi.org/10.22038/ijbms.2023.71905.15623

Temel, Y., Kucukler, S., Yıldırım, S., Caglayan, C., & Kandemir, F. M. (2020). Protective effect of chrysin on cyclophosphamide-induced hepatotoxicity and nephrotoxicity via the inhibition of oxidative stress, inflammation, and apoptosis. Naunyn-Schmiedeberg's archives of pharmacology, 393, 325-337.

Tolou-Ghamari, Z., & Palizban, A. A. (2015). Review of sodium valproate clinical and biochemical properties. Zahedan Journal of Research in Medical Sciences, 17(8).

Tong, V., Teng, X. W., Chang, T. K., & Abbott, F. S. (2005). Valproic acid I: time course of lipid peroxidation biomarkers, liver toxicity, and valproic acid metabolite levels in rats. Toxicological Sciences, 86(2), 427-435.

Tuncer, S. Ç., Akarsu, S. A., Küçükler, S., Gür, C., & Kandemir, F. M. Effects of sinapic acid on lead acetate-induced oxidative stress, apoptosis and inflammation in testicular tissue. Environmental Toxicology.

Wang, Y. B., Ge, Z. M., Kang, W. Q., Lian, Z. X., Yao, J., & Zhou, C. Y. (2015). Rutin alleviates diabetic cardiomyopathy in a rat model of type 2 diabetes. Experimental and therapeutic medicine, 9(2), 451-455.

Wei, S.-M., Yan, Z.-Z., & Zhou, J. (2011). Protective effect of rutin on testicular ischemia-reperfusion injury. Journal of pediatric surgery, 46(7), 1419-1424.

Yardim, A., Kandemir, F. M., Ozdemir, S., Kucukler, S., Comakli, S., Gur, C., & Celik, H. (2020). Quercetin provides protection against the peripheral nerve damage caused by vincristine in rats by suppressing caspase 3, NF-κB, ATF-6 pathways and activating Nrf2, Akt pathways. Neurotoxicology, 81, 137-146.

Yıldız, M. O., Çelik, H., Caglayan, C., Genç, A., Doğan, T., & Satıcı, E. (2022). Neuroprotective effects of carvacrol against cadmium-induced neurotoxicity in rats: role of oxidative stress, inflammation and apoptosis. Metabolic brain disease, 37(4), 1259-1269.

Youssef, S. S., Ibrahim, N. K., El-Sonbaty, S. M., & El-Din Ezz, M. K. (2022). Rutin Suppresses DMBA Carcinogenesis in the Breast Through Modulating IL-6/NF-κB, SRC1/HSP90 and ER-α. Natural Product Communications, 17(9), 1934578X221118213.

Zhou, L., Chen, L., Zeng, X., Liao, J., & Ouyang, D. (2020). Ginsenoside compound K alleviates sodium valproate-induced hepatotoxicity in rats via antioxidant effect, regulation of peroxisome pathway and iron homeostasis. Toxicology and applied pharmacology, 386, 114829.

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RESEARCH ARTICLE

Effects of Tarantula cubensis Extract on Experimental Acute Spinal Cord İnjury in Rats

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ABSTRACT

Spinal cord injury (SCI) is a critical health problem that occurs after spinal trauma. In this study, the possible effects of Tarantula cubensis extract in the acute period were evaluated. For the rat trauma model, 28 Sprague dawley rats were used. All rats were divided into 4 groups (n=7); Control, injury (IG), prednisolone treatment (PG), and Tarantula cubensis extract treatment (TCG). Laminectomy was applied to all rats except the control group, and an aneurysm clamp was applied to the spinal cord for 60 seconds. BBB (Basso, Beattie, Bresnahan Locomotor Rating Scale) for clinical improvement assessment, histopathological (inflammatory response, neuron viability), and the amount of biochemical parameters 8-hydroxydeoxyguanosine (8-OHdG), myeloperoxidase (MPO), transforming growth factor beta 1 (TGF β-1), tumor necrosis factor alpha (TNF-α), interleukin 2 (IL-2) were evaluated. According to histopathological evaluations; less damage and inflammation were observed in the TCG group compared to the IG group. There was a statistical similarity between TCG and PG (p=0.005) BBB score TCG values were higher than PG and IG rats. MPO level was found to be significantly lower than TCG group IG and PG in laminectomy rats (p=0.001). TGF β-1 (ng-l), which is an anti-inflammatory cytokine, was measured at the highest level of TCG (p=0.001). TNF-α (ng-l) levels were higher in TCG, PG, and IG compared to the control group. IL-2 (ng-l) levels were decreased in TCG, PG, and control groups compared to the IG group (p=0.05). Tarantula cubensis extract has been determined neuroprotective effect by showing anti-inflammatory activity.

Keywords: Anti-inflammatory effect, Neuroprotection, Spinal cord injury, *Tarantula cubensis* extract

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Tarantula cubensis Ekstraktının Sıçanlarda Deneysel Akut Omurilik Yaralanması Üzerine Etkileri

ÖZ

Omurilik yaralanması (SCI), omurganın ciddi yaralanmalarından sonra ortaya çıkan kritik bir sağlık sorunudur. Bu çalışmada Tarantula cubensis ekstraktının akut dönemdeki olası etkileri değerlendirilmiştir. Sıçan travma modeli için 28 Sprague dawley kullanıldı. Tüm ratlar 4 gruba ayrıldı (n=7): Kontrol, hasar grubu (IG), prednizolon tedavisi (PG) ve Tarantula cubensis ekstraktı tedavisi (TCG). Kontrol grubu dışındaki tüm ratlara laminektomi uygulandı ve spinal korda 60 saniye anevrizma klempi uygulandı. Klinik iyileştirme değerlendirmesi, BBB (Basso, Beattie, Bresnahan Locomotor Rating Scale), histopatolojik (inflamatuar yanıt, nöron canlılığı) ve biyokimyasal değerlendirmede için 8- hydroxydeoxyguanosine (8-OHdG), myeloperoxidase (MPO), transforming growth faktör beta 1 (TGF β-1), tümor Nekroz Faktör Alfa (TNF-α), interleukin-2 (IL-2) değerlendirildi. Histopatolojik değerlendirmelere göre; TCG grubunda IG grubuna oranla daha az hasar ve inflamasyon gözlendi. TCG ve PG arasında istatistiksel olarak benzerlik vardı (p=0.005). BBB skoru TCG değerleri PG ve IG'ye göre yüksek bulundu. Laminektomi yapılan sıçanlarda MPO düzeyi TCG'de IG ve PG'den anlamlı düzeyde düşük bulundu (p=0.001). Antiinflamatuvar bir sitokin olan TGF β-1 (ng-l) TCG'de en yüksek seviyesinde ölçüldü (p=0.001). TNF-α (ng-l) seviyeleri TCG, PG ve IG'de kontrol grubuna göre daha yüksekti. Ancak kendi aralarında anlamlı bulunmamışlardır. IL-2 (ng-l) düzeyleri TCG, PG'de IG grubuna göre azalmıştı (p=0.05). Sonuç olarak; Tarantula cubensis ekstraktının antiinflamatuar aktivite göstererek omurilik dokusunda nöroprotektif etkisi olduğu belirlenmiştir.

Anahtar Kelimeler: Antiinflamatuar etki, Nöroproteksiyon, Omurilik yaralanması, *Tarantula cubensis* ekstraktı

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INTRODUCTION

Spinal cord injury (SCI) is a considerable health problem that can result in paralysis. This situation, which affects many people, can occur as a result of spinal injuries, especially in traffic accidents. While spontaneous recovery of SCI can be seen in non-mammalian species, this is very limited in humans (Bloom, 2014). Leukocyte infiltration and hemorrhage may occur due to primary damage to the nerve tissue and increased neuron damage. Therefore, tissue stabilization and nerve tissue regeneration are the main goals after injury. Steroids are currently used as a first choice in the treatment of spinal cord patients (Bracken et al. 1992; Walters et al. 2013). These drugs suppress the inflammatory response and reduce the leukocyte infiltration into the damaged tissue. However, it was determined in experimental studies that they did not provide sufficient improvement in treatment and were ineffective at high doses after trauma (Tator and Koyanagi, 1997). It has also been stated that steroids can cause hyperglycemia in metabolic diseases and worsen pneumonia situations (Sultan et al. 2020). The fact that the current treatment options do not result in full recovery in SCI makes it necessary to apply new treatment protocols and drugs. It has been reported that some useful results regarding SCI have been obtained in previous studies. These; Schwann cells differentiated from adipose stem cells (Zaminy et al. 2013). growth factor treatment (Chehrehasa et al. 2014), anti-CD 11cell treatment (Geremia et al. 2017), Cyclooxygenase-2 (Cox-2) selective inhibitör parecoxib treatment (Yuksel et al. 2019), human bone marrow-derived stem cells treatment (Munter et al. 2019), melatonin hormone (Zhang et al. 2019), platelet rich plasma(Salarinia et al. 2017) and quetiapine treatment (Aytar et al. 2018). However, the unique structure of the nerve tissue and the variability of trauma responses do not always produce positive outcomes in humans.

Tarantula cubensis extract is used especially in veterinary medicine. It is known that it has favorable outcomes on postpartum complications, and foot and breast diseases, and it is used in oral papillomatosis with its immunomodulatory effects (Coskun, 2017). *Tarantula cubensis* extract D6 has been reported to have positive effects on peripheral sciatic nerve damage (Kizilay et al. 2019), wound healing (Gul Satar et al. 2017), and aflatoxin-induced injury in rats (Karabacak et al. 2015). It is necessary to present new approaches to spinal cord injury, which is a very serious health problem. This study examined the possible effects of *Tarantula cubensis* extract in the treatment of acute SCI in rats.

MATERIALS AND METHODS

Animal Study

This experimental study was performed with the permission of Adnan Menderes University Animal Experiments Local Ethics Committee (ADU-HADYEK 64583101/2018/004). For the animal study 220-250 g weight 28 female Sprague Dawley rats were randomly divided into 4 groups (n=7). These were Control (CG), Injury group (IG), Prednisolone treatment group (PG), and *Tarantula cubensis* extract treatment group (TCG). Laminectomy was applied to all groups except the control group. Methylprednisolone (Precord liyo, Kocak Pharma/Turkey) was intraperitoneally administered at a dose of 1 mg/kg and Tarantula cubensis extract (Theranekron D6, Ricter Pharma/Austria) was administered intraperitoneally (IP) at 0.3 ml/animal (Kizilay et al. 2019). An equal volume of physiological saline was injected into the control and injury groups. In the treatment groups (PG and TCG), drug administrations were performed at the 4th hour after injury. The urinary bladders were emptied by applying handle massage every 6 hours to the rats who underwent surgical procedures until the end of the 72 th hours of the study. The reason why male animals were not used in the study was urinary incontinence problems that could develop after SCI.

Laminectomy

The laminectomy procedure was performed as stated by previous studies (Rivlin and Tator, 1978). Ketamine (50mg/kg/bw) and xylazine (5 mg/kg/bw) anesthesia was applied to the rats who underwent a surgical procedure. The dorsum of rats were cleaned with betadine solution following to clipping the hairs. After the skin incision, resection of the muscle and fascia tissues was performed. Thoracic (T 7-9) spine-level laminectomy was performed without damaging the dura mater by using the clip compression method. In clip compression application, an aneurysm clamp (Mizuho Aneurysm Clip, Mizuho, Japan) was applied for 60 seconds (Figure 1). The area was then closed by suturing the muscle and skin. After applying antiseptic to the skin, the rats were observed in clean cages. There was mortality observed after the surgical procedures. Paracetamol (100 mg/kg/bw) was added to the drinking water of the operated rats until the first 12 hours to reduce pain.

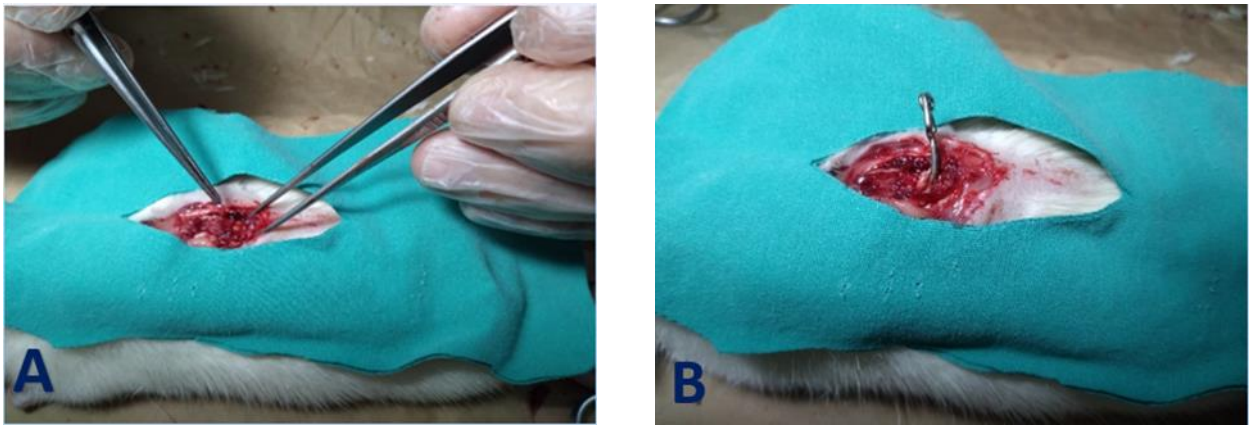

Figure 1: Laminectomy procedure on spinal cord injury (**A**) and application of aneurysm clamp for 60 seconds (**B**) in a rat model. (**A**); Following the general surgery principles, the spinal cord is opened in rats under anesthesia until the spinal cord is seen. Laminectomy is completed without damaging the dura mater. (**B**); Aneurysm clamp is applied for 60 seconds. Afterward, the operation was completed using the appropriate suture material. All surgical procedures were completed in accordance with animal welfare.

Biochemical analyzes

At the end of the study (72nd hour), intracardiac blood samples were taken and serums were separated with a refrigerated centrifuge device (Sigma, 3K30). Samples were stored at -20 ˚C degrees until analysis. A commercial test kit was used to determine the total protein level in blood serum samples (A2300, Archem Health Ind. Co., Turkey). Myeloperoxidase (MPO) activity, 8- Hydroxydeoxyguanosine (8-OHdG), TNF-α (Tumor Necrosis Factor Alpha), IL-2 (Interleukin 2), and Transforming growth factor beta 1 (TGF beta-1) levels were measured in blood serum. A commercial elisa test kit was used for MPO activity (Colorimetric, Cat No: ab105136). For measuring 8-OHdG Elisa Kit (Cat no: E-EL-0028) also TGF beta 1 ELISA Kit (Cat no: ab119558), TNF-α ELISA Kit Bt-lab (Cat no: E0764Ra), IL-2 Bt-lab ELISA Kit (Cat no: E0123Ra) was used. All tests were analyzed by a subject expert on 96 Elisa plates with 3 replicates of each sample. ELISA tests; microplate reader: BIOTEK ELX800-Auto strip washer: BIO TEK EL X 50 it was done on the device.

Physical examination and Basso, Beattie, Bresnahan (BBB) Locomotor Rating Scale

Locomotor testing was performed at 24, 48, and 72 hours after SCI trauma. In the evaluations, muscle and joint movements, paraplegia level, reflexes, general condition, and movement codifications were examined. The result of the evaluation, the BBB test with a scale of 0-21 was used (Basso et al. 1995). The examinations were video recorded for 1 minute. The results were evaluated by 2 different experts.

Histopathology evaluation

All rats were euthanized by the cervical dislocation method under anesthesia (Ketamine and Xylazine combination) 72 hours after the operation. The spinal cords were harvested immediately, and tissue segments (4-6 cm long) were placed in 10% formaldehyde solution. Serial transverse sections were made from the laminectomy areas from the materials after 24 hours of fixation, the pieces were placed in cassettes and routinely followed for 16 hours in an automatic tissue tracking device. After routine follow-up, serial sections of 4 µm were taken from the samples embedded in paraffin blocks with a microtome (Thermo Shandon HM 355S). Prepared slides were stained with a commercial kit of Hematoxylin-Eosin staining. Samples were examined with a light microscope (Olympus BX53, Japan). Photo samples were recorded with a high-resolution video camera (Olympus BX61, Japan).

Histopathological evaluation; the samples were examined by three expert pathologists. The degree of ischemic damage and leukocyte leak in motor neurons in the ventral horns of the spinal cord was evaluated. Neurons showing cellular swelling or loss of eosinophilic cytoplasm, nucleus, and Nissl bodies were considered damaged. Neurons with thin chromatin, prominent nucleolus, and cytoplasmic Nissl bodies were defined as alive. The viability index was calculated as the ratio between the number of viable motor neurons in the entire spinal cord section and the number of all motor neurons for each rat (viability index = Number of viable neurons / total number of neurons). In addition, the inflammatory response was graded semi-quantitatively by calculating the number of leukocytes seen infiltrating the tissue in randomly selected gray matter areas: '0' for none, '1' for less than 20, '20 to 50' for '2', "3" for more than 50 detected leukocytes (Boga et al. 2006).

Statistical analysis

Biochemical parameters were expressed as the mean ± SE (standard error) for the 7 rats in each group. SPSS for Windows Version 20 was used for statistical analysis of different groups. Statistical significance among groups was tested using the Kruskal-Wallis test for parameters (pathological parameters) that were not normally distributed and using one-way ANOVA for normally distributed parameters (other parameters). A significant difference is accepted when $p<0.05$ (Conover, 1980).

RESULTS

Biochemical Analyzes Results

According to the results of 8-OHdG blood serum levels, a statistically significant difference was found between the treatment groups and the control group ($p=0.04$). In the tests performed with the ELISA assay, the highest result was found in TCG. There was also a significant difference between the group's PG and TCG.

MPO activity increased with laminectomy and its level decreased in the treated groups. One of the most important results we found in the tests is MPO activity. Because TCG levels decreased statistically significantly in PG and IG groups (Table 1).

TGF β-1 level was the lowest in the control group. It was determined that the level of laminectomy was increased in groups (IG, PG, and TCG). TGF β-1 level was similar between PG and IG, the highest level was determined in TCG ($p=0.001$).

There was no statistically significant difference between TNF-α blood levels PG, IG, and TCG. These values were found to be higher than the control group ($p=0.025$). IL-2 levels of PG, TCG, and control groups showed a statistically significant decrease in the IG group ($p=0.05$).

Table 1. Biochemical parameters from blood serum taken from rats at the end of the study (72nd hour). 8-hydroxy-2'-deoxyguanosine damage marker (8-OHdG), myeloperoxidase (MPO) activity, transforming growth factor beta-1 (TGF β-1) levels, TNF-α (Tumor Necrosis Factor Alpha), IL-2 (Interleukin 2)

Groups (n=7)	Parameters				
	8-OHdG (ng/l)	**MPO (ng/ml)**	**TGF β-1 (ng/l)**	**TNF-α (ng/l)**	**IL-2 (ng/l)**
Control	5.90±0.07^{c}	0.41±0.02^{d}	57.62±1.42^{c}	35.95±3.44^{b}	26.38±5.42^{b}
Injury	5.94±0.09^{c}	2.27±0.08^{a}	62.38±0.95^{b,c}	73.49±4.22^{a}	46.37±8.35^{a}
Prednizolon	6.95±0.50^{b}	1.50±0.05^{b}	68.43±4.04^{b}	64.96±6.83^{a}	28.20±3.03^{b}
Tarantula cubensis	10.01±0.37^{a}	1.21±0.06^{c}	79.08±2.30^{a}	68.77±8.18^{a}	25.56±4.28^{b}
p	0.040	0.001	0.001	0.025	0.050

a, b, c, d: Different letters in the same column indicate statistically significant difference.

Histopathological Results

Spinal cord ventral horns in animals in the control group had a high viability index, and the inflammatory response detected in gray matter was mild. Diffuse ischemic harm was observed in the ventral horns of spinal cords from animals in the IG group, consistent with the low viability index. In the same samples, the inflammatory response in the gray matter was severe. The viability index of the spinal cords taken from the animals belonging to the PG and TCG groups was at similar rates, and neuronal ischemic damage was observed to be lower (focal) in these groups. Although these results were lower than the control group, they were statistically significantly higher than the Injury group (Figure 2). The lowest rate of inflammatory response was seen in TCG after the control group (Table 2). The statistical similarity in both groups indicates anti-inflammatory activity.

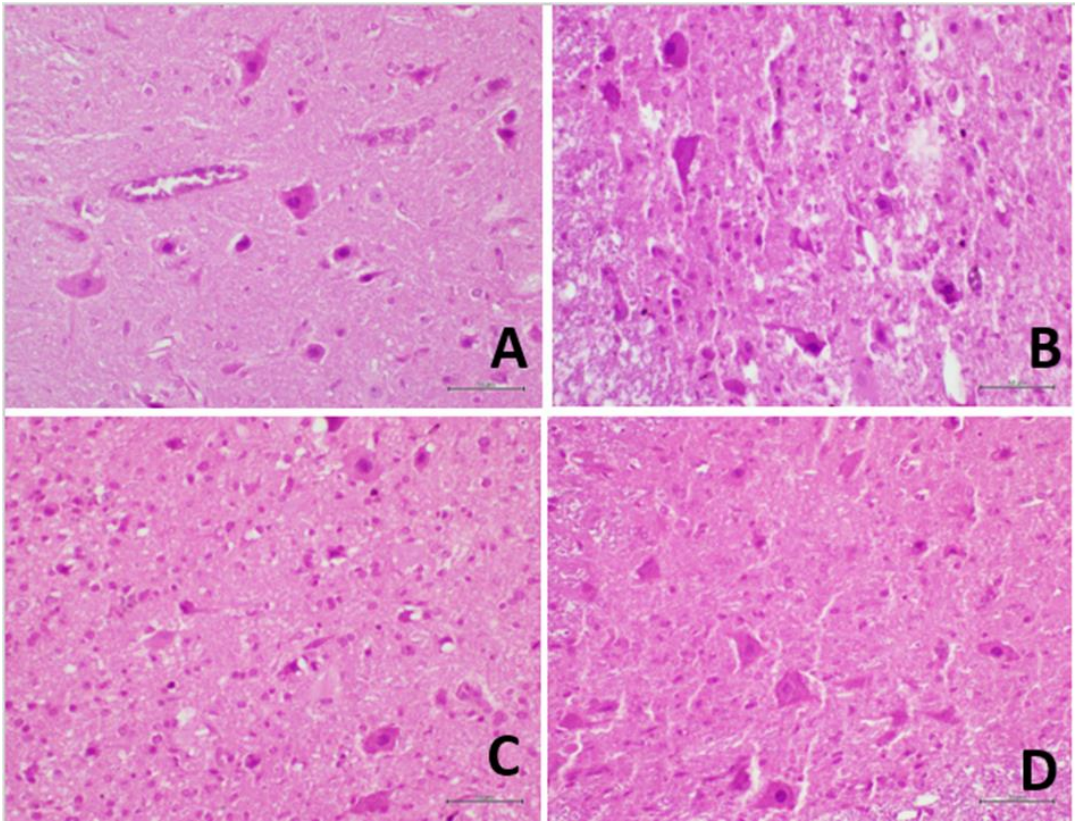

Figure 2: Hematoxylin-eosin staining (HxE, 400x magnification) in rat spinal cords after 72 hours. **(A)** Control group, normal spinal nerve formations, non-inflammation and damage. **(B)** Injury group (IG), high rate of leukocyte infiltration and inflammation, decrease in viable motor neurons and nissl bodies, cavitation onsets. **(C)** Prednisolone treatment group (PG), less inflammation, cavitation. **(D)** Tarantula cubensis extract treatment group (TCG), low inflammation rate, low leukocyte rate. There are similarities in histopathology evaluation between the PG and TCG groups.

Table 2. Histopathological evaluation of spinal cord tissue. Viability index = number of viable neurons /total number of neurons. Inflammatory response; **'0'** for none, **'1'** for less than 20, 20 to 50 for **'2'**, **"3"** for more than 50 leukocytes.

Groups (n=7)	**Parameters**	
	Neuron Viability (%)	**Inflammatory response**
Control	95.00±0.77[a]	1.00±0.00 [c]
Injury group	54.00±1.41 [c]	2.60±0.24 [a]
Prednisolone group	70.40±1.72 [b]	1.60±0.24 [b]
Tarantula cubensis group	74.00±2.44 [b]	1.20±0.20 [b,c]
p	0.001	0.005

a, b, c, d: Different letters in the same column indicate statistically significant differences.

Physical Examinations and BBB Scale Results

In the BBB score evaluation, paraplegia was present in all animals in the first 24 hours, and no significant difference was observed. The values on the 24th hour are IG, PG, and TCG, respectively; It was found to be 1±0.00, 1.50±0.22, 1.66±0.21. 48 th hour values are; It was determined as 1.50±0.22, 2.66±0.33, 4.16±0.33. At the end of the study (72nd hour); It was determined as 2.33 ±0.21, 3.5 ±0.34, and 5.83±0.3 (Figure 3).

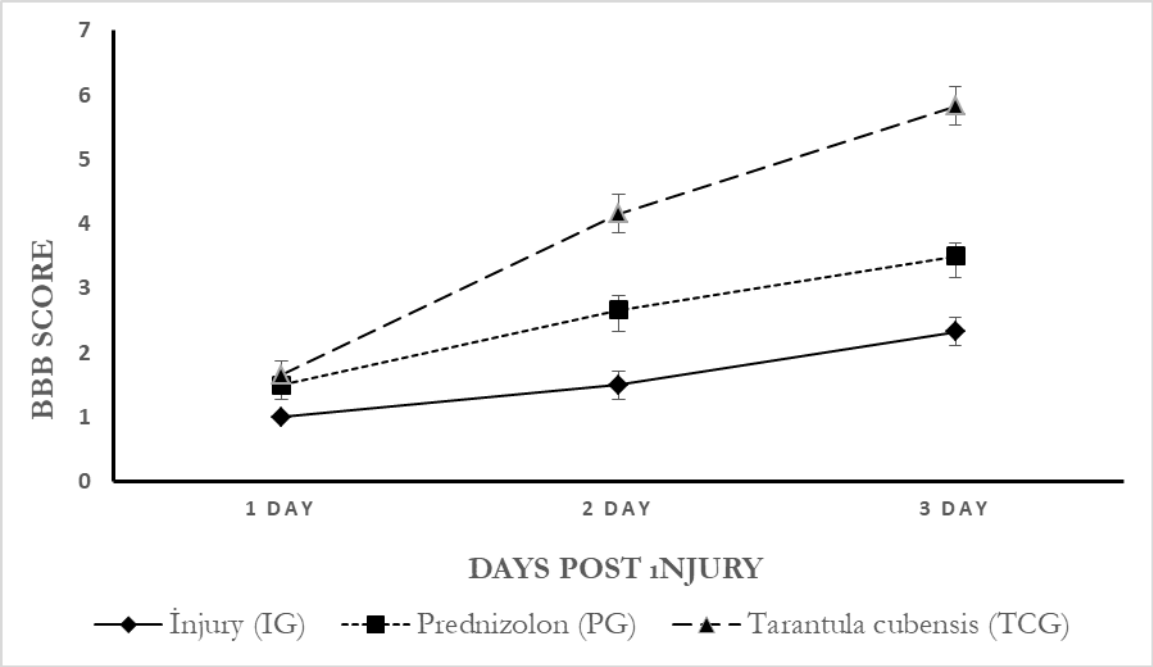

Figure 3. Clinical improvement scale applied to rats that underwent laminectomy; Beattie, Bresnahan (BBB) Locomotor Rating Scale. Tarantula cubensis extract group (TCG), Prednisolone treatment group (PG) and Injury group (IG). BBB values are (0; poor-21; healthy).

DISCUSSION

SCI has a complex damage mechanism and results in incomplete healing (Gensel and Zhang, 2015). Preventing the acute phase response in spinal cord injuries may help prevent chronic cavitations and glial scarring (McDonald et al. 2002). Although previous experimental animal studies have offered new approaches, steroids are now used as the only option (Bracken et al. 1992). The first 12 hours after SCI trauma is extremely important. It has been reported that hemorrhage, axonal necrosis, and demyelination develop after acute SCI, and leukocyte infiltration paradoxically disrupts the situation (Kakulas, 1984; Wallace et al. 1987). In this study, it was observed that there was a statistically significant similarity between the PG and TCG in the histopathological evaluation of the acute period after trauma. Likewise, there was a similar situation in the inflammatory response and neuron viability values (Table 2). Although only hematoxylin-eosin was evaluated in this study, a clear similarity was observed between PG and TCG in niss body ratios and leukocyte rates (Figure 2). İnflammation and leukocyte infiltration were observed at a lower rate in TCG compared to IG. In another previous study, Tarantula cubensis extracts are consistent with the results obtained in the peripheral nerve tissue trauma model (Kizilay et al. 2019). Although the mechanism of action of Tarantula cubensis extracts is unclear, appears to have neuroprotective effects on the nervous tissue.

TNF-α and IL-2 are important proinflammatory cytokines and their levels increase in inflammatory reactions (Gensel and Zhang, 2015). In this study, TNF-α showed a non-statistical decrease in TCG compared to IG. This is attributed to the fact that TNF-α remains high in the first hours of the injury and then decreases in the 72nd hour (Table 1). IL-2 levels are high in IG and low in PG and TCG, showing statistical significance ($p=0.05$). This is important evidence that prednisolone and Tarantula cubensis extract significantly reduce secondary damage and inflammation of SCI. TGF β-1 tends to increase in cases of damage or ischemia of nerve tissues (Klempt et al. 1992). It has been reported in experimental studies that it also plays a role in neuronal regeneration (Abe et al. 1996). Also, it is a cytokine with anti-inflammatory properties. TGF β-1 levels have not been examined in any experimental study before. In this study, the changes in TGF levels were statistically significant between the groups and increased in the TCG compared to the other groups. Although this indicates the role of Tarantula cubensis extracts in the recovery of neuronal damage, it requires further studies. 8-OHdG is a general marker of DNA damage and its level increases in case of damage. In this study, the blood DNA damage level was higher in the TCG in the acute period compared to the others. This can be explained by the fact that he is an alcoholic extract. Alcohol is used as a general solvent in homeopathic medicines. However, it is recommended to be used at low rates due to its cytotoxicity (Chirumbolo et al. 2015). This confirms the hypothesis of reviewing and reducing the alcohol content in homeopathic medicines. MPO activity is due to the increase in leukocytes penetrating the tissue after trauma. This situation creates more ROS (Reactive oxygen species) compounds in the environment and complicates the

recovery of axonal damage. Previous studies have shown that MPO is a very important marker in the acute period after trauma (Geremia et al. 2012). In this study, it was observed that the MPO activity, which increased significantly after trauma, was at the lowest level in the TCG, and it decreased statistically significantly even compared to the PG. This is evidence that Tarantula cubensis extracts significantly reduce leukocyte infiltration.

BBB scale has been reported to be important in clinical improvement in acute experimental SCI studies (Basso et al. 1995). In this study, no significant difference was found between the groups in the first 24 hours after laminectomy. A better improvement in TCG was observed at 48 and 72 hours compared to PG and CG. It was observed that Tarantula cubensis extract supports clinical improvement and can do this better than prednisolone (Figure 3). The only problem with SCI damage does not occur in the neuronal tissue. Post-traumatic proinflammatory response and leukocyte increase may also reveal distant tissue damage such as lung (Gris et al. 2008). In this study, the anti-inflammatory effect of Tarantula cubensis extracts and tissue responses similar to prednisolone were observed on the damaged spinal cord. In addition, it reduces MPO and IL-2 activity in the blood, increases TGF β-1 levels, and shows an important inflammatory response suppression feature. It is thought that similar effects may also occur in distant tissue damage.

CONCLUSIONS

Tarantula cubensis extract significantly reduces neuronal degeneration acute SCI, has anti-inflammatory activity, and increases clinical improvement. It is suggested that this study should be performed at different doses and durations (long-term), and the combined effects of steroids should be revealed.

Ethical Approval: This experimental study was performed with the permission of Adnan Menderes University Animal Experiments Local Ethics Committee (ADU-HADYEK 64583101/2018/004).

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Limitations the Study: In this study, Caspase 3 tissue staining could not be performed due to budget constraints.

REFERENCES

Abe, K., Chu, PJ., Ishihara, A, Saito, H. Transforming growth factor-beta 1 promotes re- elongation of injured axons of cultured rat hippocampal neurons. Brain Res. 1996; 723(1-2): 206-209. doi: 10.1016/0006-8993(96)00253-3.

Aytar, MH., Civi, S., Kaymaz, M., Ergun, E., Kaymaz, FF., Pasaoglu, A. The Effect of Quetiapine on Treatment of Experimental Acute Spinal Cord Injury. Turk. Neurosurg. 2018; 28(1):105-110, doi: 10.5137/1019-5149.JTN.17476-16.1.

Basso, DM., Beattie, MS., Bresnahan, JC. A sensitive and reliable locomotor rating scale for open field testing in rats. J. Neurotrauma 1995;12:1–21. doi: 10.1089/neu.1995.12.1

Bloom, O. Non-mammalian model systems for studying neuro-immune interactions after spinal cord injury. Exp. Neuro. 2014; 258: 130–140. doi: 10.1016/j.expneurol.2013.12.023

Boga, M., Discigil, B., Ozkisacik, EA., Gurcun, U., Badak, MI, et al. The combined effect of iloprost and N-acetylcysteine in preventing spinal cord ischemia in rabbits. European Journal of Vascular and Endovascular Surgery 2006; 31: 366–372. doi:10.1016/j.ejvs.2005.10.027

Bracken, MB., Shepard, M., Collins, WF., Holford, TR., Baskin, DS., Eisenberget, HM., et al. Methylprednisoloneor naloxone treatment after acute spinal injury: 1-year follow-up data. Results of the Second National Acute Spinal Cord Injury Study. J. Neurosurg. 1992;76: 23-31. doi: 10.3171/jns.1992.76.1.0023

Chehrehasa, F., Cobcroft, M., Young, YW., Mackay-Sim, A., Goss, B. An acute growth factor treatment that preserves function after spinal cord contusion injury. J. Neurotrauma 2014;31(21):1807-1813. doi: 10.1089/neu.2013.3294

Chirumbolo, S., Bjørklund, G. Homeopathic Dilutions, Hahnemann Principles, and the Solvent Issue: Must We Address Ethanol as a "Homeopathic" or a "Chemical" Issue? Homeopathy 2018; 107 (01): 040-044. doi: 10.1055/s-0037-1608898

Coskun, D. Veterinary Supportive Therapy: Tarantula Cubensis Alcoholic Extract, InactivedParapoxvirus Ovis and Corynebacterium Cutis Lysate, Dicle Üniv Vet Fak Derg 2017;10(1):30-3

Conover, WJ. Practical Nonparametric Statistics. 2nd Edition, 1980, John Wiley & Sons, New York.

Gensel, JC., Zhang, B. Macrophage activation and its role in repair and pathology after spinal cord injury. Brain Res. 2015;1619:1-11. doi:10.1016/j.brainres.2014.12.045

Geremia, NM., Bao, F., Rosenzweig, TE., Hryciw, T., Weaver, L., et al. CD11d antibody treatment improves recovery in spinal cord-injured mice. J. Neurotrauma. 2012; 29(3): 539–550. doi: 10.1089/neu.2011.1976

Geremia, NM., Hryciwa, T., Baoa, F., Streijger, F., Okonb, E., Lee, JHT., et al. The effectiveness of the anti-CD11d treatment is reduced in ratmodels of spinal cord injury that produce significant levels of intraspinal hemorrhage. Exp. Neurol. 2017; 295:125-134.doi: 10.1016/j.expneurol.2017.06.002

Gris, D., Hamilton, EF., Weaver, LC. The systemic inflammatory response after spinal cord injury damages lungs and kidneys. Exp. Neurol. 2008; 211(1): 259-270. doi: 10.1016/j.expneurol.2008.01.033

Gul Satar, NY., Cangul, IT., Topal, A., Kurt, H., Ipek, V., & Onel, GI. The effects of Tarantula cubensis venom on open wound healing in rats. Journal of wound care, 2017. 26(2), 66–71. https://doi.org/10.12968/jowc.2017.26.2.66

Kakulas BA. Pathology of spinal injuries. 1984; Cent. Nerv. Syst. Trauma 1, 117±129.

Karabacak, M., Eraslan, G., Kanbur, M., Sarıca, ZS. Effects of Tarantula cubensis D6 on aflatoxin-induced injury in biochemical parameters in rats. Homeopathy 2015;104(3):205-210. doi: 10.1016/j.homp.2015.02.005

Kizilay, Z., Aktas, S., Cetin, NK., Kılıc, MA., Oztürk, H. Effect of Tarantula Cubensis Extract (Theranekron) on Peripheral Nerve Healing in an Experimental Sciatic Nerve Injury Model in Rats. Turk. Neurosurg. 2019; 29(5):743-749. doi: 10.5137/1019-5149.JTN.26162-19.2

Klempt, ND., Sirimanne, E., Gunn, AJ., Klempt, M., Singh, K., Williams, C., et al. Hypoxia-ischemia induces transforming growth factor-/31 mRNA in the infant rat brain. Mol. Brain Res. 1992; 13(1-2): 93-101. doi: 10.1016/0169-328X(92)90048-G

McDonald, JW., Sadowsky, C. Spinal-cord injury. Lancet. 2002;359(9304):417-425. doi:10.1016/S0140-6736(02)07603-1

Munter, JP., Beugels, J., Munter, S., Jansen, L., Cillero-Pastor, B., Moskvin, O., et al. Standardized human bone marrow-derived stem cells infusion improves survival and recovery in a rat model of spinal cord injury. J. Neurol. Sci. 2019;402:16-29. doi: 10.1016/j.jns.2019.05.002

Rivlin, AS., Tator, CH. Effect of duration of acute spinal cord compression in a new acute cord injury model in the rat. Surg. Neurol. 1978; 10 (1):38-43.

Salarinia, R., Sadeghnia, HR., Alamdari, DH., Hoseini, SJ., Mafinezhad, A., Hosseini, M. Platelet rich plasma: Effective treatment for repairing of spinal cord injury in rat. Acta Orthop. Traumatol. Turc. 2017;51(3):254-257. doi: 10.1016/j.aott.2017.02.009

Sultan, I., Lamba N., Liew, A., Doung, P., Tewarie, I., Amamoo, JJ., et al. The safety and efficacy of steroid treatment for acute spinal cord injury: A Systematic Review and meta-analysis. Heliyon 2020; 6(2): e03414. doi: 10.1016/j.heliyon.2020.e03414.

Tator, CH., Koyanagi, I. Vascular mechanisms in the pathophysiology of human spinal cord injury. J. Neurosurg. 1997; 86 (3): 483-492. doi: 10.3171/JNS.1997.86.3.0483

Wallace, MC., Tator, CH., Lewis, AJ. Chronic regenerative changes in the spinal cord after cord compression injury in rats. 1987, Surgical. Neurology 1987; 27 (3): 209-219. doi: 10.1016/0090-3019(87)90031-0

Walters, BC., Hadley, N., Hurlbert, RJ., Aarabi, B., Dhall, SS., Gelb, DE., et al. Guidelines for the management of acute cervical spine and spinal cord injuries. 2013 update. Neurosurgery 2013; 60 (Suppl. 1): 82–91. doi: 10.1227/01.neu.0000430319.32247.7f

Yuksel, U., Bakar, B., Dincel, GC., Yildiran, FAB., Ogden, M., Kisa, U. The Investigation of the Cox-2 Selective Inhibitor Parecoxib Effects in Spinal Cord Injury in Rat. J Invest Surg. 2019; 32(5):402-413. doi: 10.1080/08941939.2017.1423423

Zaminy, A., Shokrgozar, MA., Sadeghi, Y., Norouzian, M., Heidari, HM., Piryaei, A. Transplantation of Schwann Cells Differentiated from Adipose Stem Cells Improves Functional Recovery in Rat Spinal Cord Injury. Arch. Iran. Med. 2013;6(9):533-541.

Zhang, Y., Liu, Z., Zhang, W., Wu, Q., Zhang, Y., Liu, Y., et al. Melatonin improves functional recovery in female rats after acute spinal cord injury by modulating polarization of spinal microglial/macrophages. J. Neurosci. Res. 2019;97(7):733-743. doi: 10.1002/jnr.24409.

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RESEARCH ARTICLE

Heavy Metal Quantity and Health Risk Assessments in Frozen Shrimp Samples

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ABSTRACT

In this study, it was aimed to determine the residual levels of toxic heavy metals cadmium (Cd), total mercury (THg), and lead (Pb) in a total of 48 frozen shrimp samples obtained from randomly selected sales points, and thus to evaluate the potential risks to public health associated with shrimp consumption. It was determined that the Cd level in the examined shrimp samples varied between <LOD-2.054 mg kg^{-1}/wet weight, while the mean THg and Pb concentrations were found to be 0.144±0.010 and 0.094±0.090 mg kg^{-1}/wet weight, respectively. Target Hazard Quotient (THQ) and Hazard Index (HI) values calculated for Cd, THg and Pb are <1, the Estimated Daily Intake (EDI) amount remains within the reference values of the Provisional Maximum Tolerable Daily/Weekly Intake (PTDI-PTWI) excluding Cd and metHg, and the Consumption Rates (CR_{lim}) were determined not to exceed the RfDo (Oral Reference Dose) limits. However, it was determined that the Cd and Pb concentrations detected in some samples exceeded the national/international legal limits determined for fishery products including shrimps and may pose a risk in terms of public health. It is thought that increasing the frequency of inspections by the competent authorities and monitoring the level of heavy metal contamination by including it in the annual sample plan of the enterprises that produce and sell frozen shrimp will provide significant benefits for reducing/preventing health risks.

Keywords: Bioaccumulation, heavy metal, public health, shrimp.

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Dondurulmuş Karides Örneklerinde Ağır Metal Miktarı ve Sağlık Risk Değerlendirmeleri

ÖZ

Bu çalışmada; rasgele seçilmiş satış noktalarından temin edilen toplam 48 adet dondurulmuş karides örneğinde, toksik ağır metallerden olan kadmiyum (Cd), toplam cıva (THg) ve kurşun (Pb) kalıntı düzeylerinin belirlenmesi ve böylece karides tüketimi ile ilişkili halk sağlığına yönelik potansiyel risklerin değerlendirilmesi amaçlanmıştır. İncelenen karides örneklerinde Cd düzeyinin <LOD-2,054 mg kg^{-1}/yaş ağırlık arasında değiştiği saptanırken, THg ve Pb yoğunluğu ortalamasının sırasıyla 0,144±0,010 ve 0,094±0,090 mg kg^{-1}/yaş ağırlık olduğu tespit edilmiştir. Cd, THg ve Pb için hesaplanan Hedef Tehlike Katsayısı (Target Hazard Quotient/THQ) ve Tehlike İndeksi (Hazard Index/HI) değerlerinin <1 olduğu, Tahmini Günlük Alım Düzeyi (Estimated Daily Intake/EDI) miktarının Cd ve metHg hariç Tolere Edilebilir Günlük/Haftalık Alım (Provisional Maximum Tolerable Daily/Weekly Intake/PTDI-PTWI) referans değerleri içinde kaldığı ve İzin Verilen Maksimum Balık Tüketim Oranı'nın (Consumption Rates/CR_{lim}), RfD_o (Oral Referans Doz) limitlerini aşmadığı belirlenmiştir. Ancak bazı örneklerde tespit edilen Cd ve Pb yoğunluğunun karideslerin de dahil olduğu su ürünleri için belirlenen ulusal/uluslararası yasal limitleri aştığı ve halk sağlığı açısından risk taşıyabileceği saptanmıştır. Yetkili otoritelerin denetim sıklığını arttırmaları ve dondurulmuş karides üretimi ile satışı yapan işletmelerin yıllık numune planına dahil edilerek ağır metal kontaminasyon düzeyinin izlenmesinin, sağlık risklerinin azaltılmasına/önlenmesine yönelik önemli faydalar sunacağı düşünülmektedir.

Anahtar Kelimeler: Ağır metal, biyoakümülasyon, halk sağlığı, karides.

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GİRİŞ

Tarımsal ve endüstriyel süreçler gibi antropojenik (madencilik, zirai ilaçlar, endüstriyel atıklar vb.) ve jeojenik (mineralleşmiş bölgenin doğal değişimi) faaliyetler nedeniyle su ve diğer ekosistemler kimyasal kirletici veya kirletici gruplarına (ağır metaller, kalıcı organik kirleticiler, radyonüklitler vb.) maruz kalabilmektedir (Muhammad ve Ahmad 2020; Nirmal ve ark. 2020). Toksik metaller; toksisite, biyoakümülasyon ve biyomagnifikasyon özelliklerinden dolayı su ekosistemlerini (su, sediment, biyota) en çok kirleten, su ortamlarında uzun süre bozulmadan kalabilen ve su canlılarına zarar veren en önemli kontaminantlardandır.

Ağır metaller suda çözünmüş formda, suda asılı yükler şeklinde veya dip sedimentlerde bulunmaktadır (Sabir ve ark. 2017). Bu kimyasallar akümüle olabilmeleri, besin zincirinde bileşenler arası transfer ve başka besin zincirlerine aktarılabilmesi nedeniyle ekosistem için ayrıca bir risk oluşturmaktadır. Saha ve laboratuvar araştırmalarının sonuçlarına göre balık dokularındaki ağır metal biyobirikimi metal tipi, yoğunluk ve maruz kalma süresi, sıcaklık, alkalinite, sertlik, pH, tuzluluk oranı ile bazı metaller ve organik karbon gibi su kalite parametrelerinden etkilenebilmektedir. Ayrıca habitat, beslenme alışkanlıkları, büyüklük, cinsiyet, yaşam süresi ile akuatik besin ağlarındaki konumu gibi türle ilişkili ekolojik ve fizyolojik faktörlerin de ağır metal akümülasyonuna etkisi bulunmaktadır (USEPA 2016; Miri ve ark. 2017; Solgi ve ark. 2019). Yenilebilir su canlıları tüketiminin insanların çeşitli kimyasal kirleticilerin alımında önemli bir faktör olduğu bilinmektedir (Ezemonye ve ark. 2019). Bu kontaminantlar tüketilebilir su canlıları yoluyla besin zincirine dahil olmakta ve insan sağlığı için potansiyel tehlike oluşturmaktadır (Copat ve ark. 2013; Aytekin ve ark. 2019; Nirmal ve ark. 2020).

Ağır metaller, toksisitelerine ve besin değerlerine bağlı olarak esansiyel ve esansiyel olmayan (toksik metaller) olarak sınıflandırılmaktadır. Bakır (Cu), Mangan (Mn), Demir (Fe), Çinko (Zn) ve Kobalt (Co) canlıların normal işlevlerin sürdürülmesi ve hayatta kalabilmesi için eser miktarlarda alınması gerekli metallerdendir (Muhammad ve ark. 2019). Esansiyel metallerin yeterli miktarda alınmaması durumunda organizmada işlevsel bozukluklar görülebilirken, bu metallerin yüksek konsantrasyonları ciddi sağlık sorunlarına neden olabilmektedir (Alves ve ark. 2018). Kurşun (Pb) Kadmiyum (Cd), Cıva (Hg), Nikel (Ni) ve Krom (Cr) gibi ağır metaller, potansiyel olarak toksik elementler olup insanlarda ve hayvanlarda güvenlik eşiğinin üzerinde çok küçük miktarlarda bile önemli hastalıkların (nörolojik problemler, baş ağrısı ve karaciğer ve böbrek hastalığı gibi kanserojen olmayan tehlikeler, mide hastalıkları, anoreksiya, kalp hastalıkları, hipertansiyon ve kanser vb.) ortaya çıkmasına yol açabilmektedir (Miri ve ark. 2017; Ezemonye ve ark. 2019; Muhammad ve Ahmad 2020). Toksisiteleri ve akümülasyon davranışları nedeniyle hem deniz canlılarının çeşitliliğine hem de ekosistemlere zarar verebilmekte ve daha sonra besin zinciri yoluyla insanlara geçebilmektedirler. İnsanların ağır metaller gibi toksik kimyasallara maruz kalmasının ana kaynaklarından biride kontamine gıdaların, özellikle deniz ürünlerinin diyetle alınmasıdır. Bu nedenle, insan sağlığına yönelik olası riskleri değerlendirmek için deniz organizmalarının kimyasal kalitesinin, özellikle metal seviyelerinin belirlenmesi ve periyodik olarak izlenmesi önem arz etmektedir (Nabavi ve ark. 2012; Miri ve ark. 2017).

Balıklar ve diğer su canlıları genellikle içinde yaşadıkları ortamın önemli bir biyoindikatörü olarak kabul edilirler. İnsan beslenmesinde besin zincirinin son halkasını oluşturan bu canlılar, insanlara ağır metal aktarımında önemli gruplardan biri olarak kabul edilirler (Miri ve ark. 2017; Aytekin ve ark. 2019). Metallerin su canlılarına geçişi sindirim sistemi (diyet maruziyeti) ve solungaç yüzeyi (su maruziyeti) ile olmaktadır. Daha sonra kan yoluyla karaciğer gibi diğer hedef organlara aktarılmaktadır. Eser metaller esas olarak metalotiyoninler (yüksek sistein içeriğine sahip düşük moleküler ağırlıklı proteinler) yoluyla detoksifikasyon için karaciğerde depolanmaktadır. Kaslar, genelde cıva hariç metal aglomerasyonunda önemli bir doku değildir. Kaslardaki potansiyel metal birikimlerinin incelenmesinin nedeni halk sağlığı açısından olası tehlikelerin tespitine yöneliktir (Nabavi ve ark. 2012).

Kabuklu su canlıları, dünya çapında protein açısından zengin gıda arzı sağlayan en büyük deniz ürünlerinden biridir. Karides yüksek kaliteli protein, yağ asiti, vitamin ve mineral kaynağı olup tüm dünyada yaygın olarak tüketilen bir üründür. Karidesler genellikle piyasa talebine göre kabuklu/işlenmiş şekilde taze veya dondurulmuş formlarda satışa sunulmaktadır. Küresel karides üretimi 2020'de 5,03 milyon tona ulaşırken, 2020'den 2025'e kadar %6,1 bileşik yıllık büyüme oranı ile 2025'e kadar 7,28 milyon tona çıkması beklenmektedir (Nirmal ve ark. 2020).

Ağır metaller kararlı bileşikler olması, biyolojik olarak parçalanmaması, çevresel kompartımanlarda uzun süre kalıcılıkları ve potansiyel sağlık riskleri gibi daha birçok faktörden dolayı bu kontamitantlara ilişkin küresel endişeler bulunmaktadır (Miri ve ark. 2017). Ekosistemlerin sağlığının ve sürdürülebilirliğinin değerlendirilmesine yönelik yapılan araştırmalar, çevre ve halk sağlığının korunmasına yönelik önemli bilimsel veriler sunmaktadır. Bu nedenle sucul ekosistemlerdeki zamansal varyasyonları belirlemek için ağır metal kontaminasyon seviyesinin düzenli olarak ölçülmesi çok önemlidir (Muhammad ve Ahmad 2020). Cd, Pb ve toplam cıva (THg), çevrede yaygın olarak bulunan ve önemli sağlık sorunlarına neden olabilen toksik ağır metallerdir. Bu araştırmada, dondurulmuş olarak satışa sunulan karideslerdeki Cd, Pb ve THg gibi bazı toksik kontaminantların

yoğunluğunun belirlenmesi ve tüketimle ilişkili olarak potansiyel halk sağlığı risklerinin değerlendirilmesi amaçlanmıştır.

MATERYAL ve METOT

Örneklerin Toplanması ve Analizlere Hazırlanması

Araştırmada; Nisan-Mayıs 2021 tarihinde, satış noktalarından (süpermarket, şarküteri, zincir market vs.) temin edilen, farklı markalara ait (farklı parti numaralı) 400-500 gr'lık ambalajlarda dondurulmuş formda satışa sunulan toplam 48 paket karides örneği (*n=48*) değerlendirildi. Toplanan karides örnekleri, içerisinde buz bulunan polietilen strafor kutulara konularak soğuk zincir içerisinde kısa sürede laboratuvara ulaştırıldı ve analizlere kadar derin dondurucuda (Arçelik 2501, Türkiye) -20 °C'de muhafaza edildi.

Ağır Metal Analizleri

Çalışmada kullanılacak tüm cam eşyalar bir gün boyunca HNO_3 (%30, Merck, Germany) içerisinde bekletilmiş ve ultra saf suyla (Synergy® Water Purification System, Germany) iyice durulandıktan sonra oda sıcaklığında kurutulmuştur. Dondurulmuş örnekler oda sıcaklığında bekletilerek çözünmeleri sağlanmıştır. Daha sonra homojen hale getirilen örnekler (0,5 gr) teflon tüplere aktarılmış, üzerine 9 ml Nitrik asit (%65, HNO_3, Merck, Germany)+3 ml Hidroklorik asit (%35 HCl, Merck, Germany) eklenerek mikrodalga fırınında (1450 W-45 bar) (Milestone Start D, İtalya) iki aşamada (15 dakikada 110 °C'ye yükseltme, 110 °C'de 15 dakika bekleme) yaş yakmaya alınmış ve bozundurma süreci tamamlanmıştır (USEPA 2007). Soğutulan teflon tüpler ultra saf suyla yıkanarak polipropilen santrifüj tüplerine alınmış ve yine ultra saf su ile 50 ml'ye tamamlanarak İndüktif Eşleşmiş Plazma-Optik Emisyon Spektrometresi (Inductively Coupled Plasma-Optical Emission Spectroscopy/ICP-OES, Perkin Elmer Optima 8000, USA) ile üç tekerrürlü ölçümleri yapılmıştır. THg analizi; hidrid sistem (%0,2 $NaBH_4$ (%0,05 NaOH+%3 HCl) kullanılarak yapılmıştır. Karides örneklerine ait Cd, THg ve Pb ölçüm sonuçları entegre yazılım ile mg kg^{-1}/yaş ağırlık şeklinde hesaplanmıştır. Analitik kalite kontrol parametreleri ilgili metot prosedürleri doğrultusunda gerçekleştirilmiştir. Sertifikalı referans materyali (SRM) ile (QCS-27, Belçika) ile analitik prosedürün parametreleri değerlendirilmiştir. Korelasyon katsayısı r^2>0,999, SRM ve spike yapılmış örneklere ait geri kazanım oranları %92 ile %106,4 arasında olup Cd, THg ve Pb için tespit limitlerinin (Limit of Detection/LOD) sırasıyla 0,003, 0,001 ve 0,005 olduğu belirlenmiştir (Tablo 1).

Sağlık Risk Değerlendirmeleri

Bu çalışmada, karides tüketimine yönelik potansiyel sağlık risklerinin belirlenmesi amacıyla Tahmini Günlük Alım Düzeyi (Estimated Daily Intake/EDI), Hedef Tehlike Katsayısı (Target Hazard Quotient/THQ), Tehlike İndeksi (Hazard Index/HI) ve İzin Verilen Maksimum Balık Tüketim Oranı (Consumption Rates/CR_{lim}) değerlendirilmiştir.

EDI; gıdadan insanlara gıda kontaminant transferinin tahmini için yaygın olarak kullanılan bir indekstir ve tüketilen gıdanın miktarına, tüketim süresine ve kontaminasyon düzeyine bağlıdır (Solgi ve ark. 2019).

THQ; uzun süreli maruz kalma, yutulan miktar ve vücut ağırlığı ile ilişkili boyutsuz bir risk indeksidir. Kimyasal kirleticilere uzun süre maruz kalmayla ilişkili potansiyel sağlık risklerinin tahmini için USEPA tarafından geliştirilmiştir. Maruz kalma süresi ve sıklığı, alınan miktar ve vücut ağırlığıyla ölçülen konsantrasyon ile oral referans doz arasındaki oran olarak tanımlanmaktadır. THQ, ilgili toksik elemente maruz kalmanın neden olduğu kanserojen olmayan sağlık riskini göstermektedir. THQ<1 ise, kanserojen olmayan sağlık etkileri beklenmemektedir. Bununla birlikte THQ>1 ise, maruz kalma seviyesinin güvenli referans sınırdan daha yüksek olduğunu ve potansiyel bir sağlık riski olasılığını göstermektedir (Yu ve ark. 2020; USEPA 2021).

Oral Referans doz (RfD_o); insan popülasyonuna (hassas alt gruplar dâhil) belirli bir süre boyunca kayda değer yan etki riski olmaksızın günlük oral maruz kalma tahmini olarak tanımlanmaktadır ve değerlendirilen eser elemente özgü bir değerdir (USEPA 2011).

HI veya Toplam Tehlike İndeksi (Total Hazard Index/THI); her gıda türü için değerlendirilen unsurların bireysel hedef tehlike oranlarının toplamıdır. HI, tüketilen gıdada potansiyel olarak toksik olan birkaç elemente aynı anda maruz kalınabileceği varsayımı üzerinden yapılan değerlendirmedir (USEPA 1989; Antoine ve ark. 2017).

Analizler sonucunda karides numunelerinde saptanan Cd, THg ve Pb yoğunluklarına göre EDI, THQ ve HI değerleri aşağıdaki formüllere göre hesaplanmıştır (Copat ve ark. 2014; Antoine ve ark. 2017; Solgi ve ark. 2019; Çiftçi ve ark. 2021; USEPA 2023a; USEPA 2023b; Varol ve Kaçar 2023).

Analiz edilen ağır metallerin EDI_{Cd}, EDI_{iHg}, EDI_{metgHg} ve EDI_{Pb} sonuçları, karides örneklerindeki ortalama metal yoğunluğu ve gram cinsinden günlük alım miktarına göre belirlenmiştir.

$$\text{EDI (mg kg}^{-1}\text{/gün)} = \frac{\text{C} \times \text{F}_{\text{IR}}}{\text{BW}}$$

EDI (*Estimated Daily Intake*): Öğün başına Tahmini Günlük Alım Miktarı

C (*Metal concentration*): Metal konsantrasyonu (ortalama, mg kg^{-1}/yaş ağırlık)

F_{IR} (*Fish Ingestion Rate*): g/gün/kişi cinsinden günlük balık tüketim miktarı

(17,81 g/gün/kişi; TEPGE 2022)

BW (*Body Weight*): Vücut Ağırlığı, yetişkinler için ortalama70 kg (Varol ve Sünbül 2020)

$$THQ = \frac{EF \times ED \times C \times F_{IR}}{RfD_o \times BW \times AT_n} \times 10^{-3}$$

EF (*Exposure Frequency*): Yıl boyunca maruz kalma sıklığı (350 gün/yıl; USEPA 1991)
ED (*Exposure Day*): Maruz kalma süresi: ~26 yıl (USEPA 1989; USEPA 2011)
RfD_o (*Reference Dose*): Oral referans doz (mg kg^{-1}/gün) (Cd: 0,001 (1,0E-03), iHg: 0,0003 (3,0E-04), metHg: 0,0001 (1,0E-04), (Pb: -) mg kg^{-1} vücut ağırlığı/gün (USEPA 2021; USEPA 2023a).
AT_n ($AT_{noncancer}$, *Average Exposure Time for Noncarcinogens*): Kanserojen Olmayanlar İçin Ortalama Maruz Kalma Süresi (AT_n; 365 gün/yıl × 26 yıl; USEPA 1989)

$$HI = \sum_{i=1}^{n} THQ \left(HI = THQ_{Cd} + THQ_{THg} + THQ_{Pb}\right)$$

CR_{lim}, tüketicileri metallerin olumsuz etkilerinden korumak amacıyla bir kirleticinin kanserojen olmayan sağlık riskleri açısından gün bazında güvenle tüketilmesi gereken maksimum balık miktarını belirlemek için kullanılmaktadır (USEPA 2000).

$$CR_{lim}(kg/gün) = \frac{RfD_o \times BW}{C_m}$$

CR_{lim}(*Consumption Rates*): İzin verilen maksimum balık tüketim oranı (g/gün)
C_m (*Metal concentration*): Metal konsantrasyonu (ortalama, mg kg^{-1}/yaş ağırlık)

Ülkemizde kişi düzeyinde günlük/aylık karides tüketim miktarına yönelik detaylı istatistiki bir veri tespit edilemediği için hesaplamalarda 2021 yılı kişi başı ortalama su ürünleri tüketim miktarı 17,81 g/kişi/gün (6,5 kg/kişi/yıl) baz alınmıştır (TEPGE 2022). Yetişkinler için ortalama vücut ağırlığı 70 kg varsayılmıştır (Varol ve Sünbül 2020). EDI değerleri, Avrupa Gıda Güvenliği Otoritesi (European Food Safety Authority/EFSA) ve Gıda Katkı Maddeleri Ortak Uzman Komitesi (Joint FAO/WHO Expert Committee on Food Additives/JECFA) tarafından önerilen Geçici Tolere Edilebilir Günlük/Haftalık Alım (Provisional Tolerable Daily/Weekly Intake/PTDI-PTWI) miktarıyla, THQ, HI ve CR_{lim} ise Amerika Birleşik Devletleri Çevre Koruma Ajansı (United States Environmental Protection Agency/USEPA) tarafından belirlenen standartlara ait verilerle karşılaştırılmıştır.

İstatistiksel analiz
İncelenen metallere ait konsantrasyonlar ortalama±standart sapma şeklinde verilmiştir. Cd, THg ve Pb arasındaki ilişki korelasyon analizi ile değerlendirilmiştir. $p<0,05$ istatistiksel olarak anlamlı kabul edilmiştir. Veri analizi SPSS (IBM, Endicott, NY, ABD) versiyon 20 kullanılarak yapılmıştır.

BULGULAR

Karides örneklerinde tespit edilen Cd, THg (iHg ve metHg) ve Pb konsantrasyonları ve diğer parametreler Tablo 1'de, Cd, THg (iHg ve metHg) ve Pb için belirlenen EDI, THQ, HI, CR_{lim} ile referans PTDI, PTWI ve PTMI değerleri Tablo 2'de verilmiştir.

Tablo 1. Karides örneklerinde Cd, THg (iHg ve metHg) ve Pb miktarı
Table 1. Cd, THg (iHg ve metHg) and Pb quantity in shrimp samples

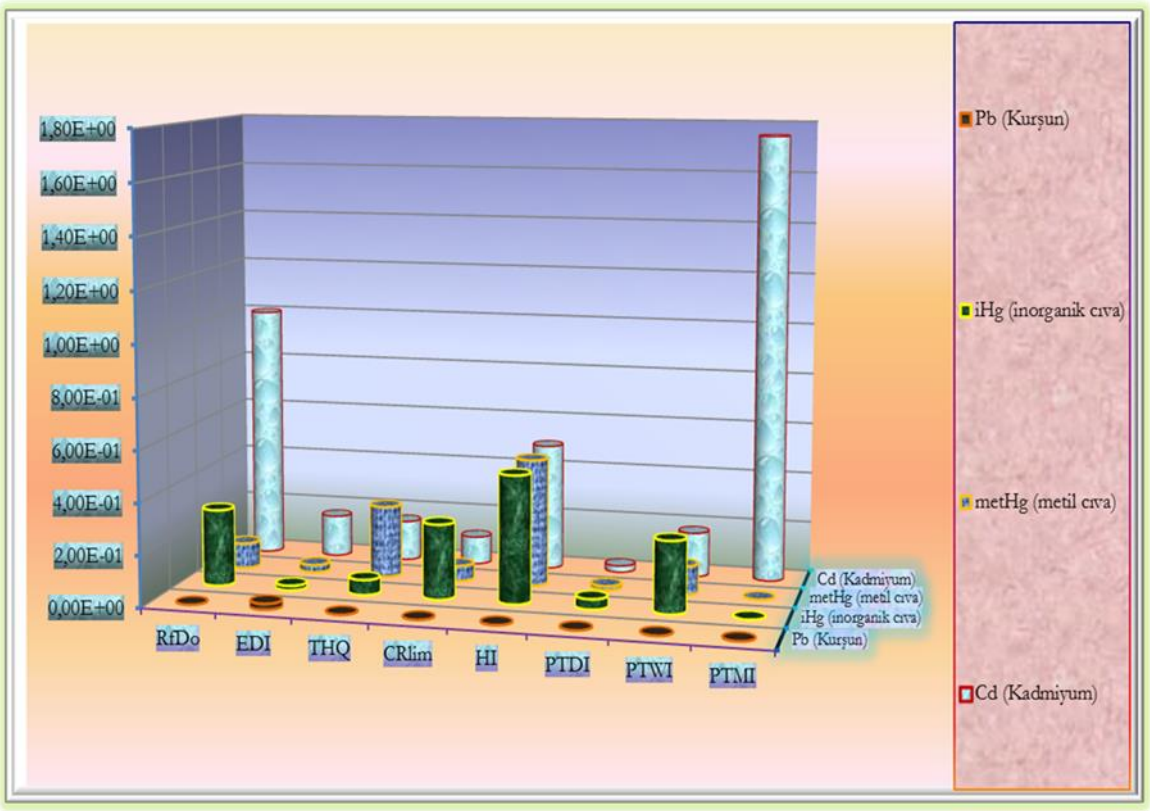
Metal	Örnek sayısı (*n: 48*)		LOD (ppm)	Konsantrasyon (mg kg^{-1} YA)	Ortalama±SS (mg kg^{-1} YA)	Maksimum Limit[a] (mg kg^{-1} YA)	>0.50 (mg kg^{-1} YA)
	(+)	(-)					
Cd	47	1	0,003	<LOD-2,054	0,643±0,490	0,50	25
THg	47	1	0,001	<LOD-0,364	0,144±0,100	0,50	*TE*
iHg				-	0,072±0,000		
metHg					0,115±0,000		
Pb	47	1	0,005	<LOD-0,620	0,094±0,090	0,30/0,50	1

(+): pozitif örnek sayısı, (-): <LOD örnek sayısı, LOD (*Limit of Detection*): Tespit Limiti, YA: Yaş Ağırlık, SS: Standart Sapma, TE: Tespit Edilemedi, a: EC (2014; 2015; 2022; 2023), JECFA (2018), TGK (2011)

Tablo 2. Karides örneklerinde Cd, THg (iHg ve metHg) ve Pb için belirlenen EDI, THQ, HI, CR_{lim}, PTDI, PTWI ve PTMI referans değerleri
Table 2. EDI, THQ, HI, CRlim, and PTDI, PTWI and PTMI reference values determined for Cd, THg (iHg ve metHg) and Pb in shrimp samples

Metal		RfD_o (mg kg^{-1}/gün)	EDI (mg kg^{-1}) *Tüketim (gün/kişi)*	THQ (mg kg^{-1}) *Tüketim (gün/kişi)*	CR_{lim} (kg/gün) *Tüketim (gün/kişi)*	HI (mg kg^{-1}) *Tüketim (gün/kişi)*	PTDI (mg kg^{-1}) (vücut ağırlığı: 70 kg)	PTWI (mg kg^{-1}) (vücut ağırlığı: 70 kg)	PTMI (mg kg^{-1}) (vücut ağırlığı: 70 kg)
Cd		1,0E-03 (0,001)	0,163	1,57E-01	0,109	4,97E-01	0,025/0,060 *(EFSA, 2011; JECFA, 2018)*	0,175 *(PTWI: 0,0025 mg kg^{-1}va) (EFSA, 2011)*	1,75 *(PTMI: 0,0250 mg kg^{-1}va) (JECFA, 2018)*
THg	iHg (THgX0.5)	3,0E-04 (0,0003)	0,018	5,90E-02	0,292		0,040 (iHg) *(Tolere Edilebilir Haftalık Alım miktarı üzerinden hesaplanmıştır)*	0,280 (iHg) *PTWI: inorganik cıva 0,004 mg kg^{-1} va) (EFSA, 2012a; JECFA, 2018)*	PTMI belirlenmemiştir *(EFSA, 2012a; JECFA, 2018)*
	metHg (THgX0.8)	1,0E-04 (0,0001)	0,029	2,81E-01	0,061		0,016 (metHg) *(Tolere Edilebilir Haftalık Alım miktarı üzerinden hesaplanmıştır)*	0,112 (metHg) *(PTWI: metil cıva 0,0016 mg kg^{-1} va) (EFSA, 2012a; JECFA, 2018)*	PTMI belirlenmemiştir *(EFSA, 2012a; JECFA, 2018)*
Pb		RfD_o belirlenmemiştir *(USEPA, 2021) (USEPA, 2023a)*	0,023	-	-	-	-	PTWI belirlenmemiştir *(EFSA, 2010; JECFA, 2018)*	PTMI belirlenmemiştir *(EFSA, 2010; JECFA, 2018)*

RfD_o (Reference Dose): Oral Referans Doz, EDI (Estimated Daily Intake): Tahmini Günlük Alım, THQ (Target Hazard Quotient): Hedef Tehlike Katsayısı, HI (Hazard Index): Tehlike İndeksi, PTDI (Provisional Maximum Tolerable Daily Intake): Tolere Edilebilir Günlük Alım, PTWI (Provisional Tolerable Weekly Intake): Tolere Edilebilir Haftalık Alım, PTMI (Provisional Tolerable Monthly Intake): Tolere Edilebilir Aylık Alım, va: vücut ağırlığı, THg: Toplam cıva, iHg: İnorganik cıva, metHg: Metil cıva



Şekil 1: Pb, iHg, metHg ve Cd için Referans doz (RfD_o, µg kg^{-1}/gün), karides tüketimine yönelik belirlenen Tahmini Günlük Alım Düzeyi (EDI, mg kg^{-1}/gün), Tahmini Hedef Tehlike Katsayısı (THQ), İzin verilen maksimum balık tüketim oranı (CR_{lim}, kg/gün), Tehlike İndeksi (HI) ile Tolere Edilebilir Günlük/Haftalık/Aylık Alım PTDI/PTWI/PTMI (mg kg^{-1}/vücut ağırlığı) referans değerleri

Figure 1: Reference dose (RfDo, µg kg^{-1}/day) for Pb, iHg, metHg and Cd and Estimated Daily Intake Level (EDI, mg kg^{-1}/day) for shrimp consumption, Estimated Target Hazard Coefficient (THQ) , Maximum allowable fish consumption rate (CRlim, kg/day), Hazard Index (HI) and Tolerable Daily/Weekly/Monthly Intake PTDI/PTWI/PTMI (mg kg^{-1}/body weight) reference values

TARTIŞMA

Dondurulmuş karideslerde ağır metal miktarının belirlendiği bu çalışmanın sonuçlarına göre ağır metal yoğunluğunun ortalama değerler üzerinden Cd>THg>Pb şeklinde olduğu saptanmıştır. İncelenen örneklerde Cd konsantrasyonu <LOD-2,054 mg kg^{-1}/yaş ağırlık ile en yüksek yoğunluklu ağır metal olurken, THg ve Pb düzeyinin sırasıyla ortalama 0,144±0,100 ve 0,094±0,090 mg kg^{-1}/yaş ağırlık olduğu tespit edilmiştir (Tablo 1).

Cd, Hg ve Pb "Tehlikeli Maddelerin Öncelik Listesi" içerisinde rapor edilen en toksik 10 madde arasında tanımlanan ağır metallerdir (ATSDR 2013). Dünya Sağlık Örgütü (World Health Organization/WHO), USEPA, JECFA ve EFSA tarafından insanlar için toksik maddelere yönelik tolere edilebilir maruz kalma (tolere edilebilir alım referans dozu ve sağlık risk faktörleri) için sağlığa dayalı bir çok kılavuz yayınlanmıştır (Copat ve ark. 2013).

Türk Gıda Kodeksi Bulaşanlar Yönetmeliği'nde farklı balık türlerinde Cd, Hg ve Pb için yasal limitler 0,05 ile 0,50 mg kg^{-1}/yaş ağırlık arasında değişirken, kabuklularda (*Crustaceans*) (başlı gövde kısmı hariç karın ve karın uzantısı kas eti) bu metaller için limit değer 0,50 mg kg^{-1}/yaş ağırlık olacak şekilde düzenlenmiştir (TGK 2011).

Avrupa Birliği direktiflerinde kabuklularda (*Crustaceans*) (başlı gövde kısmı hariç karın ve karın uzantısı kas eti) Cd, Hg ve Pb miktarına yönelik maksimum düzeyin 0,50 mg kg^{-1}/yaş ağırlık olarak belirlendiği görülmektedir (EC 2014; EC 2015; EC 2022; EC 2023). JECFA, balık etlerinde kadmiyum için herhangi bir limit değer belirtmemiş olup cıva ve kurşun için sırasıyla en fazla 0,50 ve 0,30 mg kg^{-1}/yaş ağırlık olacak şekilde sınırlamalar getirmiştir (JECFA 2018).

Tüm karides örneklerinde THg miktarının, kabuklular için TGK (2011), EC (2022) ve EC (2023) ile balık etlerine yönelik JECFA (2018) tarafından yapılan düzenlemelerdeki limit değerlerin altında olduğu saptanmıştır (Tablo 1). 25 örnekte Cd düzeyinin ve bir (1) örnektede Pb seviyesinin (0,620 mg kg^{-1}/yaş ağırlık) TGK (2011), EC (2014), EC (2015) ve EC'na (2023) göre tüketilebilirlik sınırını (0,50 mg kg^{-1}/yaş ağırlık) aştığı, JECFA'ne (2018) göre ise 1 (bir)

örneğin Pb açısından limit değerin üzerinde olduğu tespit edilmiştir (Tablo 1). Araştırma sonuçlarımıza benzer şekilde Aytekin ve ark. (2019), araştırmalarında karides örneklerinde tespit edilen Cd (6,52-8,33 μg/g kuru ağırlık) ve Pb (22,18-62,75 μg/g kuru ağırlık) düzeylerinin çeşitli sağlık kuruluşları tarafından belirlenmiş insan tüketimi için kabul edilebilir değerlerin üzerinde olduğunu bildirmişlerdir.

Yapılan bir araştırmada karideslerde saptanan Cd ve Pb yoğunluğunun 0,93 ve 22,39 mg kg^{-1} olarak saptandığını ve uluslararası düzenlemelere göre belirlenen limitleri aştığı bildirilmiştir (Ezemonye ve ark. 2019). Başka bir araştırmada ise farklı karides türlerinde 2,127-2,802 mg kg^{-1} Cd ve 1,294-2,723 mg kg^{-1} konsatrasyonlarında Pb varlığı tespit edildiği, örneklerdeki ağır metal düzeyinin özellikle JECFA'ya göre tolere edilebilir sınırı aştığı, ağır metal konsantrasyonlarının sudan tortuya ve nihayet karideslere doğru arttığı ve biyolojik birikimin görüldüğü belirtilmiştir (Herliwati ve ark. 2022). Yu ve ark. (2020), farklı karides türlerinde Cd ve Pb miktarını sırasıyla 0,001-0,236 ve <LOD-0,330 mg kg^{-1} olduğunu belirlerken; Yipel ve Tekeli (2022), araştırmalarında Cd ve Pb yoğunluğunun tespit sınırı altında (<LOD) kaldığını belirtmişlerdir. Farklı bulguların örneklerin temin edildiği aquatik ortamdaki metal kontaminasyon yoğunluğundan kaynaklanabileceği değerlendirilmektedir. Metaller genellikle karidesler tarafından doğal habitatı içerisinde yer alan besinlerle birlikte sulardan alınmakta, dolaşımla vücuda dağılmakta ve sonunda hedef organlarda birikmektedir (Agah ve ark. 2009).

Kadmiyum, mineral yataklarında ve çevrede düşük konsantrasyonlarda yaygın olarak bulunan doğal olarak oluşan bir metaldir. Kadmiyumun birincil endüstriyel kullanımları pillerin, pigmentlerin, plastik stabilizatörlerin, metal kaplamaların, alaşımların ve elektroniklerin imalatı içindir. Son zamanlarda kadmiyum, güneş pillerinde ve renkli ekranlarda kullanılmak üzere nanopartiküllerin (kuantum noktaları) üretiminde kullanılmaktadır. Karidesler diğer kabuklu deniz canlıları gibi doğal kadmiyum toplayıcılarıdır. Kadmiyum, suda yaşayan hayvanlarda biyolojik işlevi olmayan non-esansiyel bir metaldir. Yüzey suyunda çözünen minerallerin (öncelikle kalsiyum ve daha az ölçüde magnezyum) miktarı olan su sertliği, kadmiyumun toksisitesini etkileyen önemli bir su kalitesi parametresi olup yumuşak sularda sert suya göre daha akut toksik olduğu bilinmektedir (USEPA 2016; JECFA 2018). Gıda ve sigara, genel nüfus için kadmiyum maruziyetinin iki ana kaynağını oluşturmaktadır. Kadmiyum kontaminasyonu böbrek, karaciğer gibi bir çok gıdada özellikle en yüksek konsantrasyonları kabuklu deniz canlılarında bulunmaktadır. Kadmiyumun insanlarda biyolojik yarı ömrü yaklaşık olarak 10-30 yıl arasında olduğu bilinen ve insanlarda teratojen kanserojen etkileri olan en toksik elementlerden biridir. İnsanlarda aşırı Cd alımı böbrek yetmezliği ve kısırlığa neden olabilmektedir (Yildirim ve ark. 2009; USEPA 2016).

USEPA, Pb'yi çoğu yaşam biçimi için potansiyel olarak tehlikeli ve toksik olarak sınıflandırmaktadır. Pb zehirlenmesi genellikle en yaygın çevresel sağlık tehlikeleri içerisinde ilk sırada yer almaktadır. İşitme bozuklukları, anemi, böbrek yetmezliği, zayıflamış bağışıklık sistemi, düşük doğum ağırlıkları, ölü doğumlar ve abort, erken doğumlar, yüksek kan ve idrar kurşun seviyeleri Pb zehirlenmesinin en sık görülen semptomlarıdır (Yildirim ve ark. 2009; Nabavi ve ark. 2012).

Araştırma sonuçlarımıza göre karides örneklerinde tespit edilen toplam Hg (THg) düzeyinin <LOD-0,364 ve ortalama 0,144±0.010 mg kg^{-1}/yaş ağırlık olduğu tespit edilmiştir (Tablo 1). İstatistiksel analizler açısından THg ve Pb arasında negative yönlü bir korelasyon (-0,359) olduğu belirlenmiştir ($p<0,05$). Yu ve ark. (2020); farklı türlere ait karides örneklerinde Hg miktarının <LOD-0,860 mg kg^{-1} arasında olduğunu, Sultana ve ark. (2022); kültür karideslerinde Hg yoğunluğunu ortalama 0,02±0,006 mg kg^{-1} olarak tespit edildiğini bildirmişlerdir. Balıklarda bulunan toplam cıvanın yaklaşık %75-90'ı tehlikeli organik form (metilcıva, CH_3Hg^+) şeklinde bulunmaktadır. EFSA verilerinde göre balık ve diğer deniz ürünleri, tüketicilerin metilcıva'ya maruz kalmasına en çok katkıda bulunan gıdalar olduğu belirtilmektedir. Yetişkin popülasyonun diyet yoluyla metilcıva maruziyetinin haftalık ortalama 0,24 μg kg^{-1} civarında olduğu bildirilmektedir (EFSA 2012a; Hong ve ark. 2012).

Kabuklu deniz canlıları için inorganik Hg için dönüşüm faktörü olarak 0,5 kullanılırken CH_3Hg için dönüşüm faktörü olarak 0,8 kullanılmaktadır (EFSA 2012b; Hong ve ark. 2012; Omeragic ve ark. 2020). Bu değerlendirmeye göre araştırmamızda karides örneklerindeki ortalama iHg ve metHg miktarının sırasıyla 0,072 ve 0,115 mg kg^{-1}/yaş ağırlık olabileceği varsayılmıştır. Cıva, farklı kimyasal formlarda (elemental-metalik cıva, inorganik cıva bileşikleri, metilcıva ve diğer organik bileşikler) bulunan nörotoksik bir elementtir. İnsanların metil cıvaya maruz kalmasının ana yolu, dokularında cıvanın oldukça zehirli bir formu olan yüksek düzeyde metilcıva içeren balık ve kabuklu deniz canlılarının tüketilmesidir. Metil cıva özellikle sinir sistemi ve gelişmekte olan beyin için toksik olduğu için hamilelik sırasında maruz kalma metilcıva toksisitesi için en kritik dönem olarak kabul edilmektedir (EFSA 2012a). Deniz organizmalarının boyutu, balık dokularının metal içeriklerinde çok önemli bir rol oynamaktadır (Dang ve Wang 2012). Bu durum özellikle cıva için oldukça belirgindir. Ancak büyüklükle birlikte ağır metal konsantrasyonlarında görülen güvenilir istatistiksel artış eğilimi, diğer metaller için yeteri kadar belirgin değildir (Canlı ve Atlı 2003).

Deniz organizmalarındaki kirletici içeriğini değerlendirmenin en önemli avantajlarından biri de insan diyet maruziyetini ve potansiyel sağlık problemlerinin tahmin edilmesidir (Traina ve ark.

2019). Kanser riski, bir kişinin yaşamı boyunca potansiyel kanserojenlere maruz kalması nedeniyle kanser geliştirme olasılığı olarak değerlendirilmektedir (USEPA 2016). Cd; grup 1 (insanlar için kanserojen) ve Pb; grup 2A (insanlar için muhtemel kanserojen) kanser riski taşırken Hg ve iHg; grup 3, metHg ise grup 2B (insanlar için muhtemelen kanserojen) kanserojen kimyasallar olarak sınıflandırılmaktadır (IARC 2022). Araştırma bulgularımıza göre EDI_{Cd} ve EDI_{metHg} miktarının, PTDI/PTWI referans değerlerini aştığı görülürken EDI_{iHg} miktarının ise limit düzeylerin altında kaldığı belirlenmiştir (Tablo 2, Şekil 1). Bu durum incelenen örneklerin Cd ve metHg açısından insan sağlığına yönelik potansiyel risk taşıyabileceğini göstermektedir.

Gıda maddesindeki elementler için bireysel THQ'lar tek başına birden (<1) düşük olsa bile, tüketimin kümülatif etkisi olumsuz sağlık etkilerine neden olabilir. İki veya daha fazla ağır metale bu şekilde maruz kalma, potansiyel risklerin additif etkisine yol açabilir (Antoine ve ark. 2017). Bazı toksik metaller arasında sinerjik etkinin varlığı bilinmektedir. Örneğin çocuklarda As ve Pb toksisitesinde birincil hedefin merkezi sinir sistemi olduğu ve bu durumda zihinsel hasar olasılığının arttığı görülmektedir (Tokatlı ve Ustaoğlu 2021). HI>1 ise, kanserojen olmayan olumsuz sağlık sorunları potansiyeli bulunmaktadır. HI≤1,0, önemsiz yan etkilerin tahmin edildiğini ve HI>1,0 kronik toksik etkilerin olası olduğunu ifade etmektedir (USEPA 1989; Antoine ve ark. 2017).

Karides örneklerinde tek ve/veya toplam metal yoğunluğunun (THQ<1 ve HI: 0,49) kanserojen olmayan sağlık riskleri açısından güvenlik marjı içerisinde kaldığı görülmektedir (Tablo 2, Şekil 1). Bu veriler kanserojen olmayan sağlık risklerinin ortaya çıkma ihtimalinin düşük olduğunu göstermektedir. THQ değerinin 1'den büyük olması, maruz kalma seviyesinin güvenli referanslı sınırdan daha yüksek olduğunu ve potansiyel bir sağlık riski oluşturabileceğini göstermektedir (Copat ve ark. 2014). Referans alınan güvenli sınırdan daha yüksek günlük maruz kalma seviyeleri, olumsuz sağlık etkilerine, iki veya daha fazla ağır metale bu şekilde maruz kalma, potansiyel risklerin additif etkisine yol açabilmektedir (Yu ve ark. 2020). Yapılan çalışmalarda toksik metallerle kontamine balık ve kabuklu deniz canlıları tüketimine yönelik nonkanserojen etki tespit edilemediğine yönelik veriler bulunmaktadır (Ezemonye ve ark. 2019; Arisekar ve ark. 2022). CR_{lim}, 70 kg ağırlığındaki bir yetişkinin bir günde güvenle tüketebileceği maksimum balık miktarını (kg) temsil etmektedir. Karides örneklerinde incelenen toksik metaller için CR_{lim} değerlerinin (≥61 g/gün-metHg) kişi başı günlük balık tüketim oranı (17,81 g) üzerinden kıyaslandığında kanserojen olmayan sağlık riskleri açısından herhangi bir tehlike oluşturmadığı görülmüştür (Tablo 2, Şekil 1).

SONUÇ

Elde edilen bulgular, karides tüketimine ilişkin halk sağlığı açısından olası risklerin değerlendirilmesine yönelik ağır metal kontaminasyonu hakkında güncel veriler sunmakta olup toksik metal kirliliğini önlemek için önlemler alınması gerektiğini göstermektedir. Araştırmada elde edilen bulgulara bakıldığında, bazı örneklerde saptanan toksik metal (Cd ve Pb) seviyelerinin TGK (2011), EC (2014), EC (2015), EC (2023) ve diğer bazı sağlık kuruluşları (JECFA) tarafından insan tüketimi için belirlenen limit değerleri (0,30-0,50 mg kg^{-1}/yaş ağırlık) aştığı görülmektedir. Bu veriler nedeniyle insanların karides tüketimi yoluyla bu toksik elementlere maruz kalma durumu ve olası risklerin değerlendirilmesi için devamlı şekilde periyodik izleme çalışmaları yapılmasının faydalı olacağı düşünülmektedir. Hem THQ hem de HI sonuçlarının 1'den düşük olması, tüketiciler açısından kanserojen olmayan sağlık etkilerinin ortaya çıkma olasılığının zayıf olduğunu göstermektedir. Taze, işlenmiş ve dondurulmuş karides üretimi yapan işletmelerin İyi Üretim Uygulamaları (Good Manufacturing Practice/GMP) ve/veya Tehlike Analizi ve Kritik Kontrol Noktaları (Hazard Analysis and Critical Control Point/HACCP) gibi gıda güvenliği kontrol sistemleri içerisinde ürünlerdeki ağır metal yoğunluğunun sürekli olarak izlenmesi, çevre ve su ekosistemlerinde her türlü metal kontaminasyonu açısından önleyici tedbirlerin alınması, gıda güvenliği kontrolünün güçlendirilmesi ve potansiyel sağlık risklerinin önlenmesi açısından olumlu katkıları olacaktır.

Yazar Katkıları: Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan etmişlerdir.

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

Proje Destek Bilgileri: Bu çalışmada herhangi bir kurumdan finansal destek alınmamıştır.

Teşekkür: İstatistiksel analizlerin yapılmasına katkıda bulunan Sayın Doç. Dr. Memiş BOLACALI'ya teşekkürlerimizi sunarız.

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KAYNAKLAR

Agah, H., Leermakers, M., Elskens, M., Fatemi, S. M. R., & Baeyens, W. (2009). Accumulation of trace metals in the muscle and liver tissues of five fish species from the Persian Gulf. Environmental Monitoring and Assessment, 157, 499-514. https://doi.org/10.1007/s10661-008-0551-8.

Agency for Toxic Substance and Disease Rigestry (ATSDR) (2013). Priority List of Hazardous Substances that Will Be the Subject of Toxicological Profiles. p. 30333, Atlanta, Georgia, USA.

Alves, R. N., Maulvault, A. L., Barbosa, V. L., Fernandez-Tejedor, M., Tediosi, A., Kotterman, M., ... & Marques, A. (2018). Oral bioaccessibility of toxic and essential elements in raw and cooked commercial seafood species available in European markets. Food Chemistry, 267, 15-27. https://doi.org/10.1016/j.foodchem.2017.11.045.

Antoine, J. M., Fung, L. A. H., & Grant, C. N. (2017). Assessment of the potential health risks associated with the aluminium, arsenic, cadmium and lead content in selected fruits and vegetables grown in Jamaica. Toxicology Reports, 4, 181-187.

Arisekar, U., Shakila, R. J., Shalini, R., Jeyasekaran, G., Padmavathy, P., Hari, M. S., & Sudhan, C. (2022). Accumulation potential of heavy metals at different growth stages of Pacific white leg shrimp, Penaeus vannamei farmed along the Southeast coast of Peninsular India: A report on ecotoxicology and human health risk assessment. Environmental Research, 212, 113105.

Aytekin, T., Kargın, D., Çoğun, H. Y., Temiz, Ö., Varkal, H. S., & Kargın, F. (2019). Accumulation and health risk assessment of heavy metals in tissues of the shrimp and fish species from the Yumurtalik coast of Iskenderun Gulf, Turkey. Heliyon, 5(8), e02131. https://doi.org/10.1016/j.heliyon.2019.e02131.

Canlı, M., & Atlı, G. (2003). The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. Environmental Pollution, 121(1), 129-136. https://doi.org/10.1016/S0269-7491(02)00194-X.

Copat, C., Arena, G., Fiore, M., Ledda, C., Fallico, R., Sciacca, S., & Ferrante, M. (2013). Heavy metals concentrations in fish and shellfish from eastern Mediterranean Sea: consumption advisories. Food and Chemical Toxicology, 53, 33-37. https://doi.org/10.1016/j.fct.2012.11.038.

Copat, C., Vinceti, M., D'Agati, M. G., Arena, G., Mauceri, V., Grasso, A., ... & Ferrante, M. (2014). Mercury and selenium intake by seafood from the Ionian Sea: A risk evaluation. Ecotoxicology and Environmental Safety, 100, 87-92. https://doi.org/10.1016/j.ecoenv.2013.11.009.

Çiftçi, H., Çalışkan, Ç. E., & Öztürk, K.. (2021). Bazı Balık Türlerinde İz ve Toksik Metal Düzeylerinin Belirlenmesi ve İnsan Sağlığı Riskinin Değerlendirilmesi. Journal of Advanced Research in Natural and Applied Sciences, 7(2), 219-233. https://doi.org/10.28979/jarnas.883611.

Dang, F., & Wang, W. X. (2012). Why mercury concentration increases with fish size? Biokinetic explanation. Environmental Pollution, 163, 192-198. https://doi.org/10.1016/j.envpol.2011.12.026.

European Commission (EC). (2014). Commission Regulation (EU) No 488/2014 of 12 May 2014 amending Regulation (EC) No 1881/2006 as regards maximum levels of cadmium in foodstuffs (Text with EEA relevance). The Official Journal of the European Union.

European Commission (EC). (2015). Commission Regulation (EU) 2015/1005 of 25 June 2015 amending Regulation (EC) No 1881/2006 as regards maximum levels of lead in certain foodstuffs (Text with EEA relevance). The Official Journal of the European Union.

European Commission (EC). (2022). 2022/617 of 12 April 2022 amending Regulation (EC) No 1881/2006 as regards maximum levels of mercury in fish and salt (Text with EEA relevance). The Official Journal of the European Union.

European Commission (EC). (2023). Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006 (Text with EEA relevance). The Official Journal of the European Union.

European Food Safety Authority (EFSA). (2010). Panel on Contaminants in the Food Chain (CONTAM); Scientific Opinion on Lead in Food. EFSA Journal, 8(4):1570. [151 pp.]. doi:10.2903/j.efsa.2010.1570.

European Food Safety Authority (EFSA). (2011). Panel on Contaminants in the Food Chain (CONTAM); Scientific Opinion on tolerable weekly intake for cadmium. EFSA Journal, 9(2):1975. [19 pp.] doi:10.2903/j.efsa.2011.1975.

European Food Safety Authority (EFSA). (2012a). EFSA Panel on Contaminants in the Food Chain (CONTAM); Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA Journal, 10(12):2985. [241 pp.] doi:10.2903/j.efsa.2012.2985.

European Food Safety Authority (EFSA). (2012b). EFSA Scientific Committee. Scientific panels and units in the absence of actual measured data. EFSA Journal, 2012; 10: 2579. doi: 10.2903/j.efsa.2012.2579.

Ezemonye, L. I., Adebayo, P. O., Enuneku, A. A., Tongo, I., & Ogbomida, E. (2019). Potential health risk consequences of heavy metal concentrations in surface water, shrimp (Macrobrachium macrobrachion) and fish (Brycinus longipinnis) from Benin River, Nigeria. Toxicology Reports, 6, 1-9. https://doi.org/10.1016/j.toxrep.2018.11.010.

Herliwati, H., Rahman, M., Hidayat, A. S., Amri, U., & Sumantri, I. (2022). The Occurrences of Heavy Metals in Water, Sediment and Wild Shrimps Caught from Barito Estuary, South Kalimantan, Indonesia. HAYATI Journal of Biosciences, 29(5), 643-647. https://doi.org/10.4308/hjb.29.5.643-647.

Hong, Y. S., Kim, Y. M., & Lee, K. E. (2012). Methylmercury exposure and health effects. Journal of Preventive Medicine and Public Health, 45(6), 353. 10.3961/jpmph.2012.45.6.353.

International Agency for Research on Cancer (IARC) (2022). Agents Classified by the IARC Monographs, Volumes 1–132/. https://monographs.iarc.fr/agents-classified-by-the-iarc/.

Joint FAO/WHO Expert Committee on Food Additives (JECFA) (2018). Joint FAO/WHO food standards programmed codex committee on contaminants in foods. 12th Session Utrecht, The Netherlands, 12-16 March 2018, CF/12 INF/1, 1-169.

Miri, M., Akbari, E., Amrane, A., Jafari, S. J., Eslami, H., Hoseinzadeh, E., ... & Taghavi, M. (2017). Health risk assessment of heavy metal intake due to fish consumption in the Sistan region, Iran. Environmental Monitoring and Assessment, 189, 1-10. https://doi.org/10.1007/s10661-017-6286-7.

Muhammad, S., & Ahmad, K. (2020). Heavy metal contamination in water and fish of the Hunza River and its tributaries in Gilgit–Baltistan: evaluation of potential risks and provenance. Environmental Technology & Innovation, 20, 101159. https://doi.org/10.1016/j.eti.2020.101159.

Muhammad, S., Ullah, R., & Jadoon, I. A. (2019). Heavy metals contamination in soil and food and their evaluation for risk assessment in the Zhob and Loralai valleys, Baluchistan province, Pakistan. Microchemical Journal, 149, 103971. https://doi.org/10.1016/j.microc.2019.103971.

Nabavi, S. F., Nabavi, S. M., Latifi, A. M., Eslami, S., & Ebrahimzadeh, M. A. (2012). Determination of trace elements level of pikeperch collected from the Caspian Sea. Bulletin of Environmental Contamination and Toxicology, 88, 401-405. https://doi.org/10.1007/s00128-011-0513-7.

Nirmal, N. P., Santivarangkna, C., Rajput, M. S., & Benjakul, S. (2020). Trends in shrimp processing waste utilization: An industrial prospective. Trends in Food Science & Technology, 103, 20-35. https://doi.org/10.1016/j.tifs.2020.07.001.

Omeragic, E., Marjanovic, A., Djedjibegovic, J., Turalic, A., Causevic, A., Niksic, H., ... & Sober, M. (2020). Arsenic, cadmium, mercury, and lead in date mussels from the Sarajevo fish market (Bosnia and Herzegovina): a preliminary study on the health risks. Turkish Journal of Veterinary & Animal Sciences, 44(2), 435-442. https://doi.org/10.3906/vet-1908-13.

Sabir, M. A., Muhammad, S., Umar, M., Farooq, M., & Fardiullah, N. A. (2017). Water quality assessment for drinking and irrigation purposes in upper Indus basin, northern Pakistan. Fresenius Environ Bull, 26(6), 4180-4186.

Solgi, E., Alipour, H., & Majnooni, F. (2019). Investigation of the concentration of metals in two economically important fish species from the Caspian Sea and assessment of potential risk to human health. Ocean Science Journal, 54, 503-514. https://doi.org/10.1007/s12601-019-0024-8.

Sultana, S., Hossain, M. B., Choudhury, T. R., Yu, J., Rana, M. S., Noman, M. A., ... & Arai, T. (2022). Ecological and Human Health Risk Assessment of Heavy Metals in Cultured Shrimp and Aquaculture Sludge. Toxics, 10(4), 175. https://doi.org/10.3390/toxics10040175.

Tarımsal Ekonomi ve Politika Geliştirme Enstitüsü (TEPGE). (2022). Ürün Raporu, Su Ürünleri. TEPGE YAYIN NO: 355, Ankara.

Tokatlı, C., Ustaoğlu, F. (2021). Meriç delta balıklarında toksik metal birikimlerinin değerlendirmesi: muhtemel insan sağlığı riskleri. Acta Aquatica Turcica, 17(1), 136-145. https://doi.org/10.22392/actaquatr.769656.

Traina, A., Bono, G., Bonsignore, M., Falco, F., Giuga, M., Quinci, E. M., ... & Sprovieri, M. (2019). Heavy metals concentrations in some commercially key species from Sicilian coasts (Mediterranean Sea): Potential human health risk estimation. Ecotoxicology and Environmental Safety, 168, 466-478. https://doi.org/10.1016/j.ecoenv.2018.10.056.

Türk Gıda Kodeksi (TGK). (2011). Türk Gıda Kodeksi Bulaşanlar Yönetmeliği. RG 29 Aralık 2011, 28157 (3. Mükerrer). Ankara.

United State Environmental Protection Agency (USEPA). (1989). Risk Assessment Guidance for Superfund. Volume I: Human Health Evaluation Manual (Part A) (p. 205). Interim Final. Office of Emergency and Remedial Response. EPA/540/1-89/002.

United State Environmental Protection Agency (USEPA). (1991). Human health evaluation manual, supplemental guidance: "standard default exposure factors". OSWER Directive. 1991;9285:6–03.

United State Environmental Protection Agency (USEPA). (2000). Guidance for assessing chemical contaminant data for use in fish advisories, Volume 2: Risk assessment and fish consumption limits. (EPA 823-B-00–008). Third edition. United States Environmental Protection Agency, Washington, DC. November 2000.

United State Environmental Protection Agency (USEPA). (2007). "Method 3051A (SW-846): Microwave Assisted Acid Digestion of Sediments, Sludges, and Oils," Revision 1. Washington, DC.

United State Environmental Protection Agency (USEPA). (2011). Exposure Factors Handbook: 2011 Edition. National Center for Environmental Assessment, Washington, DC; EPA/600/R-09/052F. Available from the National Technical Information Service, Springfield, VA, and online at http://www.epa.gov/ncea/efh.

United State Environmental Protection Agency (USEPA). (2016). Aquatic Life Ambient Water Quality Criteria. EPA-820-R-16-002, Office of Water, Washington DC, USA.

United State Environmental Protection Agency (USEPA). (2021). Integrated Risk Information System. Regional Fish Consumption Screening Levels. https://www.epa.gov/risk/regional-fish-consumption-screening-levels-spring-2021.

United State Environmental Protection Agency (USEPA). (2023a). Regional Screening Level (RSL) Summary Table (TR=1E-06, HQ=1) May 2023. [Erişim tarihi: 15.05.2023].

United State Environmental Protection Agency (USEPA). (2023b). Regional screening levels (RSLs)–equations May 2023. https://www.epa.gov/risk/regional-screening-levels-rsls-equations#fish. [Erişim tarihi: 15.05.2023].

Varol, M., Sünbül, M. R. (2020). Macroelements and toxic trace elements in muscle and liver of fish species from the largest three reservoirs in Turkey and human risk assessment based on the worst-case scenarios. Environmental Research, 184, 109298. https://doi.org/10.1016/j.envres.2020.109298

Varol, M., Kaçar, E. (2023). Bioaccumulation of metals in various tissues of fish species in relation to fish size and gender and health risk assessment. Current Pollution Reports, 1-11. https://doi.org/10.1007/s40726-023-00263-w.

Yildirim, Y., Gonulalan, Z., Narin, I., & Soylak, M. (2009). Evaluation of trace heavy metal levels of some fish species sold at retail in Kayseri, Turkey. Environmental Monitoring and Assessment, 149, 223-228. https://doi.org/10.1007/s10661-008-0196-7

Yipel, M., Tekeli, İO (2022). Essential and non-essential metal concentrations in shrimps from Iskenderun bay, Türkiye. Harran Üniversitesi Veteriner Fakültesi Dergisi, 11(2), 257-262. https://doi.org/10.31196/huvfd.1197900.

Yu, B., Wang, X., Dong, K. F., Xiao, G., & Ma, D. (2020). Heavy metal concentrations in aquatic organisms (fishes, shrimp and crabs) and health risk assessment in China. Marine Pollution Bulletin, 159, 111505. https://doi.org/10.1016/j.marpolbul.2020.111505.

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RESEARCH ARTICLE

The Effects of Different Slaughter Ages and Gender on Some Meat Quality Characteristics, Texture and Sensory Evaluation Values in Japanese Quails

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ABSTRACT

The aim of this study to determine the effects of different slaughter age and gender on some meat quality characteristics, texture and sensory analysis values in quails. For this purpose, carcasses of Japanese quails of different sexes and different slaughter ages (4 and 8 wks) were obtained from a private enterprise. These carcasses were brought to the laboratory under the cold chain. Analyzes were performed 24 hours after slaughter. Colour, cooking loss, and texture analysis were performed on the left side of skinless breast meat in carcasses. pH and electrical conductivity were determined in the right part of breast meat. Leg meats were used for sensory analysis. While the gender factor created a significant difference in the pH value ($p<0.05$) and flavor score ($p<0.05$) of quail meat the slaughter age had a significant difference both in the pH value ($p<0.001$) and the color values (a* and b* values; $p<0.05$). The difference between the groups in the texture values of breast meat was not statistically significant. In the sensory analysis evaluation, it was determined that only the difference between the groups in the flavor score of the gender factor was significant ($p<0.05$).

Key words: Gender, Japanese quails, meat quality, sensory evaluation, slaughter age

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Japon Bıldırcınlarında Farklı Kesim Yaşı ve Cinsiyetin Bazı Et Kalite Özellikleri, Tekstür ve Duyusal Değerlendirme Değerlerine Etkisi

ÖZ

Bu çalışmanın amacı, farklı kesim yaşı ve cinsiyetin bıldırcınlarda bazı et kalite özellikleri, tekstür ve duyusal analiz değerleri üzerindeki etkilerini belirlemektir. Bu amaçla özel bir işletmeden farklı cinsiyette ve farklı kesim yaşlarında (4 ve 8 haftalık) Japon bıldırcınlarının karkasları temin edilmiştir. Bu karkaslar soğuk zincir altında laboratuvara getirildi. Analizler kesimden 24 saat sonra yapılmıştır. Karkasta derisiz göğüs etinin sol tarafında renk, pişme kaybı ve tekstür analizleri yapıldı. Göğüs etinin sağ kısmında pH ve elektrik iletkenliği belirlendi. Duyusal analiz için bacak etleri kullanıldı. Cinsiyet faktörü bıldırcın etinin pH değerinde ($p<0,05$) ve lezzet skorunda ($p<0,05$) anlamlı bir fark yaratırken, kesim yaşı hem pH değerinde ($p<0,001$) hem de renk değerlerinde (a* ve b* değerleri; $p<0,05$) önemli bir fark oluşturmuştur. Göğüs etinin tekstür değerlerinde gruplar arasındaki fark istatistiksel olarak anlamlı değildir. Duyusal analiz değerlendirmesinde sadece cinsiyet faktörünün lezzet puanında gruplar arasındaki farkın anlamlı olduğu belirlendi ($p<0,05$).

Anahtar kelimeler: Cinsiyet, duyusal değerlendirme, et kalitesi, Japon bıldırcınları, kesim yaşı

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INTRODUCTION

While the production of poultry meat was 68.64 million tons in the 2000s, it reached 132.39 million tons in 2019 (Ritchie et al. 2019). This great increase in production shows the importance of the share of the poultry industry in meeting the protein source demand of the increasing population. In addition to being a protein source with high biological value like other meat products, it is noteworthy that especially iron and zinc minerals have high bioavailability from red meat (Barroeta 2007).

Although chicken meat has the largest share in the sector as a source of poultry meat, quail meat has gained importance in recent years. In particular, Japanese quails have been up-to-date economically for meat and eggs in the commercial poultry industry. The fact that it is put on the market more for consumption at the age of 5 weeks, it reaches early sexual maturity, and the need for lower feed and space requirements than domestic poultry are among the factors that increase its importance (Hrncar et al. 2014). Japanese quails are mostly fed for eggs in the Southeast of Asia, while it is fed for both meat and eggs in Europe and America. (Minvielle 2004).

The chemical composition of Japanese quail carcasses (at 35 to 42 days of age) is 68% water, 19% protein, 10% fat, and 3% mineral (Genchev et al. 2008; Vargas-Sanchez et al. 2019). The thin skin of quail meat and the low amount of fat between the tissues may make it recommended for a low-fat diet. It is also easy to present and not difficult to cook (Abou-Kassem et al. 2019).

Breed, gender and slaughter age are among the factors that are effective in determining the meat quality of poultry (Flecher 2002). Among these factors, gender and slaughter age can affect the desired body weight, completion of muscle development, development of muscle content (muscle fat distribution), reaching organoleptic structure, and also the characteristics used in technology (Poltowicz and Doktor 2012).

The factors that affect the consumers' choice of poultry meat and then their satisfaction are primarily its appearance and texture. In the appearance, bruises and hemorrhagic areas may be noticed along with the color. Myofibrillar protein denaturation, connective tissue content and juiciness level of meat are effective in texture development (Flecher 2002; Nusairat et al. 2022).

In a study evaluating carcass characteristics and meat quality of Japanese quails of different genders and different slaughter ages (5, 6, 7 weeks); It has been reported that the tenderness, fat content, and L value of the meat of female animals are higher, while the redness and water holding capacity values of the meat are lower. It has been reported that with increasing slaughter age, the juiciness and tenderness of the meat decrease, the water holding capacity, the amount of intramuscular fat, and the redness of the meat increase (Abou-Kassem 2019).

Lukanov et al. (2021) in their study in which they examined the pH and color values in the breast muscle of meat-type quail (WG line), it was reported that the pH value was 5.75-6.26 and it was not affected by the slaughter age (28, 35 and 42. days) and gender differences. It has been also added that the biggest change in the color values of the meat is in the brightness value of the meat (L*), and the brightness decreases with increasing age. In the same study, it was stated that while the redness value (a* 5.77-11.6) decreased with the increase in slaughter age, the yellowness value (b*: 5.14-11.13) increased. It was stated that redness in female quail meats and yellowness in male quails were more pronounced.

In another study that examines the effects of different slaughter ages (33-42 days) on meat quality values in Pharaoh quails, which have a larger body weight than other quail birds, it was stated that pH15 values and water holding capacity were higher at the slaughter age of 42 days. In the color values, it was stated that the brightness of the meat was high in quails slaughtered on the 33rd day, and the redness and yellowness values increased in the elderly quails. It has been reported that the taste, juiciness, tenderness, and flavor intensity values, which are especially desired from the sensory properties of the breast meat at the age of 33 days, are higher and the aroma intensity is lower (Wılkanowska and Kokoszyńskı 2011).

In this study, the effects of different slaughter ages and gender on some meat quality characteristics, texture, and sensory analysis results were investigated in Japanese quails.

MATERIAL AND METHODS

Raw Material

This study was conducted in November 2022. For the experimental study, quails raised under the same conditions until the desired slaughter age were obtained from a local commercial farm after they were slaughtered (after 4 hours of antemortem fasting) the mechanical system (decapitation, after bleeding, plucking, and evisceration). After slaughtering, the carcasses were brought to the laboratory at +4 C without breaking the cold chain. For the study, a total of 28 quail carcasses of different sexes and different slaugter ages (7 females -7 males, 4 wks; 7 females-7 males 8 wks) were obtained.

Meat quality and texture analyzes were performed 24 hours after slaughter. Sensory evaluations were performed 48 hours later. Carcasses were kept in refrigerator conditions (+4 °C) until analysis. Color, cooking loss and texture analysis were performed on the left skinless pectoralis major muscle. pH and electrical conductivity were determined in the right

pectoralis major muscle. Leg meats were used for sensory evaluation.

pH and Electrical Conductivity Measurement

For the analysis, 10 g samples were taken from the skinless breast meat of quails and homogenized. 100 ml of distilled water was added to it. The pH (AD110 pH-Temp, Adwa, Hungary) and electrical conductivity (Orion Star A222, Thermo Scientific, Korea) of the prepared samples were measured. Measurements were made 24 hours after slaughter.

Meat Color

A Chroma meter (Konica Minolta, CR-400, Japan) was used to make breast meat color measurements. Measurements were taken from 3 different points of the quail skinless breast meat and average values were taken. L*(lightness), a* (redness), and b*(yellowness) values were calculated.

Cooking Loss

To calculate the cooking loss, 5 g of quail breast meat was weighed. Samples were placed in polyethylene bags. It was kept in a water bath (Selecta, Spain) of 80 °C for 1 hour until the internal temperature was 70 °C. After room temperature cooling, the weighings were made. Cooking loss was calculated as a percentage by taking the weight differences before and after cooking (Honikel 1998).

Texture Analyses

For texture analysis, breast meat samples were prepared as 1 cm x 1 cm x 2 cm. Samples were measured using the texture analyzer (CT3 Texture Analyzer; BrookfieldEngineering Labs Inc., Middleborough, MA, USA) according to the modification of the method described by Masoumi et al. (2022). Probe model TA44**.**

Sensory Evaluation

Sensory evaluation panelists (10 people) consisted of senior students of the Nutrition and Dietetics department. Before the analysis, the students were given training on the conduct of the sensory evaluation. For sensory evaluation, quail legs were cooked in the oven at 200°C until the internal temperature was 80°C by adding only salt. It was then presented to the panelists on plastic plates. In the forms distributed to the panelists, they were asked to evaluate the desired features (smell, colour, flavor, tenderness, juiciness, fibrous, chewability and general appreciation etc.) with numbers from 1 to 9. Very good (8 - 9), Good (6 - 7), Fair (4 - 5), Poor (2 - 3), Very poor (0 - 1) (Farag et al. 2021).

Statistical Analysis

After controlling for the normal distribution of the data two-way ANOVA test was used for the effect of slaughter age and gender in determining the difference between the groups. The sample size used in the study was determined with the G Power 3.1 power analysis software (Faul et al. 2007). The statistical analysis was performed by means of the SPSS Statistics 23.0 package software. $p<0.05$ was taken into account statistically.

RESULTS

Some meat quality values in quail breast meats are given in Table 1. Electrical conductivity values of young females and males quail meats were measured as 1182.43 and 1164, respectively. Elderly females and males quail meats electrical conductivity values were determined as 1164 and 1185. It was determined that there was no statistically significant difference between the groups in terms of gender and slaughter age.

pH values were 5.86 and 5.73 in young and old female quails meats, respectively; it was measured as 5.92, 5.78 in young and old men quails meats. It was determined that the factors of gender ($p<0.05$) and slaughter age ($p<0.001$) created statistically significant differences between the groups.

Table 1. Some meat quality values in quail breast meat

Gender	Age	Electrical conductivity	ph	Cooking loss (%)	L* Lightness	a* Redness	b* Yellowness
Female	4 wk	1182.43±142.664	5.864±0.086	26.897±2.70	57.98±4.44	7.48±1.30	5.61±1.62
	8 wk	1164.00±34.225	5.731±0.070	27.605±3.93	54.27±2.25	8.79±2.07	4.17±0.62
Male	4 wk	1233.57±299.163	5.92±0.064	26.732±4.01	56.78±1.32	7.59±0.98	5.14±0.59
	8 wk	1185.71±88.153	5.78±0.062	30.748±1.57	57.46±2.86	9.35±2.18	4.93±0.82
				p			
Gender		0.581	*	0.232	0.382	0.611	0.698
Age		0.616	**	0.064	0.188	*	*
Interaction		0.823	0.938	0.186	0.061	0.735	0.120

Values are shown as mean±SEM. *: $p<0.05$; **: $p<0.001$.

Cooking loss values were determined as 26,73 and 30,74% in young and old male quail breast meats, respectively. It was determined that both slaughter age and gender did not differ between the groups.
Brightness value (L*), which is one of the important values in meat quality, decreased in samples taken from females and increased in samples taken from males as slaughter age increased. But these differences are not statistically significant. Among the color values, it was determined that the redness value (a*) increased with increasing slaughter age in both females and males quail meats ($p<0.05$). It was determined that the difference in genders was not significant in the a* value. The yellowness value (b*) in breast meat samples was 5.61 and 4.17 in young and old females quail, respectively; 5.14 and 4.93 in young and old men quail. The decrease in b* value with the prolongation of slaughter age in both genders was found to be statistically significant between the groups ($p<0.05$).

Table 2. Texture analysis values in quail breast meat

Gender	Age	Hardness (N)	Adhesiveness (mj)	Cohesiveness	Springiness (mm)	Gumminess (N)	Chewiness (mj)
Female	4 wk	4.687±0.86	0.608±0.52	0.488±0.246	8.052±1.917	2.497±1.43	11.70±3.44
	8 wk	5.451±0.89	0.631±0.16	0.312±0.220	6.231±1.510	1.201±0.42	8.380±4.30
Male	4 wk	4.835±1.61	0.668±0.566	0.324±0.133	7.477±1.138	1.527±0.69	11.960±7.00
	8 wk	4.747±0.65	0.870±0.780	0.355±0.136	7.222±1.320	1.687±0.55	12.201±4.42
				P			
Gender		0.499	0.484	0.409	0.717	0.470	0.289
Age		0.412	0.598	0.328	0.080	0.097	0.421
Interaction		0.302	0.674	0.165	0.180	0.037*	0.353

Values are shown as mean±SEM. *:$p<0.05$

The texture values of quail breast meat are given in Table 2. Hardness values were measured as 4.68 and 4.83 N in young females and males quails, respectively. In elderly females and males quails, it was determined as 5.45 and 4.74 N, respectively. Although the adhesiveness value was numerically higher in the samples obtained from male quail, it was determined that this difference was not statistically significant. It was determined that both the slaughter age factor and the gender factor did not create a significant difference between the groups in the cohesiveness value. It was also determined that the springiness feature was higher in the young quail (8.05; 7.47 mm; $p>0.05$). Although the effect of gender and age factor on the gumminess value was not significant, the interaction between the two characteristics was found to be significant ($p < 0.05$). The chewiness value did not differ between the groups.

Table 3. Sensory analysis values in quail leg meat

Gender	Age	Colour	Smell	Flavor	Tenderness	Juiciness	Fibrous	Chewiness	Overall liking
Female	4 wk	6.40±1,34	6.00±1.94	6.90±1.10	7.30±1.76	6.30±2.21	7.00±1.15	7.60±1.89	6.20±1.47
	8 wk	6.30±1.41	6.00±1.56	6.50±1.26	6.90±1.52	6.10±2.28	7.10±1.37	7.30±1.05	6.50±1.64
Male	4 wk	6.70±1.33	6.20±1.54	7.60±1.07	7.80±0.78	6.70±2.00	7.60±0.84	8.40±0.69	6.80±1.81
	8 wk	7.70±1.33	7.30±1.33	7.70±1.63	7.10±1.85	6.60±1.95	7.00±1.76	7.30±1.94	7.40±1.64
					P				
Gender		0.056	0.288	*	0.477	0.506	0.555	0.405	0.159
Age		0.303	0.150	0.715	0.267	0.824	0.555	0.149	0.394
Interaction		0.210	0.288	0.544	0.760	0.941	0.409	0.405	0.775

Values are shown as mean±SEM. *: $p<0.05$.

Sensory analysis results in quail leg meat are given in Table 3. Although leg meat samples obtained from male elderly quail were more appreciated in terms of color and smell, these differences were not statistically significant. Flavor values were calculated as 7.60 and 7.70 for young and elderly male quails, respectively. In the samples obtained from female quail, these values were observed to have lower scores. Differences between the groups due to the gender factor were significant ($p < 0.05$). Although tenderness, juiciness, and chewiness values were more appreciated in samples obtained from young quail, these differences were not statistically significant. It was determined that the gender and age factors were not important in the fibrous value. Although meat samples obtained from elderly and male quail received higher scores as overall liking, it was determined that these differences were not statistically significant.

DISCUSSION

Quality is one of the important factors in meat production. At the same time, it is a priority for the consumer and ensures the sale of the product. pH and color values are important in determining the quality of meat (Qiao et al. 2001). In this study, it was determined that the effects of gender and age factors

were important in pH measurements made in M. pectoralis superficial muscle in quails. It was measured that the pH value was higher in the samples obtained from young and males. In a study, it was reported that the pH value of breast meat was lower in female broilers 24 hours after slaughter. Depending on the gender difference, the glycolysis that develops in the postmortem period and the resulting difference in the accumulation of lactic acid in the muscles may cause this (Lopez et al. 2011). While the color values were not affected by the gender factor, it was determined that the effect of slaughter age on the redness (a*) and yellowness (b*) values of the meat was significant. The redness value was higher in the samples taken from the elderly and the yellowness value in the samples taken from the young.

In a study in which the effects of different slaughter ages (35-42 days) and gender on meat quality in quails were determined, it was reported that the pH24 value in male carcass meats was lower than the female carcass values. It has been also reported that the redness value is lower in female carcass meats at 35 days of age; and that the carcass characteristics of male quails at 42 days of slaughter are also better (Abreu et al. 2014).

Wilkanowska and Kokoszyński (2011) reported in their study on the effect of different slaughter ages (33-42 days) on meat quality in Pharaoh quails with large body weight; that the pH15 value was higher at the slaughter age of 42 days.

Petek et al. (2022), in their study on female quails of different genotypes and different slaughter ages (60-270 days), it was stated that the pH value was significantly higher in old quail carcasses and a* and b* values were significantly higher in meat samples of young quails. It has also been reported that a low pH value reduces the water-holding capacity.

In another study, in which different slaughter ages (8 weeks and 8 months) were applied, it was stated that the brightness value (L*) was high, and the a* and b* values were low in the meat of young quails. It has been stated that the pH value is higher in aged quail meats. In the same study, it was stated that the reason why quail meat is darker and redder than other poultry meat may be due to the increase in myoglobin pigment with increasing age (Boni et al. 2010).

In a study examining the meat quality values of quail carcasses with slaughter age greater than 45 days and less than 45 days, it was reported that there was no difference between the groups in terms of pH value and cooking loss. In addition, it has been reported that a low pH value may cause poor water capacity of myofibrillar muscle proteins, resulting in high cooking losses (Awan et al. 2017).

Unlike this study, Lukanov et al. (2021) reported that the effect of slaughter age (28; 35, and 42 days) and gender on the pH value was not significant.

The texture values of meat are an important factor for the consumer's purchasing preference. In this study, it was determined that the effects of both gender and age were not significant on quail breast meat texture values. The fact that the difference between the slaughter age groups was not high may have been effective in this.

Unlike this study, Abou Kassem et al. (2019) It has been reported that in Japanese quails of different slaughter ages (5, 6, and 7 wk) and different genders, the juiciness and tenderness values in meat decrease with the increase in slaughter age. It was stated that the gender factor was important only in the softness value in meat and this value was high in female quails. In another study, it is stated that the softness of meat decreases with age, and this may be due to the increase in fiber diameter, connective tissue, and cross-links between polypeptide chains (Reddy et al. 2017).

The sensory properties of meat, like the texture properties, play an important role in consumer preference. In a study in which different slaughter ages (33 and 42 days) were applied, it was reported that while quail carcasses slaughtered for 33 days had higher scores in sensory characteristics such as juiciness, tenderness, flavor intensity, and desirability, they scored lower in aroma intensity (Wılkanowska and Kokoszyńskı, 2011). In another study, it was stated that the flavor components were generally related to the fat content and the components increased with the prolongation of the cutting age (Reddy et al. 2017).

In this study, it was determined that there was no significant difference between age and gender in sensory characteristics. It was determined that only the effect of the gender factor on the flavor factor was significant and the meat of male quail scored higher. In this regard, It may be that the musculature of male animals is better than females of the same age genetically, which affects the development of flavor factor and texture development.

CONCLUSION

As a result, in this study, it was determined that the effect of different slaughter age factors on pH and some color values (a* and b*) was significant, while the effect of gender factors on pH and sensory characteristics only on flavor score was determined. The low difference between the slaughter ages in this study may have been effective in the similarity in the data obtained from the groups.

At the same time, when evaluated in terms of similar factors, it is important result that the results of texture analysis and sensory analysis support each other.

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

Authors' Contributions: ÖVA contributed to the project idea, design and execution of the study. ÖVA and EY contributed to the acquisition of data. ÖVA analysed the data. ÖVA drafted and wrote the manuscript. ÖVA reviewed the manuscript critically. All authors have read and approved the finalized manuscript.

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

REFERENCES

Abou-Kassem, D. E., El-Kholy, M. S., Alagawany, M., Laudadio, V., & Tufarelli, V. (2019). Age and sex-related differences in performance, carcass traits, hemato–biochemical parameters, and meat quality in Japanese quails. Poultry Science, 98, 1684–1691. http://dx.doi.org/10.3382/ps/pey543.

Abreu, L. R. A., Boari, C. A., Pıres, A. V., Pınheiro, S. R. F., Oliveria, R. G., Oliveria, K. M., Gonçalves, F. M, & Oliveria, F. R. (2014). Carcass evaluation and meat quality of slaughtered meat quails at different ages. Revista Brasileira de Saúde e Produção Animal, 15(1), 131-140. http://www.rbspa.ufba.br ISSN 1519 9940.

Awan, F. N., Shah, A. H., Soomro, A. H., Barahm, G. S., & Tunio, S. G. (2017). Carcass yield and physico-chemical characteristics of Japanese quail meat. Pakistan Journal of Agriculture, Agricultural Engineering and Veterinary Sciences, 33(1), 111-120.

Barroeta, A. C. (2007). Nutritive value of poultry meat: relationship between vitamin E and PUFA. World's Poultry Science Journal, 63(2), 277-284. http://doi.org/10.1017/ S0043933907001468.

Boni, I., Nurul, H., & Noryati, I. (2010). Comparison of meat quality characteristics between young and spent quails. International Food Research Journal, 17, 661-666.

Farag, M. M., Abd-El-Aziz, N. A., & Ali, A. M. (2021). Preparing and evaluation of new nutritious products from quail meat. Food and Nutrition Science, 12, 889-898. https://doi.org/10.4236/fns.2021.129066.

Faul, F., Erdfelder, E., Lang, A. G., & Buchner, A. (2007). G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav. Res. Methods, 39(2), 175-191.

Fletcher, D. L. (2002). Poultry meat quality. World's Poultry Science Journal, 58, 131-145.

Genchev, A., Mihaylova, G., Ribarski, S., Pavlov, A., & Kabakchiev, M. (2008). Meat quality and composition in Japanese quails. Trakia Journal of Sciences, 6, 72-82.

Honikel, K. O. (1998). Reference methods for the assessment of physical characteristics of meat. Meat Science, 49(4), 447-457. https://doi.org/10.1016/S0309-1740(98)00034-5.

Hrncar, C., Hanusova, E., Hanus, A., & Bujko, J. (2014). Effect of genotype on egg quality characteristics of Japanese Quail (Coturnix Japonica). Slovak Journal of Animal Science, 47(1), 6-11.

Lopez, K. P., Schilling, M. W., & Corzo, A. (2011). Broiler genetic strain and sex effects on meat characteristics. Poultry Science, 90, 1105–1111 https://doi.org/10.3382/ps.2010-01154.

Lukanov, H., Pavlova, I., & Genchev, A. (2021). Effect of different fattening period duration on meat productivity of domestic quails. Agriculturel Scıence and Technology, 13(4), 370-377. https://doi.org/ 10.15547/ast.2021.04.060.

Masoumi, B., Abbasi, A., Mazloomi, S. M., & Shaghaghian, S. (2022). Investigating the Effect of Probiotics as Natural Preservatives on the Microbial and Physicochemical Properties of Yogurt-Marinated Chicken Fillets. Journal of Food Quality, Article ID 5625114.

Minvielle, F. (2004). The Future of Japanese quail for research and production. World's Poultry Science Journal, 60, 500-507. https://doi.org/10.1079/WPS200433.

Nusairat, B., Tellez-Isaias, G., & Qudsieh, R. (2022). An overview of poultry meat quality and myopathies. Ed.Guillermo Tellez-Isaias. Broiler Industry Intechopen. 10.5772/intechopen.98143

Petek, M., Abdourhamane, I. M., & Ardıçlı, S. (2022). Comparison of physical meat quality properties of young and spent female quail of different genetic stock. Eurasian Journal of Veterinary Sciences, 38:2, 108-114. https://doi.org/10.15312/EurasianJVetSci.2022.371.

Poltowicz, K., & Doctor, J. (2012). Effect of slaughter age on performance and meat quality of slow-growing broiler chickens. Annals of Animal Science, 12(4), 621–631. https://doi.org/ 10.2478/v10220-012-0052-0.

Qiao, M., Fletcher, D. L., Smith, D. P., & Northcutt, J. K. (2001). The effect of broiler breast meat color on ph, moisture, water-holding capacity, and emulsification capacity. Poultry Science, 80, 676-680.

Reddy, N. A., Reddy, K. K., Kumar, M. S., Krishnaiah, N., & Rao, V. K. (2017). Studies on Effect of Sex and Age on Physico-Chemical and Organoleptic Qualities of Rajasri Chicken. International Journal of Current Microbiology and Applied Sciences, 6, 406–415. http://dx.doi.org/10.20546/ijcmas.2017.604.046.

Ritchie, H., Rosado, P., & Roser, M. (2019). Meat and dairly production. https://ourworldindata.org/meat-production

Vargas-Sanchez, R. D., Ibarra-Arias, F. J., Martinez, B. M. T., Sanchez-Escalante, A., & Torrescano-Urrutia, G. M. (2019). Use of natural ingredients in Japanese quail diet and their effect on carcass and meat quality A-rewiev. Asian-Australasian Journal of Animal Science, 232, 1641-1656. https://doi.org/10.5713%2Fajas.18.0800.

Wılkanowska, A., & Kokoszyńskı, D. (2011). Comparıson of slaughter value ın pharaoh quaıl of dıfferent ages. Journal of Central European Agriculture, 12(1), 145-154. https://doi.org/10.5513/JCEA01/12.1.891.

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RESEARCH ARTICLE

Investigation of Some Biochemical Parameters and Oxidative Stress Levels in Chuckar Partridges (Alectoris chuckar) in Different Housing Conditions

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ABSTRACT

Oxidative stress occurs as a result of an imbalance between free radicals and antioxidants in the organism's body. In this study, the effects of different growing conditions on oxidative stress levels in chuckar partridge were investigated. The research material was formed by 60 male breeding male chuckar partridges of similar age and weight, bred at the Şuhut Partridge Production Station affiliated to the V. Regional Directorate of Nature Conservation and National Parks. Blood was taken from partridges and some biochemical and oxidative stress parameters were checked. As a result of the research, glucose, triglyceride and total cholesterol, urea and creatinine values from biochemical parameters, MDA values, which are lipid peroxidation markers, were lower in partridges housed in voles than in partridges housed in cages ($p<0.05$), glutathione, SOD and CAT activities and beta carotene and vitamin A values were found to be higher ($p<0.05$). It was determined that Aspartate aminotransferase, ALT and ALP activities, enzyme and total protein levels were not affected by both hosting methods ($p>0.05$). As a result of the research, it was determined that creatinine and urea values, which are parameters showing kidney functions, were lower in the partridges housed in the cage compared to those in the volier, liver enzymes did not change and oxidative stress occurred. As a result, it was concluded that in terms of oxidative stress level, it is more appropriate to keep the chuckar partridges in volies, and it would be beneficial to support them with antioxidants when they are housed in a cage system.

Keywords: Biochemical parameters, chuckar partridge, housing, management, oxidative stress,

Farklı Barındırma Koşullarındaki Kınalı Kekliklerde (Alectoris chuckar) Bazı Biyokimyasal Parametreler ve Oksidatif Stres Düzeylerinin Araştırılması

ÖZ

Oksidatif stres, canlının bedenindeki serbest radikaller ve antioksidanlar arasındaki dengesizlik sonucu oluşur. Bu çalışmada kınalı kekliklerde farklı yetiştirilme koşullarının oksidatif stres düzeyleri üzerine etkileri araştırıldı. Araştırma materyalini Doğa Koruma ve Milli Parklar V. Bölge Müdürlüğüne bağlı Şuhut Keklik Üretme İstasyonunda doğaya salım yapmak üzere yetiştirilen 60 adet, benzer yaş ve ağırlıkta damızlık erkek kınalı keklik oluşturdu. İki gruba ayrılan ve 30 adedi kafes sisteminde 30 adedi de voliyerlere salınarak 3 hafta bakılan kınalı kekliklerden kan alınarak bazı biyokimyasal ve oksidatif stres parametrelerine bakıldı. Araştırma sonucunda biyokimyasal parametrelerden glukoz, trigliserid ve total kolesterol, üre ve kreatinin değerlerinin, Lipid peroksidasyon belirteci olan MDA değerlerinin voliyerde barındırılan kekliklerde kafeste barındırılan kekliklere göre daha düşük düzeyde ($p<0.05$), glutatyon, SOD ve CAT aktiviteleri ile beta karoten ve A vitamini değerlerinin ise daha yüksek düzeyde olduğu ($p<0.05$) tespit edildi. Aspartat aminotransferaz, ALT ve ALP aktiviteleri enzim ile total protein düzeylerinin ise her iki barındırma metodundan etkilenmediği ($p>0.05$) belirlendi. Araştırma sonucunda kafeste barındırılan kekliklerde voliyerdekilere göre böbrek fonksiyonlarını gösteren parametrelerden kreatinin ve üre değerlerinin düşük olduğu, karaciğer enzimlerinin değişmediği ve oksidatif stresin oluştuğu tespit edildi. Sonuç olarak oksidatif stres düzeyi açısından kınalı kekliklerin voliyerlerde barındırılmalarının daha uygun olduğu, kafes sisteminde barındırıldıkları koşullarda ise antioksidanlarla desteklenmelerinin faydalı olacağı kanaatine varıldı.

Anahtar Kelimeler: Bakım, barındırma, biyokimyasal parametreler, kınalı keklik, oksidatif stres

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GİRİŞ

Stres bir canlı üzerinde çeşitli faktörlerin etkisi ile oluşurken, stresin varlığı kanın bileşimini de etkilemektedir. Bu durum ise hematolojik ve biyokimyasal parametrelerin stres varlığının tespitinde kullanılabilirliğine imkân tanımaktadır (Yılmaz 2000). Sağlıklı bir organizmada normal metabolizma sonucunda oluşan süperoksit anyonu, hidrojen peroksit ve hidroksil radikali gibi reaktif oksijen radikalleri ile antioksidatif savunma sistemi arasında bir denge vardır (Storz ve Imlayt 1999). Bu dengenin, oksijen radikallerinin lehine bozulmasıyla ortaya çıkan duruma oksidatif stres adı verilmekte ve buna bağlı olarak canlı organizmada hücresel ve moleküler doku hasarı görülmektedir (Birben ve ark. 2012). Oksidatif stres hayvanlarda fizyolojik ve biyokimyasal olayların aksamasına ve sonuçta düşük verim ve kaliteli yetiştiriciliğin yapılamamasına sebep olmaktadır (Macit ve Akbulut 2015). Keklikler, Galliformis takımının Phasianidae familyasından köken alan Alectoris (A.), Perdix (P.) ve Ammoperdix den gelen orta ebatta, kalın gövdeli, kısa kuyruklu kuş türlerine verilen ortak isimdir (Özçelik 1995; Kırıkçı ve Çetin 1999; Keskin ve ark. 2002). Keklik yetiştiriciliği yapılan işletmelerde iyi bakım ve besleme koşulları hayvanların refah ve yaşama güçleri üzerinde etki eden önemli faktörlerdir. Özellikle barınak koşulları hayvanlarda fizyolojik ve biyokimyasal olayların aksamasına ve sonuçta düşük verim ve kaliteli yetiştiriciliğin yapılamamasına sebep olmaktadır. Oksidatif stres, hücresel ve moleküler doku hasarı oluşum mekanizmalarının bir parçası olarak hastalıkların şekillenmesinde rol oynayarak canlının yaşama gücünü etkiler. Oksidatif stresin tespit edilmesi doğaya salım amacı ile yapılan keklik yetiştiriciliğinde daha da önem kazanmaktadır. Yapılan bir çalışmada (Özbey ve Esen 2007) kınalı kekliklerde barındırma koşullarının bazı biyokimyasal ve oksidatif stres parametrelerini etkilediği ortaya konulmuştur. Bu araştırma entansif koşullarda yetiştiriciliği yapılan kınalı kekliklerin farklı barındırılma koşullarında oksidan/antioksidan durumunun incelenmesi ve oksidatif stres düzeylerinin ölçülmesi amacıyla yapıldı.

MATERYAL VE METOT

Çalışmada Afyon Kocatepe Üniversitesi Hayvan Deneyleri Etik Kurulundan (AKUHAYDEK) 19.11.2020 tarih ve 49533702/323 sayı ile Etik Kurulu onayı ve gerekli izinler alınmıştır. Doğa Koruma ve Milli Parklar V. Bölge Müdürlüğüne bağlı Şuhut Keklik Üretme İstasyonunda doğaya salım yapmak üzere yetiştirilmekte olan kınalı kekliklerden 60 adet benzer yaş ve ağırlıkta damızlık erkek kınalı keklik araştırma materyalini oluşturmuştur. Üretim istasyonunda keklik üretimi kafes sistemi ile yapılmakta ve her bir kafes bölmesine 2 dişi bir erkek keklik konarak üreme sezonu kafes içerisinde tamamlanmaktadır. Üreme sezonu Mayıs ayının son haftasında tamamlanmakta ve keklikler voliyerlere (50x60 m^2) alınarak yeni bir üretim sezonuna kadar bu bölmelerde yaşamlarını devam ettirmeleri sağlanmaktadır. Bu araştırma çerçevesinde üretim sezonu sonrası 35 adet erkek keklik (5 âdeti yedek olarak bırakılmıştır) kafes sisteminde 3 hafta kadar daha barındırılmaya devam ettirildi ve diğer erkekler ise voliyerlere salındı. Her iki grupta aynı rasyon içeriği ile beslendi, önlerinden su eksik edilmedi. Farklı olan tek faktör barındırma koşullarıdır. Kan alım aşamasında ön keşif çalışma için 10 Haziran 2021 tarihinde DKMP 5. Bölge Keklik Üretim İstasyonu sorumlu Veteriner Hekimi Ali Gezer eşliğinde Şuhut Keklik Üretme İstasyonunda bulunan keklik grupları kontrol edildi. Kan numunesi toplama çalışması ise yine istasyon sorumlusu veteriner hekim eşliğinde 30 Haziran 2021 tarihinde gerçekleştirildi.

Kan örneklerini toplarken her iki gruptan 35'er adet keklik taşıma kafeslerine yerleştirildi ve önce voliyerlerdeki kekliklerden sonra da kafeslerde bulunan kekliklerden kanat altı venlerinden 1,5 cc civarında kan alınarak hemogram tüplerinde toplandı. Kan alımı aynı kişi tarafından aynı günde ve aynı şartlarda sağlandı. Toplamda her iki gruptan 30 ar adet kan örneği alındı ve herhangi bir hayvan zayiatı şekillenmedi. Kan alınan tüm keklikler voliyerlere salındı. Alınan kanlar laboratuvar ortamına ulaştırılana kadar soğuk ortamda muhafaza edildi. Voliyerlerde ve katlı kafes sistemi gibi farklı koşullarda barındırılan 60 adet kınalı keklikte oksidan/antioksidan durumunun incelenmesi ve oksidatif stres düzeylerinin belirlenmesi amaçlanan bu çalışmada hemogram tüplerine alınan tam kan örnekleri iki kısma ayrıldı. Bir kısmıyla beklemeksizin tam kanda malondialdehit (MDA), glutatyon (GSH) düzeylerinin ölçümü gerçekleştirildi. Diğer kısım ise öncelikle plazmaları 3000 rpm'de 10 dakika santrifüj edilerek ayrıldı ve plazmalar 1.5 ml'lik ependorf tüplere alınarak analizleri yapılıncaya kadar -80ºC'de saklandı. Kalan eritrositler SOD ve CAT aktivitelerinin ölçümü için hazırlandı. Bunun için 30 dakika içerisinde 4 ^{0}C'de 15 dakika 3500 g'de santrifüj edilerek eritrosit ve plazma ayrıldı. Çökelen eritrositler üç kez izotonik salinle yıkandı ve kabarık tabaka uzaklaştırıldı. Daha sonra izotonik salin ve eritrositler aynı hacimde ilave edilerek -20C'de saklandı (Winterbourn ve ark., 1975). Plazmadan aspartat aminotransferaz (AST), alanin aminotransferaz (ALT), alkalen fosfataz (ALP), üre, kreatinin, total protein, glukoz, trigliserid, total kolesterol, A vitamini ve β-karoten düzeyleri gibi biyokimyasal parametrelerin ölçümü yapıldı. Oksidatif stres faktörleri açısından değerlendirilme metotları ise aşağıda belirtildi.

Plazmada Biyokimyasal Parametrelerin Ölçümü

Kekliklerin plazmasından AST, ALT ve ALP enzim aktiviteleri üre, kreatinin, total protein, glukoz, trigliserid ve total kolesterol düzeyleri spektrofotometrik olarak temin edilen Human (Max-Planck-Ring, Wiesbaden, Germany) marka kitlerle ölçüldü. Ayrıca plazma A vitamini ve β-karoten düzeyleri Suzuki ve Katoh (1990)'un metoduna göre spektrofotometrik olarak belirlendi. Spektrofotometrik ölçümler Shimadzu 1601 UV-VIS spektrofotometresi (Tokyo, Japan) kullanılarak gerçekleştirildi.

Tam Kanda Malondialdehit Düzeylerinin Ölçümü

Lipid peroksidasyon (LPO) için önemli bir belirteç olan MDA, tam kanda Draper ve Hardley (1990)'in metoduyla belirlendi. Metotların prensibi MDA ile tiyobarbitürik asit (TBA)'in reaksiyonu sonucu ortaya çıkan rengin spektrofotometrik ölçümüne dayanır ve bu rengin absorbansı spektrofotometrik olarak 532 nm dalga boyunda ölçüldü. MDA düzeyi MDA-TBA kompleksinin absorbans katsayısıyla hesaplanır ve kanda nmol/ml ifade edildi.

Tam Kanda Glutatyon Düzeylerinin Ölçümü

Glutasyon düzeyleri tam kanda Beutler ve ark. (1993) tarafından tanımlanan metot kullanılarak ölçüldü. Optik dansite spektrofotometrede 412 nm dalga boyunda ölçüldü. Sonuçlar kanda nmol/ml olarak ifade edildi.

Eritrosit Lizatında Süperoksid Dismutaz Aktivitelerinin Ölçümü

Eritrosit lizatında süperoksit dizmutazın (SOD) antioksidan enzim aktiviteleri Sun ve ark. (1988)'nin metoduna göre ölçüldü. Süperoksit radikallerinin ortamda bulunan nitroblue tetrazolyumu (NBT) mavi renkli formazona indirgemesiyle elde edilen absorbans, spektrofotometrik olarak 560 nm dalga boyunda belirlendi. SOD aktivitesi eritrosit için U/mgHb olarak ifade edildi.

Eritrosit Lizatında Katalaz Aktivitelerinin Ölçümü

Eritrosit lizatında CAT aktiviteleri Sinha (1972)'nın metoduna göre belirlendi. Tampon çözelti içinde bulunan H_2O_2 örnekte bulunan CAT etkisi ile yıkımlanır ve bunun spektrofotometredeki (570 nm) absorbansında azalma meydana gelir. Bu azalma hızı CAT aktivitesi ile orantılıdır. Katalaz aktivitesi eritrosit için U/mgHb olarak ifade edildi.

Hemoglobin Konsantrasyonları Ölçümü

Hemoglobin (Hb) Drabkin ve Austin (1935)'e göre siyanomethemoglobin metoduyla kolorimetrik olarak belirlendi.

İstatistiksel Analiz

Araştırmadan elde edilen sonuçlar, SPSS for windows 22.0 paket programında analiz edildi. Voliyerlerde ve kafes sisteminde barındırılan kekliklerin incelenen kan parametreleri açısından karşılaştırılmasında bağımsız örneklemler için t testi (independent samples t test) uygulandı. İstatistiksel anlamlılık için $p<0.05$ kabul edildi.

BULGULAR

Kekliklerden alınan plazma örneklerindeki biyokimyasal parametreler Tablo 1'de gösterildi. Biyokimyasal parametrelerden glukoz, trigliserid ve total kolesterol değerlerinin kafeste barındırılan kekliklerde yüksek düzeyde olduğu buna karşın voliyerde barındırılan kekliklerde ise bu parametrelerin daha düşük düzeyde olduğu belirlendi ($p<0.05$). Bunun yanı sıra böbrek fonksiyonlarının belirteçlerinden olan üre ve kreatinin değerlerinin kafeste barındırılan kekliklerde voliyerde barındırılanlara göre yüksek düzeyde olduğu tespit edildi ($p<0.05$). Kekliklerden alınan plazma örneklerinde analizi yapılan AST, ALT ve ALP enzim aktiviteleri ile total protein düzeylerinin ise her iki barındırma metodundan etkilenmediği belirlendi ($p>0.05$). Kekliklerden alınan kan, plazma ve eritrosit örneklerinden belirlenen oksidatif stres parametrelerine ait değerler Tablo 2'de gösterildi. Lipid peroksidasyon belirteci olan MDA değerlerinin kafeste barındırılan kekliklerde yüksek düzeyde olduğu buna karşın voliyerde barındırılan kekliklerde ise bu parametrelerin daha düşük düzeyde olduğu belirlendi ($p<0.05$). Ayrıca, antioksidan açıdan önemli rol oynayan GSH, hücre içi antioksidan enzimlerden olan SOD ve CAT aktiviteleri ile antioksidan etkinlikleri bilinen beta karoten ve A vitamini değerlerinin kafeste barındırılan kekliklerde voliyerde barındırılanlara göre düşük düzeyde olduğu tespit edildi ($p<0.05$).

Tablo 1. Voliyerlerde ve kafeste yetiştirilen kınalı kekliklerden alınan plazma örneklerinde alanin aminotransferaz (ALT), aspartat aminotransferaz (AST), alkalin fosfataz (ALP), total protein, üre, kreatinin, glukoz, trigliserid ve total kolesterol düzeyleri (n:60)

Table 1. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, urea, creatinine, glucose, triglycerides and total cholesterol levels in plasma samples taken from chuckar partridges grown in voliers and cages (n:60)

Parametre	Grup	N	Ortalama	Standart Sapma	p değeri
ALT (U/L)	Voliyerde	30	14,08	1,91	0,070
	Kafeste	30	15,67	1,85	
AST (U/L)	Voliyerde	30	150,09	19,91	0,065
	Kafeste	30	160,07	19,27	
ALP (U/L)	Voliyerde	30	221,76	15,87	0,083
	Kafeste	30	225,86	11,96	
Total Protein (g/l)	Voliyerde	30	4,46	0,37	0,179
	Kafeste	30	4,86	1,55	
Üre (mg/dl)	Voliyerde	30	5,15	0,66	0,000*
	Kafeste	30	8,35	1,72	
Kreatinin (mg/dl)	Voliyerde	30	2,37	1,07	0,000*
	Kafeste	30	5,04	1,25	
Glukoz (mg/dl)	Voliyerde	30	176,78	8,38	0,000*
	Kafeste	30	216,21	5,88	
Trigliserid (mg/dl)	Voliyerde	30	39,32	8,39	0,005*
	Kafeste	30	47,07	11,90	
Total Kolesterol (mg/dl)	Voliyerde	30	79,36	14,47	0,010*
	Kafeste	30	89,02	13,44	

Tablo 2. Voliyerde ve kafeste barındırılan kınalı kekliklerden alınan kan ve plazma örneklerinde malondialdehid (MDA), glutatyon (GSH), süperoksid dismutaz (SOD), katalaz (CAT), beta karoten ve A vitamini düzeyleri (n:30)

Table 2. The levels of malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), beta-carotene and vitamin A in blood and plasma samples taken from chuckar partridges housed in volier and cage (n:30)

Parametre	Grup	n	Ortalama	Standart Sapma	p değeri
MDA(nmol/mL)	Voliyerde	30	5,68	0,97	0,000
	Kafeste	30	7,54	0,84	
GSH (nmol/mL)	Voliyerde	30	27,76	3,81	0,000
	Kafeste	30	22,72	2,47	
SOD (U/mgHb)	Voliyerde	30	48,79	5,83	0,000
	Kafeste	30	24,13	6,46	
CAT (U/mgHb)	Voliyerde	30	55,09	13,29	0,000
	Kafeste	30	33,90	10,60	
Beta Karoten (µg/dl)	Voliyerde	30	9,63	2,73	0,000*
	Kafeste	30	6,19	2,18	
A vitamini (µg/dl)	Voliyerde	30	0,89	0,25	0,000*
	Kafeste	30	0,57	0,20	

TARTIŞMA

Kanatlılarda biyokimyasal parametreler ırk, cinsiyet, yaş, hayvanların fizyolojik durumları, geçirdiği enfeksiyonlar ve stres faktörleri gibi durumlardan sıklıkla etkilenmektedir. Yapılan araştırmalar özellikle barındırma koşullarının bu parametreleri etkilediğini göstermektedir. Özbey ve Esen (2007), yetiştirme sistemlerinin ve stok yoğunluğunun kaya kekliklerinde (Alectoris graeca) bazı kan parametrelerine etkisini araştırdıkları çalışmalarında; normal yerde ve 3 farklı yoğunlukta (15, 20 ve 25'li) olacak şekilde kafeste yetiştirilen 18 haftalık erkek kaya kekliklerinde ALP aktivitelerinin, total protein, total kolesterol, trigliserit, üre ve glikoz düzeylerinin istatistiksel olarak önemli düzeyde ($p<0.05$) AST ve ALT enzim aktivitelerinin sayısal olarak arttığını belirtmişlerdir. Bu çalışma sonunda kekliklerin yetiştirme sistemlerine ve stoklama yoğunluğuna tepkilerinin farklı olduğu ve zemin sisteminden kafes sistemine geçtiği ve stoklama yoğunluğunun artmasının kan parametrelerinde önemli değişikliklere neden olduğu rapor edilmiştir. Özhan ve ark. (2016), yer ve kafes sistemlerinde yetiştirilen etlik piliçlerin bazı biyokimyasal parametrelerinde değişiklilerin olduğunu, araştırmada 30 etlik piliçlerde serum glukoz, çok düşük yoğunluklu lipoprotein (VLDL -kolesterol) ve ürik asit düzeyinin önemli ölçüde arttığını, serum total kolesterol, trigliserit ve protein düzeyleri ile ALP ve kreatin kinaz enzim aktivitelerinde önemli farklılıkların olmadığını sonuç olarak, kafes sisteminin etlik piliçlerde kan parametrelerini olumsuz etkilediğini saptamışlardır. Bu araştırmalara benzer şekilde yapılan bu çalışmada, glukoz, trigliserid, total kolesterol, üre ve kreatinin değerlerinin kafeste barındırılan kekliklerde yüksek düzeyde olduğu buna karşın voliyerde barındırılanlarda ise bu parametrelerin daha düşük düzeyde olduğu belirlendi. Bu durum kafeste barındırmaya göre voliyerde barındırmanın keklik refahına olumlu katkı sağlayarak biyokimyasal kan parametrelerini daha ılımlı bir seviyede tuttuğunu göstermektedir.

Oksidatif stres, pro-oksidan maddelerin üretimi ile oksidatif hasara yol açan antioksidan savunma seviyesi arasındaki dengesizliktir (Valko ve ark 2007). Lipid peroksidasyonunun son ürünü olan MDA oksidatif stresin önemli bir göstergesidir. Lipid peroksidasyonun artışına paralel olarak artan stresin önlemesi amacıyla antioksidanlardan GSH düzeyleri ile antioksidan enzim aktivitelerinin (SOD ve CAT) azalması da söz konusu olmaktadır (Gawel ve ark 2004). Özellikle uygun olmayan ortam ve koşullarda yetiştirilen kanatlı hayvanlarda MDA değerlerinin arttığı rapor edilen bir çalışmada (Şimşek ve ark. 2014) etlik piliç üretiminde kullanılan yer ve kafes sistemlerinin performansı, bazı oksidatif stres parametreleri ve karkas kusurları üzerine etkileri araştırılmıştır. Aynı çalışmada iki yer ve iki kafes kümesi yaz, sonbahar ve kış sezonları süresince eş zamanlı olarak takip edilmiş ve alınan serum örneklerinde MDA seviyelerinin kafes sisteminde yetiştirilen hayvanlarda daha yüksek seviyede olduğu belirtilmiştir. Yapılan bir diğer çalışmada (Alonso-Alvarez ve ark. 2010). Geniş bir yaş aralığında (1-8 yaş) olan toplam 288 kırmızı bacaklı kekliklerden (*Alectoris rufa*) yaşlı hayvanların daha az yavru ürettiği ve orta yaşlı bireylere göre eritrositlerde daha yüksek seviyelerde oksitlenmiş GSH ve peroksitlenmiş lipidlerin oluştuğu rapor edilmiştir. Sunulan çalışmada kafes ortamında yetiştirilen hayvanlarda MDA düzeylerinin arttığı, buna karşın GSH düzeyi, SOD ve CAT aktivitelerinin ise azaldığı belirlenmiştir. Bu durum kafes ortamında yetiştirilen hayvanlarda oksidatif stresin oluştuğunu ve ortaya çıkan serbest radikaller ve peroksitlerle reaksiyona giren GSH düzeylerinin, SOD ve CAT aktivitelerinin ise azaldığını göstermektedir.

Beta-karoten, singlet oksijeni bozunmadan söndürebilir ve peroksil, hidroksil ve süperoksit radikalleri gibi serbest radikallerle reaksiyona girer. Karotenoidlerin DNA, lipidler ve proteinlerde oksidatif hasarı önlediği veya azalttığı yapılan birçok çalışmada gösterilmiştir (Agarwal ve ark. 2012; Chapman 2012; Saunders ve ark. 2008). Benzer şekilde sunulan çalışmada kafes koşullarında yetiştirilen hayvanların beta karoten ve vitamin A düzeylerinin azaldığı gözlenmiş ve artan oksidatif stres düzeylerini dengelemede beta karoten ve vitamin A kullanıldığını göstermiştir.

SONUÇ

Sonuç olarak yapılan çalışmada iki farklı koşulda barındırılan kınalı kekliklerde biyokimyasal ve oksidatif stres parametrelerin etkilendiği, kafes sisteminde artan oksidan statünün engellenmesinin gerekliliği görülmüştür. Bundan dolayı kafes yetiştiriciliğinde oksidatif stresin önlenmesinde mümkünse periyodik izlemelerin yapılması ve dışarıdan gerekli antioksidan takviyelerinin verilmesinin yararlı olacağı ön görülmektedir.

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KAYNAKLAR

Agarwal, M., Parameswari, R. P., Vasanthi, H. R., & Das, D. K. (2012). Dynamic action of carotenoids in cardioprotection and maintenance of cardiac health. Molecules, 17(4), 4755-4769.

Alonso-Alvarez, C., Pérez-Rodríguez, L., García, J. T., Vinuela, J., & Mateo, R. (2010). Age and breeding effort as sources of individual variability in oxidative stress markers in a bird species. Physiological and Biochemical Zoology, 83(1), 110-118.

Beutler, E., Duron, O., Kelly, B.M. (1963). Improved method for the determination of blood glutathione. J Lab Clin Med 61:882–888.

Birben, E., Sahiner, U. M., Sackesen, C., Erzurum, S., & Kalayci, O. (2012). Oxidative stress and antioxidant defense. World allergy organization journal, 5, 9-19.

Chapman, M. S. (2012, March). Vitamin a: history, current uses, and controversies. In Seminars in cutaneous medicine and surgery (Vol. 31, No. 1, pp. 11-16). WB Saunders.

Drabkin, D.L., Austin, J.H. (1935). Spectrophotometric studies. II. Preparations fromwashed 112, blood cells; nitric oxide hemoglobin and sulfhemoglobin. J Biol Chem:51–65.

Draper, H. H., & Hadley, M. (1990). [43] Malondialdehyde determination as index of lipid Peroxidation. In Methods in enzymology (Vol. 186, pp. 421-431). Academic press.

Durak, M. H., Gökalp, E., & Gürgöze, S. (2016). Kınalı Kekliklerde (Alectoris Chukar) Bazı Hematolojik ve Biyokimyasal Parametreler İle Mineral Madde Düzeyleri Üzerine Cinsiyetin Etkisi. Dicle Üniversitesi Veteriner Fakültesi Dergisi, (1), 22-27.

Gaweł S, Wardas M, Niedworok E, Wardas P (2004) Malondialdehyde (MDA) as a lipid peroxidation marker. Wiad Lek, 57, 453-455.

Keskin, E., Çöl, R., Keçeci, T., İpek, H., & Önder, F. (2002). Konya bölgesinde yetiştirilen kaya kekliklerinde (Allectoris gracea) bazı hematolojik parametreler. Veteriner Bilimleri Dergisi. Eurasian Journal of Veterinary Sciences, 18(3-4), 23-27.

Kırıkçı, K., & Çetin, O. (1999). Keklik yetiştiriciliği. Türk Vet Hek Derg, 11(1-2), 15-18.

Macit, S., & Akbulut, G. (2015). Diabetes mellitus ve oksidatif stres. Beslenme ve Diyet Dergisi, 43(1), 59-65.

Özbey, O., & Esen, F. (2007). The effects of breeding systems and stocking density on some blood parameters of rock partridges (Alectoris graeca). Poultry science, 86(2), 420-422.

Özçelik, M. (1995). Kuşlar Dünyası, Bilim ve Teknik. 328, 66-73.

Özek K, Bahtiyarca Y. (2004). Effects of sex and protein and energy levels in the diet on the blood parameters of the chukar partridge (Alectoris chukar). Br Poult Sci, 45(2): 290-293).

Özhan N, Şimşek Ü.G., Özçelik M. (2016). Comparison of floor and cage housing systems in terms of some welfare assessments in broiler, Ankara Üniv Vet Fak Derg, 63, 317-322.

Perez-Rodriguez, L., Mougeot, F., Alonso-Alvarez, C., Blas, J., Viñuela, J., & Bortolotti, G. R. (2008). Cell-mediated immune activation rapidly decreases plasma carotenoids but does not affect oxidative stress in red-legged partridges (Alectoris rufa). Journal of Experimental Biology, 211(13), 2155-2161.

Sinha, A. K. (1972). Colorimetric assay of catalase. Analytical biochemistry, 47(2), 389-394.

Storz, G., & Imlayt, J. A. (1999). Oxidative stress. Current opinion in microbiology, 2(2), 188-194.

Sun, Y. I., Oberley, L. W., & Li, Y. (1988). A simple method for clinical assay of superoxide dismutase. Clinical chemistry, 34(3), 497-500.

Suzuki, J., & Katoh, N. (1990). A simple and cheap methods for measuring serum vitamin A in cattle using only a spectrophotometer. The Japanese Journal of Veterinary Science, 52(6), 1281–1283.

Şimşek, Ü. G., Erişir, M., Çiftçi, M., & Seven, P. T. (2014). Effects of cage and floor housing systems on fattening performance, oxidative stress and carcass defects in broiler chicken. Kafkas Vet Fak Derg, 20, 727-733.

Valko M., D. Leibfritz, J. Moncola, M.T.D. Cronin, M. Mazura, and J. Telser. (2007). Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 39:44–84.

Winterbourn CC, Hawkins RE, Brian M, Carrell RW.(1975). The estimation of red cell superoxide dismutase activity. The Journal of laboratory and clinical medicine. 85(2): 337-341.

Yılmaz B. (2000). Kan "Fizyoloji" 116- 135, Feryal Matbaacılık, Ankara.

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RESEARCH ARTICLE

The Effect of N-Acetylcysteine Use on Endoplasmic Reticulum Stress in the Kidney Tissues of Obese Rats

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ABSTRACT

Endoplasmic reticulum (ER) stress has recently acquired increasing importance in the pathogenesis of obesity-associated kidney disease. N-acetylcysteine, otherwise known as NAC, is an antioxidant that works directly and indirectly by increasing the production of antioxidants in cells. A diet consisting of 60% calories from fat was used to establish the obesity model for the present investigation. In the NAC and obesity + NAC (ObNAC) groups, NAC was administered by intragastric tube at 150 mg/kg for eight weeks. GRP78 and PERK expressions were determined immunohistochemically in sections collected from kidney tissues at the end of the experiment. The GRP78 H score was significantly higher in the obese group than in the control, NAC, and ObNAC groups ($p<0.01$). The ObNAC group H-score was significantly lower than that of the obese group ($p < 0.01$) but was not different from the control and NAC groups. The obese group PERK H-score was also significantly higher than the control, NAC, and ObNAC groups ($p<0.01$). In the ObNAC group, the H-score was significantly lower than that in the obese group ($p<0.01$) and significantly higher than those in the control and NAC groups ($p<0.01$). Increasing changes in stress markers may be improved by NAC application, since obesity induced by a high-fat diet activates ER stress in kidney tissue.

Keywords: ER stress, GRP78, N-acetylcysteine, Obesity, PERK

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Obez Sıçanların Böbrek Dokularında N-Asetilsistein Kullanımının Endoplazmik Retikulum Stresi Üzerine Etkisi

ÖZ

Endoplazmik retikulum (ER) stresi son zamanlarda obezite ile ilişkili böbrek hastalığının patogenezinde artan bir önem kazanmıştır. N-asetilsistein (NAC), hücrelerde antioksidan üretimini artırarak doğrudan ve dolaylı olarak çalışan bir antioksidandır. Çalışmada, kalorinin %60'ını yağdan elde eden bir diyet ile obezite modeli oluşturuldu. NAC ve obezite + NAC (ObNAC) gruplarında NAC intragastrik tüp ile 150 mg/kg dozunda sekiz hafta süreyle uygulandı. Deney sonunda elde edilen böbrek dokularından alınan kesitlerde GRP78 ve PERK ekspresyonları immünohistokimyasal olarak belirlendi. GRP78'in H skoru obez grubunda kontrol, NAC ve ObNAC gruplarına göre anlamlı olarak yüksekti ($p<0.01$). ObNAC grubundaki H skoru, obez grubundan önemli ölçüde düşüktü ($p < 0.01$). Ayrıca bu grubun skoru kontrol ve NAC gruplarıyla benzerdi. Obez grubunda PERK H skoru kontrol, NAC ve ObNAC gruplarına göre anlamlı olarak yüksekti ($p<0.01$). ObNAC grubunda H skoru obez grubuna göre anlamlı olarak düşük ($p<0.01$), kontrol ve NAC gruplarına göre anlamlı olarak yüksekti ($p<0.01$). Yüksek yağlı diyet ile oluşan obezite böbrek dokusunda ER stresine neden olduğundan stres belirteçlerinde artan değişikliklerin NAC uygulaması ile iyileştirilebileceği düşünülebilir.

Anahtar kelimeler: ER stres, GRP78, N-asetilsistein, Obezite, PERK

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INTRODUCTION

More than 1.9 billion individuals worldwide are overweight, more than 650 million of who are clinically obese, and the numbers are rising annually. Progressively greater attention has therefore been paid to obesity-related glomerulopathy ever since obesity emerged as a widespread problem (Lu et al. 2020). High-fat diet (HFD)-induced obesity causes several complicated health issues worldwide. Human and animal research shows that obesity is linked to type 2 diabetes, cardiovascular disease, non-alcoholic fatty liver disease, and chronic kidney disease (Promsan et al. 2022). Obesity has been shown to cause both functional and histological changes in the kidneys, making them aberrant in appearance (Xu et al. 2017). Excessive HFD consumption in kidney cells can generate reactive oxygen species (ROS). Excessive levels of ROS in the kidney induce renal cell injury and dysfunction via the activation of multiple pathways, including oxidative stress, apoptosis, and impaired autophagy (Pengrattanachot et al. 2020, Promsan et al. 2022). Obesity may potentially represent a risk factor for end-stage renal disease, even after controlling for hypertension and diabetes, according to many experimental animal and human studies (Munusamy et al. 2015). Obesity is the result of a complex relationship between genetic and environmental stimuli that affect cell metabolism and homeostasis, including mitochondrial defect, dysregulated mitophagy, autophagy, and endoplasmic reticulum (ER) stress. Obesity is both a cause and an effect of an ER stress response (Ajoolabady et al. 2021).

The structure and function of the ER are complex. It plays important roles in the production, folding, changing, and transport of proteins and in the formation and distribution of phospholipids and steroids. For example, it stores calcium ions in its lumen and slowly releases these into the cytoplasm (Schwarz and Blower 2016). Any disruption in these activities will lead to ER stress and the accumulation of misfolded proteins (Zhao et al. 2018). The unfolded protein response (UPR), an adaptive mechanism, restores ER homeostasis in response to ER stress. Improved protein folding and UPR signaling increase the expression of proteins involved in the ER folding machinery. It also attenuates general protein translation, thus lightening the strain on the ER (Hetz and Papa 2018). Also known as ER-associated degradation, terminally misfolded proteins are moved from the ER to the cytoplasm and destroyed by the proteasome. Nevertheless, UPR signaling will initiate cell death pathways if the return of ER homeostasis is significantly prolonged (Adams et al. 2019, Lee and Ozcan 2014, Lin et al. 2019). The UPR, a cellular stress response originating from the ER, is controlled by three major sensors, inositol-requiring enzyme 1 (IRE1), protein kinase RNA-activated (PKR)-like ER kinase (PERK), and activating transcription factor 6 (ATF6). In the absence of stress, these three ER stress sensors are bound and rendered inactive by the ER-localized chaperone, also known as glucose-regulated protein 78 (GRP78/BIP), in the ER lumen. The accumulation or increase of misfolded proteins in the ER lumen activates the GRP78/BIP and the three sensors (Almanza et al. 2019).

Obesity occurs through a complex interaction of genetic and environmental factors that disrupt cellular homeostasis and metabolism. These include mitochondrial dysfunction, abnormal mitophagy, abnormal macroautophagy/autophagy, and abnormal ER homeostasis. Obesity may be both a cause and an effect of an unregulated ER stress response, according to recent research (Ajoolabady et al. 2022). Obesity may also disrupt the ER protein-folding mechanism, a phenomenon known as ER stress. GRP78, the resident ER chaperone, is disrupted as a result, thus exacerbating renal tubular damage. Experimentally induced ER stress in renal tubules and podocytes has been shown to activate autophagy and to cause cell damage (Munusamy et al. 2015). Excess free fatty acids (FFAs) accumulate in adipose tissues and ectopically in other organs, causing lipotoxicity and contributing to the development of obesity-related kidney damage. Endoplasmic reticulum stress occurs in tissues with abnormal deposits of FFA. More recent developments in lipid metabolism raise the possibility of ER stress as a common molecular mechanism in the etiology of disorders associated with hyperlipidemia (Li et al. 2019).

N-acetylcysteine (NAC) is a mucolytic agent that contains the thiol group and that is the subject of increasing research due to its potential anti-inflammatory and antioxidant effects. In addition, NAC is on the list of essential medicines maintained by the World Health Organization, and its well-established history of being safe makes it an appealing candidate for treating a diverse range of conditions. It is therefore interesting to note that a growing body of experimental data supports the therapeutic advantages of NAC therapy in managing obesity-related problems (Dludla et al. 2019). Derived from the amino acid L-cysteine, NAC is a precursor to glutathione in mammals. Studies have shown that it exhibits anti-hyperglycemic activity by protecting the β-cells of diabetic Zucker diabetic fatty rats, diabetic mice, and diabetic CD1 mice that have been alloxan-induced to become diabetic (Ho et al. 1999, Tanaka et al. 1999, Sarvani et al. 2017). Additionally, NAC reduces kidney damage induced with streptozotocin or iomeprol in mice and protects male Sprague-Dawley rats against the development of insulin resistance caused by hyperglycemia (Sarvani et al. 2017).

This study therefore investigated the expression of the ER stress indicators GRP78 and PERK in the kidney tissue of rats subjected to obesity. The potential protective effects of NAC against ER stress resulting from obesity in kidney tissue were also investigated and tested.

MATERIAL AND METHODS

Animals, Ethical Approval and Experimental Design

Sixteen 12-week-old Wistar albino female rats (250 ±50 g) were randomly assigned to each of four equal groups. The animals were allowed ad libitum access to food and water under standard conditions at 24±1 °C. Kastamonu University Animal Experiments Local Ethical Committee authorized and monitored all animal treatments (no. 2023/18). The control group received a regular diet (10% kcal) for eight weeks. The NAC group received a conventional diet (10% kcal) plus 150 mg/kg NAC (SigmaAldrich, Merck, Germany) via the intragastric route for eight weeks. The obese group received an HFD (60% kcal) (Arden Research and Experiment Company, Ankara, Turkey) for eight weeks for the induction of obesity. The obesity + NAC (ObNAC) was given an HFD (60% kcal) for eight weeks plus 150 mg/kg NAC via intragastric tube. At the end of the experiment, xylazine-ketamine (IP, 10mg/kg-50mg/kg) anesthesia was applied to examine kidney tissues after cervical dislocation. Tissues were fixed in 10% neutral buffered formaldehyde for 24 h and embedded in paraffin following routine tissue procedures.

Immunohistochemistry Procedure

Five micrometer-thick paraffin-embedded sections were placed onto poly-lysine slides and subjected to deparaffinization and rehydration. The sections were kept in sodium citrate buffer (pH=6.0) under moist heat and pressure for antigen retrieval. They were then incubated in 3% hydrogen peroxide (hydrogen peroxide 30% Merck: 108597) solution for 20 minutes and washed with PBS solution for 15 minutes. Ten percent standard goat serum blocking solution was next applied at room temperature for 10 minutes. The sections were subsequently incubated overnight at 4 °C with primary antibodies (Table 1). Following washing with PBS, the slides were incubated with a secondary antibody (TP-125-HL, Thermo Fisher Scientific, USA). The antigen-antibody complex was then demonstrated with the AEC chromogen. Gills hematoxylin was used as a counterstain, and the sections were covered. The sections were examined under a light microscope and photographed (Zeiss Axiolab 5, Jena, Germany).

Table 1. Antibodies used in immunohistochemical staining as the primary antibodies

Primary antibodies	IHC Dilution	Code	Company
GRP78/BIP Polyclonal antibody	1/200	11587-1-AP	Proteintech Group
PERK/EIF2AK3 Polyclonal antibody	1/200	24390-1-AP	Proteintech Group

Semiquantitative Scoring

GRP78 and PERK immunoreactivities were measured using a semiquantitative scoring technique based on intensity of staining (IS). This was evaluated as absence of staining (-), mild staining (++), medium staining (+++), and high staining. The average of the results from two different researchers was used to calculate the IS of immunoreactions in cells. A semiquantitative assessment of IS and the percentage of positive cells was also used to calculate the sections' immunohistochemistry scores (H-score). Finally, all antibody expression levels were compared statistically using the median H-score (Tatar et al. 2023).

Statistical Analysis

The statistical analyses were performed using the SPSS 26.0 (IBM SPSS Statistics, IBM Corporation, Chicago, IL) software for MAC. The data were analyzed for normality using the Shapiro-Wilks test. Kruskal Wallis tests were used to compare the groups. The data were presented as the median, and the interquartile range [Me (Q25–Q75)] and $p<0.05$ was recognized as statistically significant.

RESULTS

GRP78 Immunohistochemistry

GRP78 immunoreactivity was mainly localized in proximal and distal tubules and the mesangial areas of glomeruli in the obese group. This area also exhibited intense immunoreactivity compared to the other groups (Table 2). The H-score of this group was also significantly higher than those in the control, NAC, and ObNAC groups ($p<0.01$). However, in the ObNAC group, GRP78 immunoreactivity was weakly present in both the proximal tubule (PXT) and distal tubule (DT) regions but not in glomerular compartments (in either glomerular capillaries or Bowman's space) (Figure 1). This group's H-score was lower than that of the obese group ($p < 0.01$), but did not differ significantly from the control and NAC groups (Table 4).

PERK Immunohistochemistry

While no PERK immunoreactivity was detected in the control and NAC groups, very strong immunoreactivity was seen in the obese group cortex and medulla. Specifically, PERK in the obese group was localized in the proximal and distal tubules,

mesangial areas of the glomeruli, and medullary regions (Table 3). The H-score of this group was also significantly higher than those of the control, NAC, and ObNAC groups ($p<0.01$) (Table 4). In the ObNAC group, PERK immunoreactivity was moderate in the PXT, DT and medullary regions. At the same time, it was weakly expressed in the glomerular capillaries and medullar area (Figure 2). The H-score of this group was significantly lower than that of the obese group ($p<0.01$), but was also significantly higher than those of the control and NAC groups ($p<0.01$) (Table 4).

Table 2. Semiquantitative analysis of GRP78 immunoreactivity

GRP78 immunoreactivity	Groups			
Parameters	**Control**	**NAC**	**Obese**	**ObNAC**
Distal tubules	+	+	+++	+
Proximal tubules	+	+	+++	+
Glomeruli	+	+	+++	+
Medulla	-	-	-	-

-: No staining; +: weak positive; ++: moderate positive; +++: strong positive.

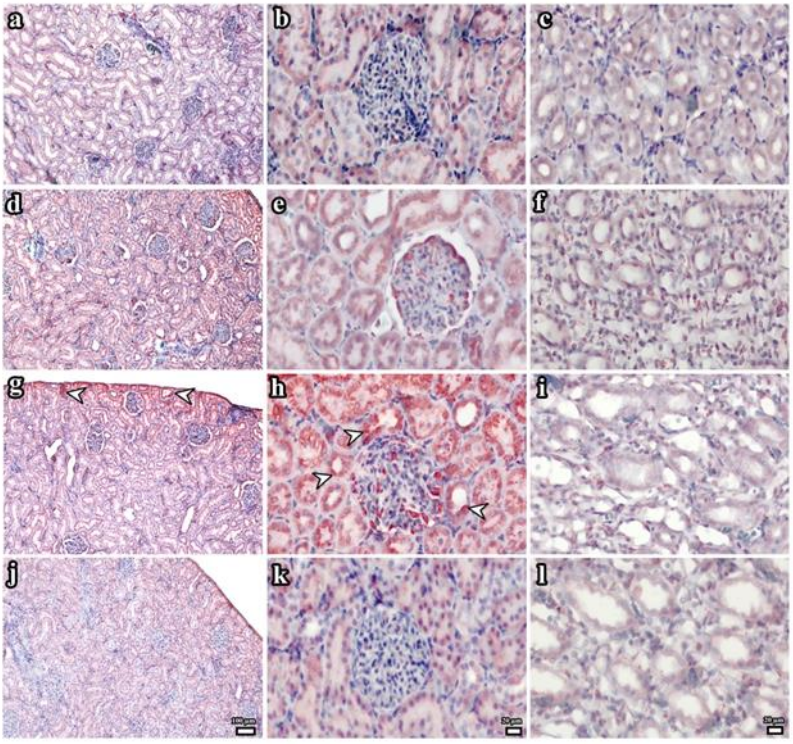

Figure 1: GRP78 expression analyses using immunohistochemistry (IHC) in kidney tissues. (a-c) Representative staining of GRP78 in the control group kidneys is weakly positive in proximal and distal tubules. (d-f) The staining of GRP78 in the NAC group is weakly positive in proximal and distal tubules and glomerular capillaries. (g-i) Densely positive staining is detected in the obese group proximal and distal tubules and glomerular capillaries. (j-l) Representative staining of GRP78 in the ObNAC group is weakly positive in the proximal and distal tubules, but with no reactivity in the glomerulus. The scale bar represents 100 μm for the left and 20 μm for the middle and right columns.

Table 3. Semiquantitative analysis of PERK immunoreactivity

PERK immunoreactivity	Groups			
Parameters	**Control**	**NAC**	**Obese**	**ObNAC**
Distal tubules	**-**	**-**	**+++**	**++**
Proximal tubules	**-**	**-**	**+++**	**++**
Glomeruli	**-**	**-**	**+++**	**++**
Medulla	**-**	**-**	**+++**	**++**

-: No staining; +: weak positive; ++: moderate positive; +++: strong positive.

Table 4. Semi-quantitative analysis of GRP78 and PERK antibody results. The data were presented as the median and the interquartile range [Me (Q25–Q75)].

	GRP78 positivity score	PERK positivity score
Control	80 (75-90) [a, d]	80 (75-100) [b]
NAC	100 (80-110) [c, d]	80 (75-110) [b]
Obese	250 (230-270) [a, b, d]	240 (200-270) [a, b, d]
ObNAC	100 (90-100) [a, b, c]	165 (150-175) [c]

[a]p = 0.000; versus the control group, [b]p = 0.000; versus the NAC group, [c]p = 0.000; versus the obese group, [d]p = 0.001; versus the ObNAC group, Kruskal–Wallis/Tamhane T2 test

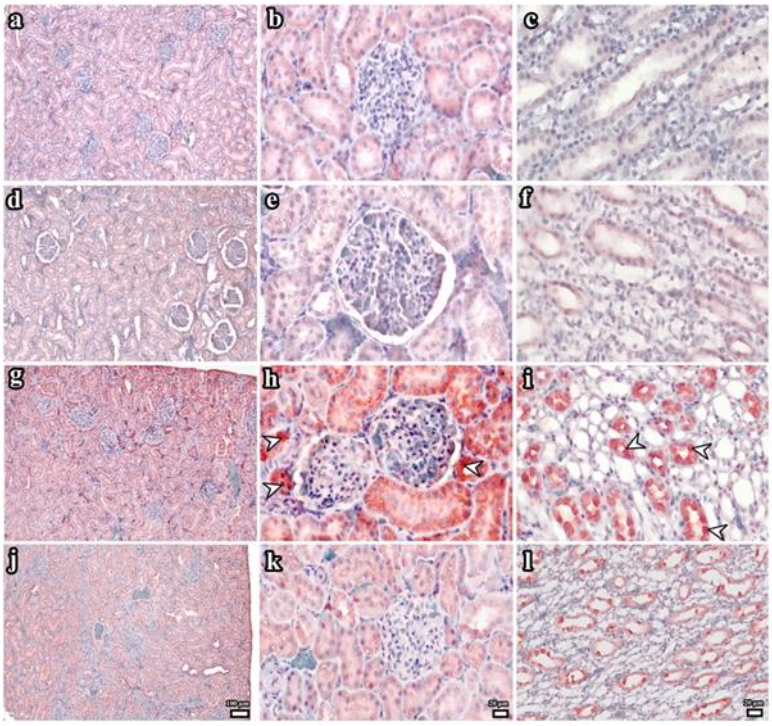

Figure 2: Analysis of PERK expression in kidney tissues by immunohistochemistry. (a-c) No immunoreactivity was detected at representative GRP78 staining in the control group. (d-f) Similarly to the control group, no GRP78 immunoreactivity is detected in the cortex or medulla in the NAC group. (g-i) In the obese group, very intense positive staining is seen in the proximal and distal tubules, and glomerular capillaries. (j-1) Representative GRP78 staining in the ObNAC group is moderately positive in the proximal and distal tubules and weak in the glomerulus and medullar region. The scale bar represents 100 μm for the left and 20 μm for the middle and right columns.

DISCUSSION

The primary purpose of this study was to investigate the hepatoprotective effect of NAC, a well-defined and safe compound, against ER stress in an obese rat model. Immunohistochemical analysis clearly showed that increased expressions of GRP78 and PERK, indicators of ER stress caused by obesity in kidney tissue, were reduced by the application of NAC. These results provide direct evidence supporting the use of NAC supplementation as an effective means of blocking obesity-related ER stress.

Despite the worldwide increase in obesity, pharmacotherapeutic alternatives for treating the disease and related disorders are still limited and inadequate. It is therefore essential to understand the molecular causes of obesity. ER stress may be used as a therapeutic target in treating the condition (Kim et al. 2013, Angelidi et al. 2022).

Obesity is a disease associated with oxidative stress and high levels of reactive oxygen species (ROS). A previous study of the association between obesity and kidney damage reported that the cafeteria diet, with its pro-oxidant effect and activation of apoptotic pathways, is capable of reducing the oxidative stress resulting from such damage (La Russa et al. 2019). Research has demonstrated that obesity generated by an HFD in several organs leads to ER stress and triggers the UPR signaling pathway (Kawasaki et al. 2012, Chen et al. 2016). ROS oxidize newly formed proteins and increase misfolded and unfolded proteins in the ER. Increases in ER stress indicators

such as GRP78, p-PERK, and p-eIF2 have been reported in mice with HFD-induced obesity (Liñares-Pose et al. 2018).

Similarly, our results also showed the presence of ER stress by revealing GRP78 activation in the kidneys of obese rats. However, although this study shows that obesity induces ER stress, we have not been able to elucidate the mechanisms involved. However, numerous studies in the literature have indicated ROS formation as the mechanism responsible for ER stress (Chong et al. 2017). In addition, ROS can act on calcium channels that are present in the ER membrane, which then increases calcium release from it. The reduced total calcium concentration in the ER lumen ultimately makes it difficult for newly produced proteins to fold correctly (Kawasaki et al. 2012, Zeeshan et al. 2016, Burgos-Morón et al. 2019, Bhattarai et al. 2021, Cui et al. 2022, Masenga et al. 2023).

A state of ER stress exists in tissues with abnormal accumulations of FFAs. ER stress is thought to represent a common molecular pathway in the pathogenesis of hyperlipidemia-related diseases (Li et al. 2019). Recent research involving obese Zucker rats, a rodent model of obesity with hypertension and metabolic syndrome, revealed kidney activation of ER stress (Wang et al. 2014). Oxidative stress and ER stress play a critical role in the pathophysiology of various kidney diseases. The renal glomeruli and tubular interstitium in both obese and diabetic animal models contain ER stress markers, suggesting a connection between chronic ER stress and kidney damage (Chen et al. 2014, Wang et al. 2014). Liu et al. (2008), determined that three hallmarks of ER-associated apoptosis, CHOP, JNK, and caspase-12, were activated in the diabetic rat kidney. Wang et al. (2014), found that the ER stress protein GRP78, eIF2α-ATF4-CHOP, caspase 12, and JNK-MAPK signaling pathways increased activation due to lipid accumulation in the kidneys of obese mice. In the same study, significant GRP78 expression was detected in both glomeruli and the tubular interstitium using the immunohistochemical method in obese control mice.

However, research has also suggested that changes in the ER membrane may also affect the activation status of IRE1 and PERK, both directly and independently of the accumulation of unfolded proteins in the ER lumen (Cherngwelling et al. 2021). The effects of ischemia-reperfusion-induced acute kidney injury worsen over time in the reperfusion phase of kidney damage. The activation of the ER stress markers GRP78 and XBP1, and CHOP expression as a result of this damage is consistent with this deleterious change (Gu et al. 2018).

Consistent with the above, our immunohistochemical results also revealed an increase in PERK activity, specifically in the kidneys of the obese group. Further studies involving ELISA and Western blotting are now needed to investigate the increase in GRP78, and PERK in kidney tissue observed in the present research. NAC has been reported to increase the rate of synthesis of the cellular antioxidant reduced glutathione (GSH) by upregulating intracellular cysteine level in several metabolic, liver, and psychiatric diseases, including obesity, in which oxidative stress and inflammation are involved (Santos et al. 2017, Tümer 2020, Tüfekci et al. 2023). NAC is a cysteine derivative with an acetyl group affixed to the nitrogen atom that can be oxidized by numerous radicals, including thiol. It also functions as a nucleophile (electron-pair donor) (Samuni et al. 2013, Elbini Dhouib et al. 2016). In addition, NAC is a precursor of reduced glutathione (Zafarullah et al. 2003). Glutathione, the cell's principal antioxidant, neutralizes reactive oxygen and nitrogen species both directly and indirectly (Dean et al. 2011). The metabolic pathways with which NAC has been found to interact include control of cell cycle and apoptosis, the development of cancer and tumors, mutagenesis, gene expression and signal transmission, immunological modulation, and mitochondrial activities (Zafarullah et al. 2003). Zhang et al. (2014), determined that NAC significantly reduced cleaved caspase 3, p53 and renal epithelial tubular cell apoptosis in a rat model of renal ischemia/reperfusion injury. Another study showed that NAC administration against hepatic ischemia-reperfusion injury significantly reduced the expression of GRP78, ATF-4, and CHOP (Sun et al. 2014). Hu et al. (2019), determined that NAC downregulates XBP1 splicing against fluoride-induced testicular apoptosis, inhibits testicular cell apoptosis by reducing IRE1α-JNK phosphorylation, and contributes to the inhibition of nuclear factor E2-related factor 2 (Nrf2)-related oxidative damage. Lee et al. (2016) also highlighted the role of NAC in ER stress-mediated diabetic nephropathy. NAC reduces levels of ER stress indicators PERK, eIF2, ATF6, GRP78, and CHOP in fatty rats. However, the molecular processes that reduce that stress are still unknown. Based on the available literature, NAC reduces oxidative stress by reducing ROS build-up and ER stress-mediated problems (Sarvani et al. 2017). HFD-derived ER stress may cause severe hepatic steatosis through the activation of the UPR pathway and the induction of apoptosis in the liver. HFD-induced hepatic steatosis and apoptosis are reversed by NAC treatment. Long-term HFD consumption by newborn offspring results in steatosis of the liver and suppresses the protective action of the UPR resulting from ER stress that leads to the hepatocyte death. NAC administration has been shown to reduce liver fat accumulation, to restore the protective effect of UPR, and to reduce hepatocyte damage and apoptosis. This antioxidant activity of NAC becomes more pronounced when it is used over an extended period (Tsai et al. 2020). Studies show that NAC exhibits a protective effect by inhibiting the apoptosis pathway associated with ER

stress (Sun et al. 2014, Lee et al. 2016). We therefore speculated that a similar protective mechanism may underlying the decrease in ER stress markers in the obese group receiving NAC in this.

CONCLUSION

The expressions of the ER stress marker chaperone GRP78 and PERK implicated in the UPR response were identified in order to investigate the mechanisms involved in kidney injury in obesity, which are currently little understood, and to show the function of NAC, a potential therapeutic target, in obesity-mediated ER stress. In the light of the results obtained, we think that NAC administration may be a useful therapeutic modality in obesity-induced ER stress. Although we did not measure ROS as an underlying mechanism of obesity-induced ER stress, other studies in the literature have considered this. ROS may therefore have triggered ER stress, which may have been ameliorated by NAC, a powerful antioxidant.

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Explanation: The data supporting the findings of this study available from the corresponding author, MT, upon reasonable request.

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REFERENCES

Adams, C. J., Kopp, M. C., Larburu, N., Nowak, P. R., & Ali, M. M. U. (2019). Structure and Molecular Mechanism of ER Stress Signaling by the Unfolded Protein Response Signal Activator IRE1. Frontiers in Molecular Biosciences, 6. https://doi.org/10.3389/fmolb.2019.00011

Ajoolabady, A., Liu, S., Klionsky, D. J., Lip, G. Y. H., Tuomilehto, J., Kavalakatt, S., Pereira, D. M., Samali, A., & Ren, J. (2022). ER stress in obesity pathogenesis and management. Trends in Pharmacological Sciences, 43(2), 97–109. https://doi.org/10.1016/j.tips.2021.11.011

Ajoolabady, A., Wang, S., Kroemer, G., Klionsky, D. J., Uversky, V. N., Sowers, J. R., Aslkhodapasandhokmabad, H., Bi, Y., Ge, J., & Ren, J. (2021). ER Stress in Cardiometabolic Diseases: From Molecular Mechanisms to Therapeutics. Endocrine Reviews, 42(6), 839–871. https://doi.org/10.1210/endrev/bnab006

Almanza, A., Carlesso, A., Chintha, C., Creedican, S., Doultsinos, D., Leuzzi, B., Luís, A., McCarthy, N., Montibeller, L., More, S., Papaioannou, A., Püschel, F., Sassano, M. L., Skoko, J., Agostinis, P., de Belleroche, J., Eriksson, L. A., Fulda, S., Gorman, A. M., & Samali, A. (2019). Endoplasmic reticulum stress signalling - from basic mechanisms to clinical applications. The FEBS Journal, 286(2), 241–278. https://doi.org/10.1111/febs.14608

Angelidi, A. M., Belanger, M. J., Kokkinos, A., Koliaki, C. C., & Mantzoros, C. S. (2022). Novel Noninvasive Approaches to the Treatment of Obesity: From Pharmacotherapy to Gene Therapy. Endocrine Reviews, 43(3), 507–557. https://doi.org/10.1210/endrev/bnab034

Bhattarai, K. R., Riaz, T. A., Kim, H.-R., & Chae, H. J. (2021). The aftermath of the interplay between the endoplasmic reticulum stress response and redox signaling. Experimental & Molecular Medicine, 53(2), 151–167. https://doi.org/10.1038/s12276-021-00560-8

Burgos-Morón, E., Abad-Jiménez, Z., Martinez de Maranon, A., Iannantuoni, F., Escribano-López, I., López-Domènech, S., & Víctor, V. M. (2019). Relationship Between Oxidative Stress, ER Stress, and Inflammation in Type 2 Diabetes: The Battle Continues. Journal of Clinical Medicine, 8(9), 1385. https://doi.org/10.3390/jcm8091385

Chen, J., Guo, Y., Zeng, W., Huang, L., Pang, Q., Nie, L., Mu, J., Yuan, F., & Feng, B. (2014). ER stress triggers MCP-1 expression through SET7/9-induced histone methylation in the kidneys of db/db mice. American Journal of Physiology-Renal Physiology, 306(8), F916–F925. https://doi.org/10.1152/ajprenal.00697.2012

Chen, Y., Wu, Z., Zhao, S., & Xiang, R. (2016). Chemical chaperones reduce ER stress and adipose tissue inflammation in high fat diet-induced mouse model of obesity. Scientific Reports, 6(1), 27486. https://doi.org/10.1038/srep27486

Cherngwelling, R., Pengrattanachot, N., Swe, M. T., Thongnak, L., Promsan, S., Phengpol, N., Sutthasupha, P., & Lungkaphin, A. (2021). Agomelatine protects against obesity-induced renal injury by inhibiting endoplasmic reticulum stress/apoptosis pathway in rats. Toxicology and Applied Pharmacology, 425, 115601. https://doi.org/10.1016/j.taap.2021.115601

Chong, W., Shastri, M., & Eri, R. (2017). Endoplasmic Reticulum Stress and Oxidative Stress: A Vicious Nexus Implicated in Bowel Disease Pathophysiology. International Journal of Molecular Sciences, 18(4), 771. https://doi.org/10.3390/ijms18040771

Cui, X., Zhang, Y., Lu, Y., & Xiang, M. (2022). ROS and Endoplasmic Reticulum Stress in Pulmonary Disease. Frontiers in Pharmacology, 13. https://doi.org/10.3389/fphar.2022.879204

Dean, O., Giorlando, F., & Berk, M. (2011). N-acetylcysteine in psychiatry: current therapeutic evidence and potential mechanisms of action. Journal of Psychiatry & Neuroscience, 36(2), 78–86. https://doi.org/10.1503/jpn.100057

Dludla, P. V., Mazibuko-Mbeje, S. E., Nyambuya, T. M., Mxinwa, V., Tiano, L., Marcheggiani, F., Cirilli, I., Louw, J., & Nkambule, B. B. (2019). The beneficial

effects of N-acetyl cysteine (NAC) against obesity associated complications: A systematic review of pre-clinical studies. Pharmacological Research, 146, 104332. https://doi.org/10.1016/j.phrs.2019.104332

Elbini Dhouib, I., Jallouli, M., Annabi, A., Gharbi, N., Elfazaa, S., & Lasram, M. M. (2016). A minireview on N -acetylcysteine: An old drug with new approaches. Life Sciences, 151, 359–363. https://doi.org/10.1016/j.lfs.2016.03.003

Gu, Y., Huang, F., Wang, Y., Chen, C., Wu, S., Zhou, S., Hei, Z., & Yuan, D. (2018). Connexin32 plays a crucial role in ROS-mediated endoplasmic reticulum stress apoptosis signaling pathway in ischemia reperfusion-induced acute kidney injury. Journal of Translational Medicine, 16(1), 117. https://doi.org/10.1186/s12967-018-1493-8

Hetz, C., & Papa, F. R. (2018). The unfolded protein response and cell fate control. Molecular cell, 69(2), 169-181. https://doi.org/10.1016/j.molcel.2017.06.017

Ho, E., Chen, G., & Bray, T. M. (1999). Supplementation of N-acetylcysteine inhibits NFκB activation and protects against alloxan-induced diabetes in CD-1 mice. The FASEB journal, 13(13), 1845-1854. https://doi.org/10.1096/fasebj.13.13.1845

Hu, Y., Wang, Y., Yan, T., Feng, D., Ba, Y., Zhang, H., Zhu, J., Cheng, X., Cui, L., & Huang, H. (2019). N-acetylcysteine alleviates fluoride-induced testicular apoptosis by modulating IRE1α/JNK signaling and nuclear Nrf2 activation. Reproductive Toxicology, 84, 98–107. https://doi.org/10.1016/j.reprotox.2019.01.001

Kawasaki, N., Asada, R., Saito, A., Kanemoto, S., & Imaizumi, K. (2012). Obesity-induced endoplasmic reticulum stress causes chronic inflammation in adipose tissue. Scientific Reports, 2(1), 799. https://doi.org/10.1038/srep00799

Kim, G. W., Lin, J. E., Blomain, E. S., & Waldman, S. A. (2013). Antiobesity Pharmacotherapy: New Drugs and Emerging Targets. Clinical Pharmacology & Therapeutics, 95(1), 53–66. https://doi.org/10.1038/clpt.2013.204

La Russa, D., Giordano, F., Marrone, A., Parafati, M., Janda, E., & Pellegrino, D. (2019). Oxidative Imbalance and Kidney Damage in Cafeteria Diet-Induced Rat Model of Metabolic Syndrome: Effect of Bergamot Polyphenolic Fraction. Antioxidants, 8(3), 66. https://doi.org/10.3390/antiox8030066

Lee, E. S., Kim, H. M., Kang, J. S., Lee, E. Y., Yadav, D., Kwon, M.-H., Kim, Y. M., Kim, H. S., & Chung, C. H. (2016). Oleanolic acid and N-acetylcysteine ameliorate diabetic nephropathy through reduction of oxidative stress and endoplasmic reticulum stress in a type 2 diabetic rat model. Nephrology Dialysis Transplantation, 31(3), 391–400. https://doi.org/10.1093/ndt/gfv377

Lee, J., & Ozcan, U. (2014). Unfolded Protein Response Signaling and Metabolic Diseases. Journal of Biological Chemistry, 289(3), 1203–1211. https://doi.org/10.1074/jbc.R113.534743

Li, B., Leung, J. C. K., Chan, L. Y. Y., Yiu, W. H., Li, Y., Lok, S. W. Y., Liu, W. H., Chan, K. W., Tse, H. F., Lai, K. N., & Tang, S. C. W. (2019). Amelioration of Endoplasmic Reticulum Stress by Mesenchymal Stem Cells via Hepatocyte Growth Factor/c-Met Signaling in Obesity-Associated Kidney Injury. Stem Cells Translational Medicine, 8(9), 898–910. https://doi.org/10.1002/sctm.18-0265

Lin, T., Lee, J., Kang, J., Shin, H., Lee, J., & Jin, D. (2019). Endoplasmic Reticulum (ER) Stress and Unfolded Protein Response (UPR) in Mammalian Oocyte Maturation and Preimplantation Embryo Development. International Journal of Molecular Sciences, 20(2), 409. https://doi.org/10.3390/ijms20020409

Liñares-Pose, L., Rial-Pensado, E., Estévez-Salguero, Á., Milbank, E., González-García, I., Rodríguez, C., Seoane-Collazo, P., Martinez-Sánchez, N., Nogueiras, R., Prieto, D., Diéguez, C., Contreras, C., & López, M. (2018). Genetic Targeting of GRP78 in the VMH Improves Obesity Independently of Food Intake. Genes, 9(7), 357. https://doi.org/10.3390/genes9070357

Liu, G., Sun, Y., Li, Z., Song, T., Wang, H., Zhang, Y., & Ge, Z. (2008). Apoptosis induced by endoplasmic reticulum stress involved in diabetic kidney disease. Biochemical and biophysical research communications, 370(4), 651-656. https://doi.org/10.1016/j.bbrc.2008.04.031

Lu, Y., Zhang, Y., Lou, Y., Cui, W., & Miao, L. (2020). Sulforaphane suppresses obesity-related glomerulopathy-induced damage by enhancing autophagy via Nrf2. Life Sciences, 258, 118153. https://doi.org/10.1016/j.lfs.2020.118153

Masenga, S. K., Kabwe, L. S., Chakulya, M., & Kirabo, A. (2023). Mechanisms of Oxidative Stress in Metabolic Syndrome. International Journal of Molecular Sciences, 24(9), 7898. https://doi.org/10.3390/ijms24097898

Munusamy, S., do Carmo, J. M., Hosler, J. P., & Hall, J. E. (2015). Obesity-induced changes in kidney mitochondria and endoplasmic reticulum in the presence or absence of leptin. American Journal of Physiology-Renal Physiology, 309(8), F731–F743. https://doi.org/10.1152/ajprenal.00188.2015

Pengrattanachot, N., Cherngwelling, R., Jaikumkao, K., Pongchaidecha, A., Thongnak, L., Swe, M. T., Chatsudthipong, V., & Lungkaphin, A. (2020). Atorvastatin attenuates obese-induced kidney injury and impaired renal organic anion transporter 3 function through inhibition of oxidative stress and inflammation. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, 1866(6), 165741. https://doi.org/10.1016/j.bbadis.2020.165741

Promsan, S., Thongnak, L., Pengrattanachot, N., Phengpol, N., Sutthasupha, P., & Lungkaphin, A. (2022). Agomelatine, a structural analog of melatonin, improves kidney dysfunction through regulating the AMPK/mTOR signaling pathway to promote autophagy in obese rats. Food and Chemical Toxicology, 165, 113190. https://doi.org/10.1016/j.fct.2022.113190

Samuni, Y., Goldstein, S., Dean, O. M., & Berk, M. (2013). The chemistry and biological activities of N-acetylcysteine. Biochimica et Biophysica Acta (BBA) - General Subjects, 1830(8), 4117–4129. https://doi.org/10.1016/j.bbagen.2013.04.016

Santos, P., Herrmann, A. P., Benvenutti, R., Noetzold, G., Giongo, F., Gama, C. S., Piato, A. L., & Elisabetsky, E. (2017). Anxiolytic properties of N -acetylcysteine in mice. Behavioural Brain Research, 317, 461–469. https://doi.org/10.1016/j.bbr.2016.10.010

Sarvani, C., Sireesh, D., & Ramkumar, K. M. (2017). Unraveling the role of ER stress inhibitors in the context of metabolic diseases. Pharmacological Research, 119, 412–421. https://doi.org/10.1016/j.phrs.2017.02.018

Schwarz, D. S., & Blower, M. D. (2016). The endoplasmic reticulum: structure, function and response to cellular signaling. Cellular and molecular life sciences, 73, 79-94. https://doi.org/10.1007/s00018-015-2052-6

Sun, Y., Pu, L. Y., Lu, L., Wang, X.-H., Zhang, F., & Rao, J. H. (2014). N-acetylcysteine attenuates reactive-oxygen-species-mediated endoplasmic reticulum stress during liver ischemia-reperfusion injury. World Journal of Gastroenterology, 20(41), 15289–15298. https://doi.org/10.3748/wjg.v20.i41.15289

Tanaka, Y., Gleason, C. E., Tran, P. O. T., Harmon, J. S., & Robertson, R. P. (1999). Prevention of glucose toxicity in HIT-T15 cells and Zucker diabetic fatty rats by antioxidants. Proceedings of the National Academy of Sciences, 96(19), 10857-10862. https://doi.org/10.1073/pnas.96.19.10857

Tatar, M., Tufekci, K. K., Uslu, S., & Öner, J. (2023). Expression of ADAMTS 1-4-8 and placental growth factor in ovary and oviduct during pregnancy in the first trimester. Anatomia, Histologia, Embryologia. 52, 619–626. https://doi.org/10.1111/ahe.12922

Tsai, C. C., Chen, Y. J., Yu, H. R., Huang, L. T., Tain, Y. L., Lin, I. C., Sheen, J. M., Wang, P. W., & Tiao, M. M. (2020). Long term N-acetylcysteine administration rescues liver steatosis via endoplasmic reticulum stress with unfolded protein response in mice. Lipids in Health and Disease, 19(1), 105. https://doi.org/10.1186/s12944-020-01274-y

Tüfekci, K. K., Bakirhan, E. G., & Terzi, F. (2023). A Maternal High-Fat Diet Causes Anxiety-Related Behaviors by Altering Neuropeptide Y1 Receptor and Hippocampal Volumes in Rat Offspring: the Potential Effect of N-Acetylcysteine. Molecular Neurobiology, 60(3), 1499–1514. https://doi.org/10.1007/s12035-022-03158-x

Tümer, N. B. (2020). N-acetyl cysteine attenuates ferroptosis mediated lung injury induced by lower limb ischaemia/reperfusion. Turkish Journal of Clinics and Laboratory, 11(4), 288–293. https://doi.org/10.18663/tjcl.769961

Wang, C., Wu, M., Arvapalli, R., Dai, X., Mahmood, M., Driscoll, H., Rice, K. M., & Blough, E. (2014). Acetaminophen Attenuates Obesity-Related Renal Injury Through ER-Mediated Stress Mechanisms. Cellular Physiology and Biochemistry, 33(4), 1139–1148. https://doi.org/10.1159/000358683

Xu, T., Sheng, Z., & Yao, L. (2017). Obesity-related glomerulopathy: pathogenesis, pathologic, clinical characteristics and treatment. Frontiers of Medicine, 11(3), 340–348. https://doi.org/10.1007/s11684-017-0570-3

Zafarullah, M., Li, W. Q., Sylvester, J., & Ahmad, M. (2003). Molecular mechanisms of N -acetylcysteine actions. Cellular and Molecular Life Sciences (CMLS), 60(1), 6–20. https://doi.org/10.1007/s000180300001

Zeeshan, H., Lee, G., Kim, H. R., & Chae, H. J. (2016). Endoplasmic Reticulum Stress and Associated ROS. International Journal of Molecular Sciences, 17(3), 327. https://doi.org/10.3390/ijms17030327

Zhang, L., Zhu, Z., Liu, J., Zhu, Z., & Hu, Z. (2014). Protective effect of N-acetylcysteine (NAC) on renal ischemia/reperfusion injury through Nrf2 signaling pathway. Journal of Receptors and Signal Transduction, 34(5), 396–400. https://doi.org/10.3109/10799893.2014.908916

Zhao, S., Liu, Y., Wang, F., Xu, D., & Xie, P. (2018). N-acetylcysteine protects against microcystin-LR-induced endoplasmic reticulum stress and germ cell apoptosis in zebrafish testes. Chemosphere, 204, 463–473. https://doi.org/10.1016/j.chemosphere.2018.04.020

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RESEARCH ARTICLE

Determination of Some Heavy Metal Concentrations in Serum of Young and Adult Cattle in the Şiran District of Gümüşhane by ICP-MS

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ABSTRACT

The purpose of this study was to evaluate certain heavy metal concentrations such as selenium (Se), manganese (Mn), zinc (Zn), aluminium (Al), vanadium (V), tin (Sn), chromium (Cr), iron (Fe), nickel (Ni), cobalt (Co), copper (Cu), arsenic (As), cadmium (Cd), and lead (Pb) in young and adult cattle in the Şiran district of Gümüşhane. For this, a total of 100 blood samples were taken from 50 young (aged 1 to 3 years old) and 50 adult (aged 4 to 6 years old) cattle slaughtered at an abattoir in the Şiran. Serum was then separated from the blood samples by centrifugation, and analysed for certain heavy metals by using the inductively coupled plasma mass spectrometry (ICP-MS) method. The concentrations of serum heavy metals in the serum of young and adult cattle breed in the Şiran were determined. When comparing young and adult cattle, the adult cattle had significantly higher ($p<0.05$) concentrations of Al, V, Ni, and Sn. No age-related accumulation ($p>0.05$) was found for the other heavy metal species analysed (Se, Mn, Zn, Cr, Fe, Co, Cu, As, Cd, and Pb). As a result, in this study, serum heavy metal concentrations and age-related bioaccumulation in cattle farmed in the Şiran district were determined for the first time. The periodic monitoring of the concentrations of these heavy metals may be helpful in improving animal health and production.

Key Words: Aluminium, Bovine, Nickel, Serum, Tin, Vanadium

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Gümüşhane'nin Şiran İlçesindeki Genç ve Yetişkin Sığırların Serumlarında Bazı Ağır Metal Konsantrasyonlarının ICP-MS ile Belirlenmesi

ÖZ

Bu çalışmanın amacı, Gümüşhane'nin Şiran ilçesindeki genç ve yetişkin sığırlarda selenyum (Se), manganez (Mn), çinko (Zn), alüminyum (Al), vanadyum (V), kalay (Sn), krom (Cr), demir (Fe), nikel (Ni), kobalt (Co), bakır (Cu), arsenik (As), kadmiyum (Cd) ve kurşun (Pb) gibi bazı ağır metal konsantrasyonlarının belirlenmesidir. Bunun için, Şiran mezbahanesinde kesilen 50 genç (1-3 yaş arası) ve 50 yetişkin (4-6 yaş arası) sığırdan toplam 100 kan örneği alınmıştır. Daha sonra kan örneklerinden santrifüj yoluyla serumlar ayrıştırılmış ve endüktif eşleşmiş plazma kütle spektrometresi (ICP-MS) yöntemi kullanılarak belirli ağır metaller açısından analiz edilmiştir. Şiran'da yetiştirilen genç ve yetişkin sığırların serumlarındaki ağır metal konsantrasyonları bu şekilde tespit edilmiştir. Genç ve yetişkin sığırlar karşılaştırıldığında, yetişkin sığırların Al, V, Ni ve Sn konsantrasyonlarının önemli ölçüde daha yüksek olduğu görülmüştür ($p<0.05$). Analizi yapılan diğer ağır metal türleri (Se, Mn, Zn, Cr, Fe, Co, Cu, As, Cd ve Pb) için ise yaşa bağlı bir birikim tespit edilmemiştir ($p>0.05$). Sonuç olarak, bu çalışmada Şiran ilçesinde yetiştirilen sığırlarda serum ağır metal konsantrasyonları ve yaşa bağlı biyoakümülasyonları ilk kez belirlenmiştir. Bu ağır metal düzeylerinin periyodik olarak izlenmesi, hayvan sağlığı ve üretimi açısından iyileştirici katkılar sağlayabilir.

Anahtar Kelimeler: Alüminyum, Kalay, Nikel, Serum**,** Sığır, Vanadyum

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INTRODUCTION

Heavy metals are considered to be extremely biologically hazardous chemicals due to their bioaccumulative properties (Paksy et al. 1997). Industrial activities such as cement production, steel industry, combined heat and power generation, glass production, waste and sewage sludge incineration plants produce large quantities of heavy metals. These metals can enter the soil, air and water in various ways. They can also be transmitted to humans and animals through vegetables, fruits, and crops grown on contaminated farmland (Wu et al. 2010, Rajaganapathy et al. 2011). Therefore, it is important to consider the environment, soil and water in which food is grown to minimize exposure to heavy metals.

The toxicity of heavy metals depends on their mobility, their concentration in soil and water, the chemical composition of the parent material, the solubility of the composition and environmental factors (Arslanbas and Baydan, 2013). Studies have shown that the accumulation of heavy metals in biological tissues and body fluids such as liver, kidney, brain, etc. of animals fed plant-based feeds in heavily industrially polluted soils can lead to tissue damage and death (Karagül et al. 2000, Ergün 2001, Beşkaya et al. 2008, Saghaei et al. 2012).

Although certain heavy metals, such as Zn, Co, Fe and Ni are necessary for living organisms in low amounts, their bioaccumulation can cause toxic effects by disrupting enzyme systems in living organisms over time (Bigersson et al. 1988, Sözgen 2000, El-Demerdash et al. 2004). Heavy metals with no biological function, such as Pb, Cd, and mercury (Hg), have been found to have toxic effects in tissues even at low concentrations (Duffus and Worth 1996, Zheljazkov and Nielsen 1996, Al-Saleh et al. 2003, Dündar et al. 2012). Studies indicate that heavy metals such as As, Cr, Hg, Cd, Pb, and Ni exhibit carcinogenic, mutagenic, and teratogenic effects in addition to their toxic effects (Boffetta et al. 1998, Quayyum and Shah 2014, Romaniuk et al. 2014, Hsueh et al. 2017, Rhee et al. 2020).

Phytochelatins in plants and metallothionein proteins in mammals also play a key role in the accumulation of heavy metals. The function of these proteins is to contribute to homeostasis by binding essential elements. However, since they also bind heavy metals taken in various ways, they can build up high amounts of these metals in tissues over time (Kägi 1991, Cobbett and Goldsbrough 2002). Moreover, the accumulation of heavy metals can also vary depending on the effects of environmental factors, resulting in varying concentrations of heavy metals in humans and animals from region to region (Ekici et al. 2015, Horasan et al. 2019). Therefore, in the present study, 14 heavy metal species (Se, Mn, Zn, Al, V, Sn, Cr, Fe, Ni, Co, Cu, As, Cd, and Pb) commonly analysed in previous studies were selected and analysed in cattle's serum raised in the Şiran district of Gümüşhane province. Additionally, age-related bioaccumulation of these heavy metals were investigated.

MATERIAL and METHODS

Sample Collection

In this study, blood samples were collected from the hearts of a total of 100 slaughtered female cattle at an abattoir in Şiran district (Figure 1) of Gümüşhane, 50 young cattle aged 1 to 3 years (17 native breeds, 17 Simmental, 16 Holstein) and 50 adult cattle aged 4 to 6 years (21 native breeds, 15 Simmental, 14 Holstein). The samples were then brought to the laboratory under a cold chain at +4 °C. In the laboratory, these samples were scratched after clotting and then centrifuged (3000 rpm for 10 min) to separate the serum.

Afterwards, the serum was collected in Eppendorf tubes, and stored at -20 °C until heavy metal analysis.

Heavy metal Analysis

The analysis of heavy metal concentrations (Se, Mn, Zn, Al, V, Sn, Cr, Fe, Ni, Co, Cu, As, Cd, and Pb) was performed using the ICP-MS (Agilent 7500a, Agilent Technologies, USA) device. The first step was the preparation of the samples for measurement. For this, 1 ml of serum from each sample was placed in Teflon cells in the microwave solubilization device. 5 ml of HNO3 (65%) was added, and after 20 minutes the device was turned on by closing the lid. After the solubilization process, the solutions were taken into 10 ml volumetric flasks and topped up with distilled water. Standards at increasing concentrations (0, 1, 5, 5, 10, 20, 20, 30, 40, and 50 ppm) were prepared from the samples ready for measurement and introduced to the ICP-MS device (Epa, 1994). After the analysis, the concentrations of heavy metals in the samples were read from the device, and the results were recorded in ppm. The method was validated by the parameters of accuracy and recovery (Se: 99.90%, Mn: 99.90%, Zn: 99.90%, Al: 99.80%, V: 99.90%, Sn: 99.90%, Cr: 99.90%, Fe: 98.20%, Ni: 99.90%, Co: 99.90%, Cu: 99.80%, As: 99.70%, Cd: 99.90%, and Pb: 99.90%), specificity, limit of detection (Se: 3.299 ppb, Mn: 0.480 ppb, Zn: 2.305 ppb, Al: 1.158 ppb, V: 0.120 ppb, Sn: 0.44 ppb, Cr: 0.730 ppb, Fe: 10.860 ppb, Ni :0.33 ppb, Co: 0.320 ppb, Cu: 1.363 ppb, As: 0.077 ppb, Cd: 0.070 ppb, and Pb: 0.200 ppb), and limit of quantitation (Se: 10.89 ppb, Mn: 1.58 ppb, Zn: 7.61 ppb, Al: 3.82 ppb, V: 0.40 ppb, Sn: 1.12 ppb, Cr: 2.41 ppb, Fe: 35.840 ppb, Ni: 1.09 ppb, Co: 1.06 ppb, Cu: 4.50 ppb, As: 0.25 ppb, Cd: 0.23 ppb, and Pb: 0.66 ppb). All chemicals used in heavy metal analysis were obtained from Sigma Chemical Company (Sigma-Aldrich, Co., Munich, Germany).

Statistical analysis

Statistical calculations were performed using SPSS 15.0 for Windows (SPSS Inc., USA). The results of this study are expressed as mean ± standard deviation (X±SD). Differences between parameters obtained from two groups (young and adult cattle) were analyzed using the Mann-Whitney U-test. A p-value of less than 0.05 was considered statistically significant**.**

Figure 1. The satellite image shows Şiran district of Gümüşhane

RESULTS

The concentrations of Se, Mn, Zn, Al, V, Sn, Cr, Fe, Ni, Co, Cu, As, Cd, and Pb in the blood samples collected from young and adult cattle were given in Table 1. The results revealed that the concentrations of Al, V, Ni, and Sn were considerably higher in adult cattle compared to young cattle ($p<0.05$). Specifically, the concentrations of Al in young cattle, were 1.682±0.951 (ranging from 0.355 to 1.884 ppm), V was 0.015±0.003 (ranging from 0.012 to 0.017 ppm), Ni was 0.022±0.016 (ranging from 0.009 to 0.038 ppm), Sn was 0.027±0.012 (ranging from 0.018 to 0.046 ppm). On the other hand, the concentrations of Al in adult cattle were 3.565±1.02 (ranging from 2.007 to 5.072 ppm), V was 0.046±0.015 (ranging from 0.026 to 0.062 ppm), Ni was 0.065±0.010 (ranging from 0.023 to 0.085 ppm), Sn was 0.066±0.029 (ranging from 0.022 to 0.103 ppm). Furthermore, there was no statistical difference between the two groups for the concentrations of Cr, Mn, Fe, Co, Cu, Zn, As, Se, Cd, and Pb in the blood
serums.

Table 1. The concentrations of some heavy metals in serum of young and adult cattle in the Şiran district of Gümüşhane (n=50).*

	Young (1-3 years old)		Adult (4-6 years old)		
Elements	**Mean±SD**	**Min–Max**	**Mean±SD**	**Min–Max**	**p value**
Al	1.682±0.951	0.355–1.884	3.565±1.02	2.007–5.072	**<0.05**
V	0.015±0.003	0.012–0.017	0.046±0.015	0.026–0.062	**<0.01**
Cr	0.580±0.016	0.037–0.090	0.069±0.028	0.051–0.094	**NS**
Mn	0.010±0.002	0.004–0.017	0.012±0.003	0.007–0.019	**NS**
Fe	1.855±0.030	1.346–2.838	1.750±0.041	1.320–3.154	**NS**
Ni	0.022±0.016	0.009–0.038	0.065±0.010	0.023–0.085	**<0.01**
Co	0.002±0.0023	0.001–0.002	0.003±0.0037	0.002–0.004	**NS**
Cu	0.573±0.273	0.347–0.935	0.679±0.208	0.509–0.911	**NS**
Zn	0.626±0.182	0.382–0.843	0.651±0.196	0.504–0.927	**NS**
As	0.018±0.009	0.013–0.040	0.021±0.011	0.014–0.050	**NS**
Se	0.134±0.039	0.023–0.266	0.150±0.061	0.032–0.428	**NS**
Cd	0.003±0.002	0.002–0.005	0.004±0.001	0.003–0.007	**NS**
Pb	0.008±0.002	0.000–0.016	0.010±0.003	0.000–0.019	**NS**
Sn	0.027±0.012	0.018–0.046	0.066±0.029	0.022–0.103	**<0.05**

***** : as ppm **NS** : not significant

DISCUSSION

Heavy metals are known to pollute the environment through their release into water, soil, and air as waste products of industrial activities. Living organisms that consume water or consume products grown in contaminated soil may be exposed to heavy metals. In this way, heavy metals collected from the external environment mostly bind to proteins and are transmitted to the body's liver through the blood. Heavy metals processed in the liver are stored there, sent to bile or returned to the bloodstream for excretion by the kidneys (Kıvrakdal 2010, Rajaganapathy et al. 2011).

Heavy metals are known to exhibit bioaccumulation characteristics, and studies on this topic are still being carried out. The extent of accumulation in living organisms varies depending on the concentration of environmental contamination and duration of exposure. Therefore, the concentration of this heavy metal accumulation may differ from region to region and from age to age. In a study conducted by Simsek et al. (2015) in Çankırı province, it was found that Al, V, Mn, Ni, As, and Sn concentrations were higher in adults Angora goats than in young ones. The present study was found the similar results regarding the age-related accumulation of Al, V, Ni and Sn in cattle of Şiran district.

Aluminium, which has a neurotoxic effect, can accumulate in cattle through various routes such as the equipment used in the barns, feed additives, drinking water, and consumed vegetable feeds (Allowaw, 2013). The high concentrations of Al observed in adult cattle in this study may be related to

There are studies investigating the toxic effects of many heavy metals and their connection with cancer the high exposure to the above conditions.

Vanadium, which is an essential element in all mammalian, has been found to act like insulin in all the tissues mainly targeted by the hormone, namely skeletal muscle and fat (Chasteen 1983, Goldwaser et al. 2000). In our study, adult cattle was found to have a higher concentration of vanadium than young cattle, and the reason for this may be that the muscles of adult cattle are much more developed.

Nickel is a heavy metal that is present in coal, petroleum, copper and steel industries, mining and combustion of fossil fuels. It can easily pass into the soil, water and atmosphere with the use of wastewater in agricultural areas. It is then absorbed into the body through inhalation, drinking water and food contaminated with this metal (Chau and Kulikovsky-Cordeiro 1995, Seven et al. 2008). In this regard, housing the animals in conditions with air, drinking water and feed rich in this metal may have resulted in a higher concentration of Ni in adult cattle of Şiran district.

Tin is found in soil and plants. Its main use is in the coating of steel to protect it and in the manufacture of biocides, which are widely used in agriculture (Alloway, 2013). In our study, the accumulation of Sn in adult cattle was found to be significantly higher than in young cattle, which may be due to their longer exposure to an environment containing Sn.

The other heavy metals (Co, Cr, Mn, Fe, Cu, Cd Zn, As, Se, and Pb) investigated in the study, are not caused significant age-related bioaccumulation in animals. This could be due to the absence of intensive industrial activity such as mining in Şiran, unlike other districts of Gümüşhane.

Heavy metals when accumulated over time can cause diseases by disrupting various physiological mechanisms (Sözgen 2000, Romaniuk et al. 2015). (Llobet et al. 2003, Hsueh et al. 2017, Rhee et al. 2020). Studies in rats and humans have also shown that certain heavy metals, such as Al, Ni, and Cd, disrupt the oxidant and antioxidant balance by increasing the production of free oxygen radicals (Gurer et al. 1998, El-Demerdash et al. 2004, Ranjbar et al. 2008).

CONCLUSION

In conclusion, this study determined for the first time heavy metals concentrations in young and adult cattle raised in the Şiran district of Gümüşhane province. Additionally, it was revealed that the serum Al, V, Ni, and Sn concentrations changed with age, and heavy metal accumulation was higher in adult cattle. As previous studies have shown, this consequently may lead to health problems in cattle and consequent a reduction of livestock production. Therefore, a regular monitoring system for heavy metal concentrations should be established, and ranchers should be knowledgeable about the threat of heavy metal contamination sources. Thanks to these precautions, heavy metal accumulation can be significantly reduced and kept under control in Şiran district. Indirectly, it will also contribute to the health of people who consume the meat of these animals. Additionally, It will be a reference for future heavy metal studies to be carried out in this region.

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

Authors' Contributions: The authors declared that they contributed equally to the article.

Ethical approval: This study is not subject to the permission of HADYEK in accordance with the

"Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees" 8 (k). The data, information and documents presented in this article were obtained within the framework of academic and ethical rules.

REFERENCES

Alloway BJ. (2013). Heavy metals in soils trace metals and metalloids in soils and their bioavailability. Environ Pollut, Netherlands: Springer. pp. 551-558.

Al-Saleh I, Shinwari N, Mashhour A. (2003). Heavy Metal Concentrations in the Breast Milk of Saudi Women. Biol Trace Elem Res, 2003; 96: 21-37.

Arslanbas E, Baydan E. (2013). Metal levels in organically and conventionally produced animal and vegetable products in Turkey. Food Addit Contam Part B Surveill. 6: 130-133.

Beşkaya A, Yıldız K, Başalan M, Us MF. (2008). Kırıkkale'de endüstri bölgesi civarında toprak, yem, su ve bu yörede yetiştirilen koyunlar ile parazitlerinde bazı ağır metallerin (Cd, Cu, Pb, Zn) belirlenmesi. Etlik Vet Mikrobiyol Derg. 19: 39-46.

Bigersson B, Sterner O, Zimerson E. (1988). Eine verständliche Einführung in die Toxikologie, Wiley-VCH, ISBN 3-527-26455-8.

Boffetta P, Garcia-Gómez M, Pompe-Kirn V, Zaridze D, Bellander T, Bulbulyan M, Caballero JD, Ceccarelli F, Colin D, Dizdarevic T, Español S, Kobal A, Petrova N, Sällsten G, Merler E. (1998). Cancer occurrence among European mercury miners. Cancer Causes and Control. 9: 591-599.

Chau YK, Kulikovsky-Cordeiro OTR. (1995). Occurrence of nickel in the Canadian environment. Environ Rev. 3: 95-120.

Chasteen ND. (1983). Copper, molybdenum, and vanadium in biological systems, Springer Berlin Heidelberg, pp. 105-138.

Cobbett C, Goldsbrough P. (2002). Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. Annu Rev Plant Biol. 53: 159-182.

Cooke M, Dennis AJ. (1985). Polynuclear aromatic hydrocarbons: mechanism, method and metabolism. Ohio Battelle Press, Columbus, USA.

Duffus JH, Worth HGJ. Fundamental toxicology for chemists, Royal Society of Chemistry Information Services, Cambridge, UK, 1996.

Dündar MŞ, Altundağ H, Kaygaldurak S, Şar V, Acar A. Çeşitli endüstriyel atik sularda ağır metal düzeylerinin belirlenmesi. SAÜ Fen Bil Enstit Derg, 2012; 16: 6-12.

Ekici H, Şimşek Ö, Arıkan Ş, Eren M, Güner B. Comparing levels of certain heavy metals and minerals and antioxidativemetabolism in cows raised near and away from highways. Turk J Vet Anim Sci, 2015; 39: 322-327.

El-Demerdash FM, Yousef MI, Kedwany FS, Baghdadi HH. Cadmium-induced changes in lipid peroxidation, blood hematology, biochemical parameters and semen quality of male rats: protective role of vitamin E and β-carotene. Food Chem Toxicol, 2004; 42: 1563-1571.

EPA. Methods for the determination of metals in environmental samples. Method 200.8 EMMC version, Revision 5. 4. EMSL Cincinnati OH 45268, 1994.

Ergün A. (2001). Mineral elementler. In, Ergün A, Tuncer DŞ (Ed): Hayvan Besleme ve Beslenme Hastalıkları. Medipress, 77-91, Ankara.

Goldwaser I, Gefel D, Gershonov E, Fridkin M, Shechter Y. (2000). Insulin-like effects of vanadium: basic and clinical implications. J Inorg Biochem. 80: 21-25.

Gurer H, Özgüneş H, Neal R, Spitz DR, Ercal N. (1998). Antioxidant effects of N-acetylcysteine and succimer in red blood cells from lead-exposed rats. Toxicol. 128: 181-189.

Horasan BY, Arık F. (2019). Assessing heavy metal pollution in the surface soils of Central Anatolia Region of Turkey. Carpathian J Earth Environ Sci. 14: 107-118.

Hsueh YM, Su CT, Shiue HS, Chen WJ, Pu YS, Lin YC, Tsai CS, Huang CY. (2017). Levels of plasma selenium and urinary total arsenic interact to affect the risk for prostate cancer. Food Chem Toxicol. 107:167-175.

Kägi JH. (1991). Overview of metallothionein. Methods Enzymol. 205: 613-626.

Karagül H, Altıntaş A, Fidancı UR, Sel T. (2000). Klinik Biyokimya, Medisan Yayın Serisi, 45: Ankara.

Kıvrakdal A. (2010). Farklı sertlik değerlerindeki sularda metal (Cu, Cr) etkisinde kalan farklı büyüklükteki balıkların (Oreochromis niloticus) ATPaz tepkilerin belirlenmesi. Yüksek Lisans Tezi. Çukurova Üniversitesi, Fen Bilimleri Enstitüsü, Adana.

Llobet JM, Falco G, Casas C, Teixido A, Domingo JL. (2003). Concentrations of Arsenic, Cadmium, Mercury and Lead in common foods and estimated daily Intake by children, adolescents, adult and seniors of Catalonia, Spain. J Agric Food Chem. 51: 838-842.

Quayyum MA, Shah MH. (2014). Comparative study of trace elements in blood, scalp hair and nails of prostate cancer patients in relation to healthy donors. Biol Trace Elem Res. 162: 46-57.

Paksy K, Rajczy K, Forgács Z, Lázár, P, Bernard A, Gáti I, Kaáli GS. (1997). Effect of cadmium on morphology and steroidogenesis of cultured human ovarian granulosa cells. J Appl Toxicol. 17: 321-327.

Ranjbar A, Khani-Jazani R, Sedighi A, Jalali-Mashayekhi F, Ghazi-Khansari M, Abdollahi M. (2008). Alteration of body totalantioxidant capacity and thiol molecules in human chronic exposure to aluminum. Toxicol Environ Chem. 90: 707-713.

Rajaganapathy V, Xavier F, Sreekumar D, Mandal PK. (2011). Heavy Metal Contamination in Soil, Water and Fodder and their Presence in Livestock and Products: A Review. J Environ Sci Technol. 4: 234-249.

Rhee J, Vance TM, Lim R, Christiani DC, Qureshi AA, Cho E. (2020). Association of blood mercury levels with nonmelanoma skin cancer in the U.S.A. using National Health and Nutrition Examination Survey data (2003–2016). Br J Dermatol. 183: 480-487.

Romaniuk A, Lyndin M, Moskalenko R, Kuzenko Y, Gladchenko O, Lyndina Y. (2015). Pathogenetic mechanisms of heavy metals effect on proapoptotic and proliferative potential of breast cancer. Interv Med Appl Sci. 7: 63-68.

Saghaei S, Ekici H, Demirbas M, Yarsan E, Tumer I. (2012). Determination of the Metal Contents of Honey Samples from Orumieh in Iran. Kafkas Univ Vet Fak Derg, 18: 281-284.

Seven T, Can B, Darende BN, Ocak S. (2008). Hava ve Toprakta Ağır Metal Kirliliği. UCBAD. 1: 91-103.

Simsek O, Ekici H, Cinar M, Atmaca N, Arikan S, Guner B. (2015). Heavy metal accumulation and oxidative-antioxidative status in angora goats depending on age. Fresenius Environ Bull. 24: 2659-2663.

Sözgen K. (2000). Ters Faz Likit Kromatografisi ile Ağır Metal Analizi. İ.Ü. Fen Bilimleri Enstitüsü Doktora Tezi. İstanbul.

Wu G, Kang H, Zhang X, Shao H, Chu L, Ruan C. (2010). A critical review on the bio-removal of hazardous heavy metals from contaminated soils: issues, progress, ecoenvironmental concerns and opportunities. J Hazard Mater. 174:1-8.

Zheljazkov VD, Nielsen NE. (1996). Effect of heavy metals on peppermint and cornmint. Plant Soil. 178: 59-66.

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RESEARCH ARTICLE

Validation of A Portable Beta-Hydroxybutyric Acid Analyser for The Diagnosis of Subclinical Ketosis in Dairy Cows

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ABSTRACT

On-farm determination of beta-hydroxybutyric acid (BHBA) with an accurate analyser is important for rapid diagnosis of subclinical ketosis (SCK) or clinical ketosis in dairy cows. The accuracy of the Taidoc BHBA portable analyser has not yet been tested and validated. The objective was the validation of the Taidoc BHBA analyser with another device Wellion (already validated) in 84 dairy cows. Whole blood was collected from the coccygeal vein and immediately analysed with Taidoc and Wellion in one drop of whole venous blood of 84 dairy cows. The averages of BHBA concentration were 0.79±0.66 and 1.09±0.51 mmol.l^{-1} for Wellion and Taidoc, respectively. The correlation coefficient was r=0.64 ($p<0.001$). Method comparison by the Passing-Bablok regression equation (y=0.0664+0.06111x) showed no agreement between the two instruments. The intercept A and slope B did not include 0.0 and 1.0, respectively. The Bland-Altman plots of agreement indicated that the mean deviation was 0.30 mmol.l^{-1} and the total deviation was 2.02 mmol.l^{-1}, which was unacceptably high. The weighted kappa value was 0.26, indicating minimal agreement. AUC, sensitivity and specificity for the diagnosis of SCK at cut-off point ≥1.2 mmol.l^{-1} BHBA were 0.78, 64.7% and 91.0%, respectively. There was no agreement between the Taidoc and the Wellion BHBA portable analyser for dairy cows and they cannot be used interchangeably. Taidoc gave much higher BHBA values than Wellion and its sensitivity and specificity for SCK diagnosis was not comparable to Wellion.

Keywords: Beta-hydroxybutyric acid, Dairy cow, Subclinical ketosis, Taidoc, Wellion

Süt İneklerinde Subklinik Ketozis Teşhisi İçin Portatif Beta-Hidroksibütirik Asit Analiz Cihazının Validasyonu

ÖZ

Süt çiftliklerinde beta-hidroksibütirik asidin (BHBA) doğru bir cihazla ölçülmesi, subklinik ketozisin (SCK) veya klinik ketozisin hızlı teşhisi için önemlidir. Taidoc BHBA taşınabilir analiz cihazının doğruluğu henüz valide edilmemiştir. Bu çalışma, Taidoc BHBA analiz cihazını valide etmek için daha önceden validasyonu yapılmış Wellion cihazı ile 84 süt ineğinde karşılaştırılarak yapılmıştır. Süt ineklerinde V. coccygea'dan alınan bir damla kan Taidoc ve Wellion ile analiz edilmiştir. BHBA konsantrasyonlarının ortalamaları Wellion ve Taidoc için sırasıyla 0,79±0,66 ve 1,09±0,51 mmol.l^{-1} olarak bulunmuştur. İki cihaz arasında korelasyon katsayısı r=0,64 ($p<0,001$) çıkmıştır. Passing-Bablok regresyon denklemiyle (y=0,0664+0,06111x) yöntem karşılaştırması, iki cihaz arasında bir uyum olmadığını gösterdi. Intercept ve slope sırasıyla 0.0 ve 1.0'ı içermemiştir. Bland-Altman uyum grafikleri, ortalama sapmanın 0,30 mmol.l^{-1} ve toplam sapmanın 2,02 mmol.l^{-1} olduğunu gösterdi ki, bu kabul edilemeyecek kadar yüksek çıkmıştır. Ağırlıklı kappa değeri 0.26 çıkmış ve bu değer minimum derecede bir uyumu göstermiştir. SCK'nın teşhisi için BHBA≥1,2 mmol.l^{-1} kesme noktasında eğri altındaki alan, duyarlılık ve özgüllük sırasıyla 0,78, %64,7 ve %91,0 çıkmıştır. Süt inekleri için Taidoc ve Wellion BHBA taşınabilir analiz cihazları arasında bir uyum bulunmamış ve cihazlar birbirlerinin yerine kullanılamazlar. Taidoc, Wellion'dan çok daha yüksek BHBA değerleri vermiştir ve SCK teşhisi için duyarlılığı ve özgüllüğü Wellion ile karşılaştırılabilir oranda değildir.

Anahtar Kelimeler: Beta-hidroksibütirik asit, Subklinik ketozis, Süt ineği, Taidoc, Wellion

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INTRODUCTION

Clinical ketosis and subclinical ketosis (SCK) are economically important metabolic diseases in dairy cows, and they are observed more frequently in the second to fourth weeks after calving (Duffield 2000; Mostert et al. 2017; Deniz et al. 2020; Steeneveld et al. 2020). The indicator for the diagnosis of ketosis is an elevated beta-hydroxybutyric acid (BHBA) concentration in the blood or milk, which in dairy cows can be analysed directly with a cow-side analyser on the farm (Voyvoda and Erdogan 2010; Berge and Vertenten 2013; Suthar et al. 2013; Khol et al. 2019; Aksoy et al. 2022). SCK is a form of ketosis without clinical signs (Duffield 2000; Suthar et al. 2013; Deniz et al. 2020; Steeneveld et al. 2020). The diagnosis of ketosis or SCK in cows is an important point on farms for immediate action, as it has a large impact on farmers' profitability due to milk yield losses costing €130-150 per SCK case (Mostert et al. 2017; Steeneveld et al. 2020) and other associated metabolic diseases such as displaced abomasum, retained placenta, mastitis, metritis, reproduction problems and early culling (Duffield 2000; Suthar et al. 2013; Raboisson et al. 2015; Deniz et al. 2020; Aksoy et al. 2022). The cut-off point for BHBA in blood has been accepted as ≥ 1.20 mmol.l^{-1} for SCK diagnosis (Voyvoda and Erdogan 2010; Khol et al. 2019; Deniz et al. 2020; Aksoy et al. 2022). Cow-side analysis of blood BHBA facilitates immediate diagnosis of SCK compared to costly laboratory serum analysis (Iwersen et al. 2009). There are many commercial blood or milk BHBA analysers on the market worldwide, but the question has been whether or not their accuracy has been validated. Many bloods or milk BHBA analysers have been validated and their accuracy tested and published (Geishauser et al. 2000; Carrier et al. 2004; Iwersen et al. 2009; Voyvoda and Erdogan 2010; Iwersen et al. 2013; Pineda and Cardoso 2015; Tatone et al. 2016; Macmillan et al. 2017; Jansen et al. 2021), including the Wellion whole blood BHBA analyser (Khol et al. 2019; Jansen et al. 2021). There are no published data on the validation and accuracy of the Taidoc cow-side BHBA analyser in dairy cows, although it is already used on the market. The aim of the present study was to test the accuracy and validation of the Taidoc cow-side portable BHBA analyser with another already validated device, Wellion, in dairy cows' whole venous blood.

MATERIAL and METHODS

This study protocol was approved by the Ethics Committee of Muğla Sıtkı Koçman University with approval number MUDEM-HADYEK, 15.09.2020, 08/20.

Eight-four dairy Holstein cows were randomly included in the study from different dairy farms in the western cities in Turkey. Twenty-one of 84 dairy cows were primiparous, the rest (n=63) were multiparous. The study cows were within one month postpartum. The study was conducted between June 2020 and October 2021. Ambient temperatures varied between 13 and 40°C during sampling. According to the farmers and veterinarians, none of the cows showed any clinical signs of disease at the time of blood collection. Blood was gently collected from the coccygeal vein (Jansen et al. 2021) into non-anticoagulant containing injector and one drop of whole blood was immediately used for the analysis of BHBA in cow-side portable analysers Wellion (WellionVet Belua, Med Trust Handels GmbH, Marz, Austria) and Taidoc (Taidoc Technology Corporation, Wugu dist. 24888 New Taipei City, Taiwan). The cut-off point of BHBA for SCK definition was set at ≥1.20 mmol.l^{-1} BHBA (Voyvoda and Erdogan 2010; Suthar et al. 2013; Khol et al.

2019; Deniz et al. 2020; Aksoy et al. 2022). Taidoc is a real-time BHBA analyser and gives the result in 5 seconds. It uses 0.7 µl whole blood for the analysis of BHBA. The minimum and maximum measurement ranges of Taidoc are 0.1 and 8.0 mmol.l^{-1} BHBA. The accuracy of Taidoc was reported by the manufacturer to be ±0.5 mmol.l-1 when ≤2.00 mmol.l-1 BHBA. The device delivers 25% more when > 2.00 mmol.l^{-1} BHBA was measured. The chemical reaction for BHBA analysis in Taidoc is based on beta-hydroxybutyrate dehydrogenase activity (electrochemical). In the present study, the cut-off points of BHBA <2.00 mmol.l^{-1} and ≥2.00 mmol.l^{-1} were also used for observation. Wellion is a portable BHBA analyser and displays the result of the analysis in 8 seconds. It uses 0.8 µl of whole blood for the analysis of BHBA. Compared to the reference method (Khol et al. 2019), the average values of BHBA analysed with Wellion and the reference method (wet biochemistry, optical spectrometry) were 0.73±0.94 and 0.71±0.93 mmol.l^{-1}, respectively. The correlation, kappa value and AUC were satisfactory for good agreement (r=0.95, 0.89, 0.99) with the reference method. BHBA concentration is analysed by BHBA dehydrogenase enzyme (electrochemical) in Wellion in a range of 0.1 to 8.0 mmol.l^{-1}. As the Wellion showed good agreement with the standard reference laboratory method (Khol et al. 2019) and demonstrated good diagnostic performance for SCK diagnosis (Khol et al. 2019; Jansen et al. 2021), the Wellion was used as the reference device in the present study.

Statistical analysis

MedCalc software (MedCalc Software Ltd Acacialaan 22, 8400 Ostend, Belgium) version 2022 was used to perform the statistical analyses. The normality of the data was checked using the Shapiro-Wilk test. Descriptive statistics as x±sd (mean and standard deviation) and the percentage of sick cows based on cut-off points were presented when needed. The correlation coefficient was calculated between Taidoc and Wellion. The Passing-Bablok regression equation (Jensen and Kjelgaard 2006; Zulle 2011) was used between Taidoc and Wellion to compare the methods. Bland Altman's Plots of Agreement Test was used to test the agreement between Taidoc and Wellion (Bland and Altman 1999; Jensen and Kjelgaard 2006; Giavarina 2015). Receiver Operating Characteristic Analysis (ROC) (Simundic 2009; Trevethan 2017) was performed to determine the sensitivity (Se), specificity (Sp) and area under the curve (AUC) of Taidoc at the cut-off point ≥1.20 mmol.l^{-1} BHBA for Wellion. Cohen's kappa statistic (Cohen 1960; Macmillan et al. 2017) was used for inter-rater reliability of variables (≤0.00 no agreement, 0.01-0.20 still or slightly, 0.21-0.40 fairly, 0.41-0.60 moderately, 0.61-0.80 substantially, 0.81-1.00 perfect agreement). However, the kappa coefficient was also interpreted according to McHugh (2012) from a medical perspective as follows: 0-20 no agreement (0-4% reliability of data), 21-39 minimal agreement, 0.40-59 weak agreement, 0.60-0.79 moderate agreement, 0.80-0.90 strong agreement and > 0.90 perfect agreement (81-100% reliability of data).

RESULTS

The averages of BHBA concentration were 0.79±0.66 mmol.l^{-1} (min: 0.10, max: 2.70 mmol.l^{-1}) and 1.09±0.51 mmol.l^{-1} (min: 0.20, max: 3.10 mmol.l^{-1}) for Wellion and Taidoc, respectively. The mean difference between Taidoc and Wellion was 0.30 mmol.l^{-1} (38% higher). The averages of BHBA concentrations and the percentage of sick cases at defined cut-off points were presented in Table 1. The correlation between Wellion and Taidoc was significant but not very high, so the correlation coefficient was r=0.64 (p<0.001) (Fig. 1). Comparison with the Passing-Bablok regression

method (y=0.663+0.611x) yielded systematic and proportional errors (Fig. 1), as the 95% CI of intercept A did not include '0' (0.547 to 0.725) and slope B did not include '1' (0.500 to 0.789). The weighted kappa coefficient was calculated to be 0.26±0.068 (95% CI: 0.129 - 0.396) indicating a low agreement. The mean and total deviations between Taidoc and Wellion were 0.30 and 2.02 mmol.l^{-1} BHBA, respectively, according to Bland-Altman plots of agreement (Fig. 2). Analysis of ROC showed that the AUC, Se and Sp of Taidoc compared with Wellion at a cut-off point ≥1.20 mmol.l^{-1} BHBA were 0.78, 64.7% and 91.0%, respectively (Fig. 3).

Table 1. Descriptive statistics of Wellion and Taidoc for defined cut-off points of beta-hydroxybutyric acid (BHBA) in whole venous blood of dairy cows.

	Wellion (W)			Taidoc (T)			Difference of mean (T-W)
	No. Cows	%	x+sd	No. Cows	%	x+sd	in %
All cows	84	100	0.79±0.66	84	100	1.09±0.51	38.0
BHBA<1.20 mmol.l^{-1}	67	79.8	0.50±0.30	59	70.2	0.84±0.21	68.0
BHBA ≥1.20 mmol.l^{-1}	17	20.2	1.90±0.52	25	29.8	1.66±0.56	-12.6
BHBA <2.00 mmol.l^{-1}	76	90.5	0.63±0.43	77	91.7	0.96±0.30	52.4
BHBA ≥2.00 mmol.l^{-1}	8	9.5	2.32±0.32	7	8.3	2.48±0.45	6.9

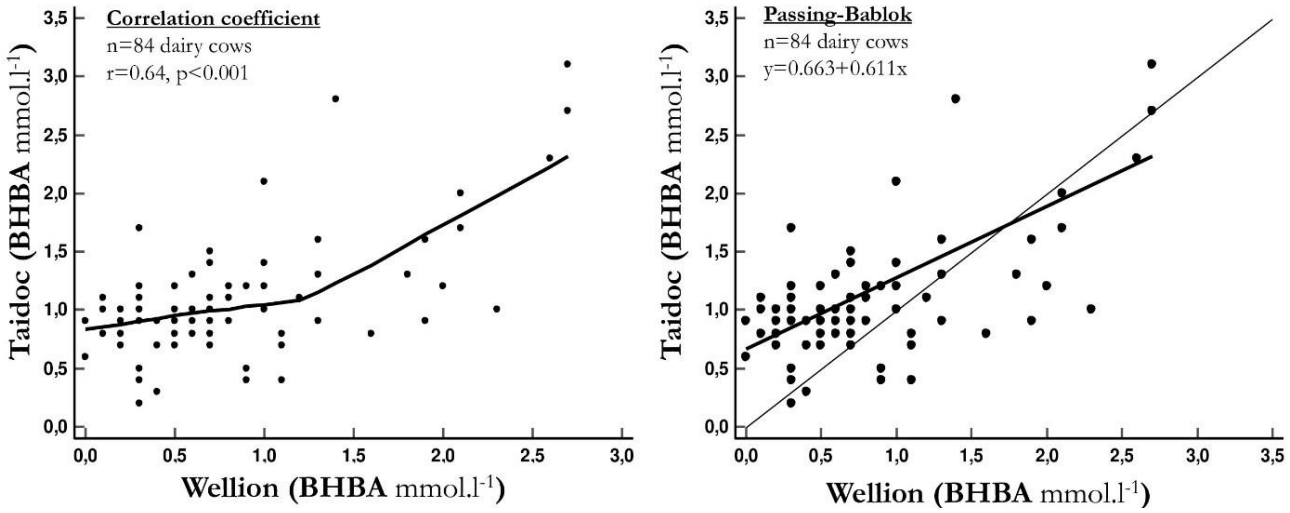

Figure 1. The correlation coefficient and Passing-Bablok regression equation between Taidoc and Wellion for the analysis of whole blood beta-hydroxybutyric acid (BHBA) concentration in dairy cows.

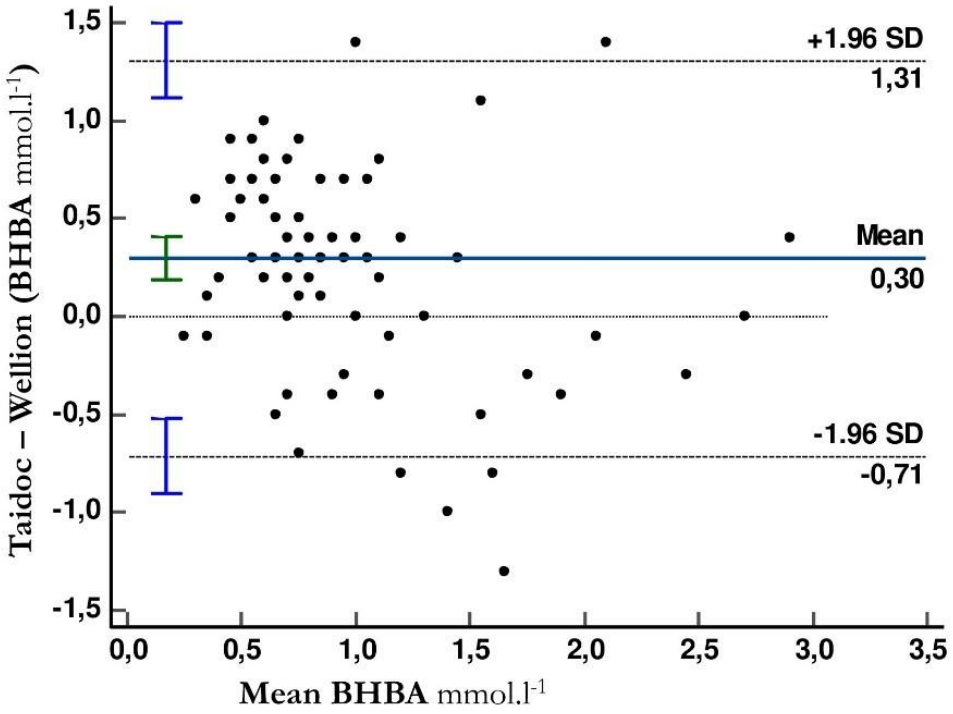

Figure 2. Bland-Altman plots of agreement between Taidoc and Wellion for the analysis of whole blood beta-hydroxybutyric acid concentration (BHBA) in dairy cows.

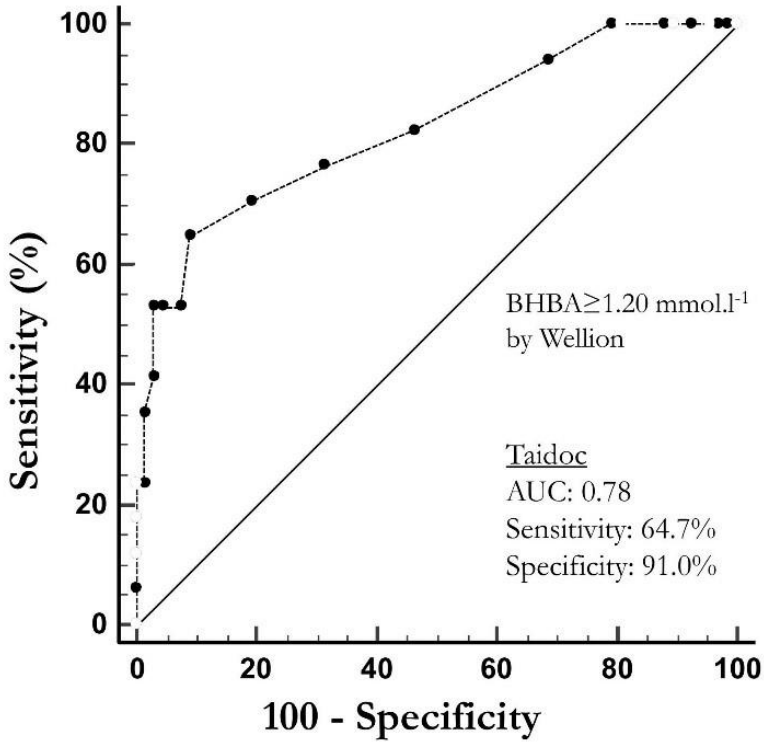

Figure 3. Receiver operating characteristics curve analysis for Taidoc based on cut-off point of beta-hydroxybutyric acid (BHBA) ≥ 1.20 mmol.l^{-1} in whole blood of dairy cows tested by Wellion. AUC: area under the curve

DISCUSSION

BHBA is an important parameter for the diagnosis of economically important SCK or clinical ketosis in dairy cows after calving (Suthar et al. 2013; Khol et al. 2019; Deniz et al. 2020). There are a number of devices that are portable and useful on the farm for the analysis of BHBA in dairy cows. However, the accuracy of BHBA analysers for the diagnosis of ketosis needs to be tested in cows using a previously validated test device or gold method (Iwersen et al. 2009; Voyvoda and Erdogan 2010; Khol et al. 2019; Jansen et al. 2021) to be sure that the disease is present or not. In the present study, Taidoc, a BHBA analyser for cows, was validated in the farms with another already validated BHBA analyser, Wellion. Both analysers were portable devices for use on farms. Wellion was previously compared to a reference method and proved consistent in both capillary and whole blood with satisfactory correlation coefficients, kappa values and sensitivity (Khol et al. 2019; Jansen et al. 2021). However, no validation study of Taidoc in dairy cows has been published to date, however a different brand of the same manufacturer (BHB-check) for BHBA analysis was validated by others (Jansen et al. 2021). The kappa coefficient showed fair agreement between Wellion and Taidoc. McHugh (2012), pointed out that the kappa value between 0.21 and 0.39 should represent minimal agreement in medical science and that the percentage agreement and the kappa value have strengths and limitations, so they should be interpreted cautiously. The kappa value of the present study was consistent with this statement. Furthermore, Khol et al. (2019), found a kappa value of 0.89 between Wellion and the reference method, indicating strong agreement. Kappa coefficient was not available in the validation study of Jansen et al. (2021). Moreover, Passing-Bablok, a powerful method agreement statistic (Jensen and Kjelgaard 2006; Zulle 2011), showed significant systematic and proportional errors in the regression equation between Wellion and Taidoc. The regression lines were incredibly far apart. This indicated no agreement between the two instruments. However, other studies did not provide Passing-Bablok regression equations when comparing two methods (Iwersen et al. 2009; Khol et al. 2019), but some others (Voyvoda and Erdogan 2010; Jansen et al. 2021) used this powerful statistic of method comparison (Jensen and Kjelgaard 2006; Zulle 2011). Many studies used the Pearson correlation coefficient to demonstrate linear correlation when comparing methods (Iwersen et al. 2009; Voyvoda and Erdogan 2010; Macmillan et al. 2017; Khol et al. 2019). Although the linear correlation coefficient and coefficient of determination were significantly high, it was not satisfactorily high between Wellion and Taidoc in the present study. The correlation coefficient between Wellion and the reference method was found to be r=0.94 and 0.95 for capillary and venous whole blood, respectively (Khol et al. 2019). Another study (Jansen et al. 2021) reported a correlation coefficient of r=0.76 for Wellion compared to the reference method; however, that was Spearman correlation coefficient. The correlation coefficient between Taidoc and Wellion was lower than reported in these studies. All samples were collected within a short time interval and analyses were performed by the same person in the present study. Therefore, factors such as temperature, handling and diurnal variations should have no influence on the results (Jansen et al. 2021). On the other hand, a significant and high correlation does not mean that the two instruments are in good agreement (Giavarina 2015; Macmillan et al. 2017), as linear regression is based on the random errors of the dependent variables. A very high correlation coefficient such as r=0.98 did not give

good agreement when analysing pO2 with two different blood gas analysers, so there were systematic and proportional errors in the Passing-Bablok regression equation (Metin et al. 2023). Therefore, Passing-Bablok or Deming regression equations (Jensen and Kjelgaard 2006; Zulle 2011) or Lin's concordance correlation coefficients (Macmillan et al. 2017) were used in the regression equation for the method comparison, as they provide information on proportional and systematic errors for both instruments. Thus, in the present study, both the linear correlation coefficient and the Passing-Bablok regression equation did not provide satisfactory results for the relationship between Wellion and Taidoc. The present study also found that the mean and total deviations between Wellion and Taidoc are very high according to Bland-Altman plots of agreement. Taidoc has systematically measured higher BHBA concentrations compared to Wellion. There was almost zero mean difference in the validation study of Wellion compared with reference method (Kohl et al. 2019), however other study (Jansen et al. 2021) found a mean of -0.269 mmol.l^{-1} difference for another BHBA analyser (BHB-check) manufactured by the same company. Taidoc's instructions for use indicate that the device measures 25% higher levels when BHBA is >2.0 mmol.l^{-1}. Since in the present study only 7 out of 84 cows had a BHBA concentration of >2.0 mmol.l^{-1}, this should not have a major impact on the mean value of the BHBA concentration. This means that Taidoc systematically gave unacceptably higher BHBA values than Wellion. Furthermore, the total deviation of 2.02 mmol.l^{-1} of confidence interval appeared unacceptable from a clinical perspective, as the BHBA cut-off point for SCK diagnosis is ≥1.20 mmol.l^{-1}, which has been accepted by many others (Voyvoda and Erdogan 2010; Suthar et al. 2013; Khol et al. 2019; Deniz et al. 2020; Aksoy et al. 2022). The BHBA value is an important indicator for the veterinarian to treat SCK or clinical ketosis, of course in accordance with the clinical manifestation. However, cows with SCK show no signs of ketosis (Duffield 2000; Suthar et al. 2013; Deniz et al. 2020), but it is still detrimental and has an economic impact on dairy farm profitability (Mostert et al. 2017; Steeneveld et al. 2020). Therefore, the disease needs to be properly identified in order to take measures for treatment and prophylaxis. Although the AUC (0.70 -0.80) in ROC analysis was sufficiently high for Taidoc indicating good diagnostic accuracy (Simundic 2009), the Se and Sp values were very low, suggesting that Taidoc did not sensitively identify the sick and healthy cows detected by Wellion. The Se value of Wellion was reported to be 89 - 98% in the capillary and venous blood of cows compared to the reference method (Khol et al. 2019). On the other side, others (Jansen et al. 2021) reported 0.97 AUC for Wellion indicating a perfect diagnostic performance. So compared to Wellion, Taidoc was not able to maintain the sufficient Se value. Thus, it provided 47% more SCK diagnoses, which could mean many false positives, and the differences in mean values of BHBA overall, at <2.00 mmol.l^{-1} and ≥2.00 mmol.l^{-1} cut-off points were 38.0%, 52.4% and 6.9% higher, respectively. This was not in accordance with the specifications of the device reported by the manufacturer. Furthermore, much higher Se (96%) (Iwersen et al. 2009), 85-90% (Voyvoda and Erdogan 2010), 98-100% (Iwersen et al. 2013; Pineda and Cardoso 2015), 94.8% (Tatone et al. 2016) have been reported for SCK diagnosis of a portable BHBA analyser depending on the cut-off points of the BHBA. In addition, a validation study found a concentration dependence of Se and Sp based on different cut-off points of the BHBA as well as the Passing-Bablok regression equation, which should be taken into account in further studies (Jansen et al. 2021). In summary, the present validation study showed that Taidoc and Wellion cannot be used interchangeably,

as Taidoc did not meet the requirements of the statistical evaluations. Taidoc provided systematically higher BHBA values than Wellion, which was unacceptably high for the correct diagnostic purpose of SCK. Although Wellion was validated with the reference standard method and showed good agreement and satisfactory diagnostic performance, it is recommended to compare both Wellion and Taidoc with a gold standard method using a wet biochemical assay. According to the results of the present study, the chips and test kits of the Taidoc device should be improved by the manufacturer for the measurement of BHBA in dairy cows.

Ethical Statement: This study protocol was approved by the Ethics Committee of Muğla Sıtkı Koçman University with approval number MUDEM-HADYEK, 15.09.2020, 08/20).

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REFERENCES

Aksoy, K., Deniz, A., Demir S., Onmaz, A.C. (2022). Blood and Milk Beta- hydroxybutyric Acid Concentrations in Diff erent Dairy Cattle Breeds and Association of Subclinical Ketosis with Postpartum Health Disorders, Culling Rate, Body Condition Score, Parity and Milk Production in Holstein. Kafkas Universitesi Veteriner Fakültesi Dergisi, 28 (2): 235-246.

Berge, A.C., Vertenten, G. (2013). A field study to determine the prevalence, dairy herd management systems, and fresh cow clinical conditions associated with ketosis in western European dairy herds. Journal of Dairy Science, 97: 2145–2154.

Bland, J.M., Altman, D.G. (1999). Measuring agreement in method comparison studies. Statistical Methods in Medical Research, 8:135-160.

Carrier, J., Stewart, S., Godden, S., Fetrow, J., Rapnicki, P. (2004). Evaluation and Use of Three Cowside Tests for Detection of Subclinical Ketosis in Early Postpartum Cows. Journal of Dairy Science, 87:3725–3735.

Cohen, J. (1960). A coefficient of agreement for nominal scales. Educational and Psychological Measurement, 20: 37-46.

Deniz, A., Aksoy K., Metin, M. (2020). Transition period and subclinical ketosis in dairy cattle: association with milk production, metabolic and reproductive disorders and economic aspects. Medycyna Weterynaryjna, 76 (9): 495-502.

Duffield, T.F. (2000). Subclinical ketosis in lactating dairy cattle. The Veterinary Clinics of North America. Food Animal Practice, 16, 231-253

Jansen, H.M., Zschiesche, M., Albers, D., Wemheuer, W., Sharifi, A.R., Hummel, J. (2021). Accuracy of Subclinical Ketosis Detection with Rapid Test Methods for BHBA in Blood in Commercial Dairy Farms. Dairy, 2: 671-683.

Jensen, A.L., Kjelgaard-Hansen, M. (2006). Method comparison in the clinical laboratory. Veterinary Clinical Pathology, 35:276-286.

Geishauser, T., Leslie, K., Tenhag, J., Bashiri, A. (2000). Evaluation of Eight Cow-Side Ketone Tests in Milk for Detection of Subclinical Ketosis in Dairy Cows. Journal of Dairy Science, Feb;83(2):296-299.

Giavarina, D. (2015). Understanding Bland Altman analysis. Biochemia Medica, 25: 141–151.

Iwersen, M., Falkenberg, U., Voigtsberger, R., Forderung, D., Heuwieser, W. (2009). Evaluation of an electronic cowside test to detect subclinical ketosis in dairy cows. Journal of Dairy Science, 92, 1–7.

Iwersen, M., Klein-Jöbstl, D., Pichler, M., Roland, L., Fidlschuster, B., Schwendenwein, I., Drillich, M. (2013). Comparison of 2 electronic cowside tests to detect sub¬clinical ketosis in dairy cows and the influence of the tem¬perature and type of blood sample on the test results. Journal of Dairy Science, 96: 7719-7730.

Khol, J.L., Freigassner, K., Stannitznig, A., Tichy, A., Wittek, T. (2019). Evaluation of a handheld device for the measurement of beta-hydroxybutyrate in capillary blood obtained by the puncture of the vulva as well as in venous whole blood in cattle. Polish Journal of Veterinary Sciences, 22: 557–564.

Macmillan, K., López, Helguera, I., Behrouzi, A., Gobikrushanth, M., Hoff, B., Colazo, M.G. (2017). Accuracy of a cow-side test for the diagnosis of hyperketonemia and hypoglycemia in lactating dairy cows. Research in Veterinary Science, Dec; 115:327-331.

McHugh, M.L. (2012). Interrater reliability: the kappa statistic. Biochemia Medica, 22(3): 276-282.

Metin, M., Aksoy, K., Pekmezci, A., Deniz, A. (2023). Comparison of the blood gas analyser Edan i15 Vet with the blood gas analyser Gem Premier 3000 for the analysis of blood gases, electrolytes, hemoglobin and hematocrit in lactating Holsteins. Medycyna Weterynaryjna, 79 (2), 83-90.

Mostert, P.F., Bokkers, E.A.M., van Middelaar, C.E., Hogeveen, H., de Boer, I.J.M. (2017). Estimating the economic impact of subclinical ketosis in dairy cattle using a dynamic stochastic simulation model. Animal, 1-10.

Pineda, A., Cardoso, F.C. (2015). Technical note: Validation of a handheld meter for measuring β-hydroxybutyrate concentrations in plasma and serum from dairy cows. Journal of Dairy Science, 98: 8818-8824.

Simundic, A.M. (2009). Measures of diagnostic accuracy: basic definitions. EJIFCC, 19:203-2011.

Steeneveld, W., Amuta, P., van Soest, F.J.S., Jorritsma, R., Hogeveen, H. (2020). Estimating the combined costs of clinical and subclinical ketosis in dairy cows. PLoSONE, 15(4):e0230448.

Suthar, V.S., Canelas-Raposo, J., Deniz, A., Heuwieser, W. (2013). Prevalence of subclinical ketosis and relationships with postpartum diseases in European dairy cows. Journal of Dairy Science, 96: 2925-2938.

Tatone, E.H., Gordon, J.L., Hubbs, J., LeBlanc, S.J., DeVries, T.J., Duffield, T.F. (2016). A systematic

review and meta-analy¬sis of the diagnostic accuracy of point-of-care tests for the detection of hyperketonemia in dairy cows. Preventive Veterinary Medicine, 130: 18-32.

Trevethan, R. (2017). Sensitivity, specificity, and predictive values, foundations, pliabilities, and pitfalls in research and practice. Frontiers in Public Health, 5:1-7.

Voyvoda, H., Erdogan, H. (2010). Use of a hand-held meter for detecting subclinical ketosis in dairy cows. Research in Veterinary Science, Dec;89(3):344-351.

Zulle, L.B. (2011). Comparison of methods: Passing-Bablok regression. Biochemia Medica, 21: 49-52.

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RESEARCH ARTICLE

Subjective Scoring Method for Evaluation of Uterine Echotexture to Detect Early Pregnancy in Holstein Cows and Heifers

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ABSTRACT

In the study, it was aimed to develop a scoring scale, graded from 1 to 5, to identify different gray tones of the ultrasonographic images of uterus, and the pregnancy determination in cows was tried to make by the scoring technique, in the first 20 days after artificial insemination. Sixty Holstein cows (n:30) and heifers (n:30) were enrolled in the study. For each animal, two ultrasonographic images were taken (totally 1320 images) starting from the day of the artificial insemination (D0) until the 20th day (D20) on every other day. At the same time that images were taken, subjective scoring were made. Then, the images were evaluated to determine mean gray value (MGV) by ImageJ (ImageJ 1.53k; NIH, ABD) software. The obtained data at the end of the evaluations were analyzed in terms of age of animals (cow or heifer), the days of examinations (alternate days between D0 and D20) and the 28th day pregnancy status (pregnant or non-pregnant). Also score rates were given by one experienced and one inexperienced independent operators to determine scoring agreement level in terms of experience on the ultrasonographic examinations. The mean MGV values indicated statistically significant differences only between days of sampling ($p<0.05$). On the other hand, according to other main effects and interactions were not concluded with statistically significant differences ($p>0.05$). It has been determined that experienced (Rater 1) and inexperienced (Rater 2) raters have agreement ($p<0.001$) in poor level ($\kappa=0.15$). Moreover, scores of the Rater 1 (0.720) were observed that had higher level correlations than Rater 2 (0,541). The gray tone scoring analysis were concluded that the mean of gray tone scoring were not differed significantly in terms of means and two-way interactions between sampling time and pregnancy status ($p>0.05$). In conclusion, it has been observed that the 256 different shades of gray which is analyzed by computer assisted image analysis could be identified by human eye in a 5 graded scale. However, the endometrium of the ovulation side uterine horn reflects the same differentiation in the presence or absence of pregnancy, in the first 20 days.

Keywords: Cow, Echotexture, Heifer, Pregnancy, Scoring, Ultrasound image

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Holstein Irkı İnek ve Düvelerde Erken Gebeliğin Belirlenmesinde Uterus Ekotekstürünün Değerlendirilmesi için Subjektif Skorlama Yöntemi

ÖZ

Çalışmada uterusun ultrasonografik görüntülerinin farklı gri tonlarını belirlemek için 1 ile 5 arasında derecelendirilen bir skorlama skalası geliştirilmesi amaçlanmış ve çalışmada ineklerde tohumlama sonrası ilk 20 gün içinde bu skorlama yöntemi ile gebelik tespitinin gerçekleştirilmesi araştırılmıştır. Çalışmada 60 adet Holstein inek (n:30) ve düve (n:30) kullanıldı. Her bir hayvandan suni tohumlamanın yapıldığı günden başlayarak 20. güne kadar gün aşırı olmak üzere en az iki adet (toplam 1320 adet) ultrasonografik uterus görüntüsü alındı. Subjektif skorlamalar bir deneyimli ve bir deneyimsiz olmak üzere iki bağımsız uygulayıcı tarafından gerçekleştirildi. Aynı görüntüler, ImageJ (ImageJ 1.53k; NIH, ABD) yazılımı ile Ortalama Grilik Değeri (MGV) değerlerinin belirlenmesi açısından analiz edildi. Veriler, hayvanların yaşı (inek veya düve), muayene günleri (D0 - D20) ve 28. gün gebelik durumu (gebe veya gebe değil) açısından değerlendirildi. Ortalama MGV değerleri sadece örnekleme günleri arasında istatistiksel olarak anlamlı farklılıklar gösterdi ($p<0,05$). Diğer ana etkilere ve etkileşimlere göre elde edilen farklılıklar istatistiksel olarak anlamsız bulundu ($p>0,05$). Deneyimli (1. skorlayıcı) ve deneyimsiz (2. skorlayıcı) skorlayıcıların uyumlarının zayıf düzeyde ($\kappa=0,15$) olduğu belirlendi ($p<0,001$). Ayrıca, 1. skorlayıcının (0,720) 2. skorlayıcıya göre (0,541) MGV değerleri ile daha yüksek korelasyona sahip olduğu belirlendi. Gri tonu skorlama ile gebelik belirlenmesi analizinde anlamlı bir farklılık bulunmadı ($p>0,05$). Sonuç olarak, bilgisayar destekli görüntü analizi ile değerlendirilen grinin 256 farklı tonunun (MGV) insan gözü tarafından 5 dereceli bir ölçekte tanımlanabildiği sonucu elde edilmiştir. Ancak gebe olan ve olmayan inek ve düvelerde uterus endometriyumunun ekotekstür yapısının suni tohumlama sonrası ilk 20 gün içinde benzer olduğu gözlemlenmiştir.

Anahtar kelimeler: Düve, Ekotekstür, Gebelik, İnek, Skorlama, Ultrason görüntüsü

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INTRODUCTION

B-Mode ultrasound is a unique device that is used for monitoring the tissues and organs by collecting the reflected ultrasound waves and converting them to visual images in a wide gray scale between 0 (absolute black) and 255 (absolute white) (Pierson and Adams; 1995; Zagzebski, 1996). The gray scale appearance of the tissues and organs is called echotexture. Recently, the echotexture evaluation of different structures such as muscle, tendon, bone, nerve, liver and mammary gland has been used to detect physiological and pathological changes (Sarwall et al., 2015; Martinoli et al., 1993; Themistokleous et al., 2022; Dietrich et al., 1999; Lee et al., 2011). Moreover, the differentiation in echogenic conditions of the genital tract in farm animals have been tried to determine according to reproductive situations such as ovarian and testicular soundness, oestrus, insemination time, and pregnancy (Camela et al., 2019; Cengiz et al; 2014; Kauffold et al., 2010; McKinnon and Carnevale, 1993).

In cattle breeding, ultrasonography is used widely. Moreover, it is an indispensable part of the reproductive examinations (Cengiz; 2017; Scully et al., 2014). The examinations mostly depend on direct observation of the interested event, which takes a long time to become visible. For example, pregnancy determination in cows could be made by observation of embryo and embryonic heartbeats. However, pregnancy detection with 95% accuracy can be done as early as on 35th day after fertilization by this method (Ribadu and Nakao, 1999). It has been reported that different ultrasonographic evaluating techniques such as uterine echotexture, dominant follicle diameter, endometrial thickness, uterine volume, amount of fluid collected in the uterus, and evaluation of uterine vascularization can be used in pregnancy prediction by analyzing ultrasonographic images (Abdalla et al., 2021; Holm et al., 2015; Mee et al., 2009; Souza et al., 2011; Stevenson et al., 2008; Vasconcelos et al., 2001). However, all of the studies conducted on echogenicity have been based on computer assisted image analysis. Although the computer assisted image analysis has the advantage of achieving objective results, the inability to use it in real-time in clinical applications is observed as the most important restraint to the widespread use of this method. Therefore, it is needed to develop a real-time subjective scoring technique to evaluate the echotexture properties of ultrasonographic images.

Echotexture consists of three different measurements known as mean gray value (MGV), contrast and homogeneity. Among these measurements, MGV is the most likely to identify by the human eye. Because the eye is known that can identify color tones (almost 20 different gray color tones) better than the contrast changes of an image or the distribution homogeneity of colors (Pierson and Adams, 1995). In the study, it has been aimed to develop a scoring scale, graded from 1 to 5, to identify different gray tones of the ultrasonographic images of the ovulation side uterine horn. Moreover, the pregnancy determination in cows and heifers has been tried to make by this scoring technique, in the first 20 days after artificial insemination.

MATERIALS and METHODS

All procedures involving study animals in the experiment were approved by Ankara University Animal Experiments Local Ethics Committee (No: 2021–20–180). A total of sixty Holstein cows (n:30) and heifers (n:30) which were determined as healthy according to general examination, body condition scoring, and reproductive examination were enrolled in the study. Animals were conducted in a presynch-ovsynch synchronization program and artificially inseminated (Giordano et al., 2016; Gordon et al., 2010). For each animal, two ultrasonographic images were taken (totally 1320 images) starting from the day of the artificial insemination (D0) until the 20th day (D20) on every other day. At the same time that images were taken, subjective scoring was made for all of the images. Then, the images were transferred and stored in a hard drive until the image analysis were done. The MGV values determined by ImageJ (ImageJ 1.53k; NIH, ABD) software. The obtained data at the end of the evaluations were analyzed in terms of Groups (cow or heifer), Sampling Time (alternate days between D0 and D20) and Pregnancy Status (pregnant or non-pregnant). Also score rates were given by one experienced (experienced on the reproductive ultrasonography more than 10 years) and one inexperienced (no experience on the reproductive ultrasonography) independent operators to determine scoring agreement level in terms of experience on the ultrasonographic examinations.

Ultrasound images were collected from the ovulation side uterine horn on the mentioned days by 6,5-9,5 mHz linear rectal probe attached to a portable B-mode ultrasonography device (CTS 800, SIUI, China). It was noted that all images were taken from the same area, with the same probe position, and maximum contrast provided as much as possible. Standard settings of the ultrasound device were used throughout the study. For each of the imaging procedure, probe was located on the dorsal cavity of the related region of the uterus on the dorsoventral position, and longitudinal images were taken. Images were determined as the regions including all of the endometrium and stratum vascularis layers in the hyperechoic serosa lines were in the image.

The gray tone of the uterus tissue in the images were scored between the grades of 1 and 5 at the same time the images were taken according to white point accumulation in the interested area (Figure 1).

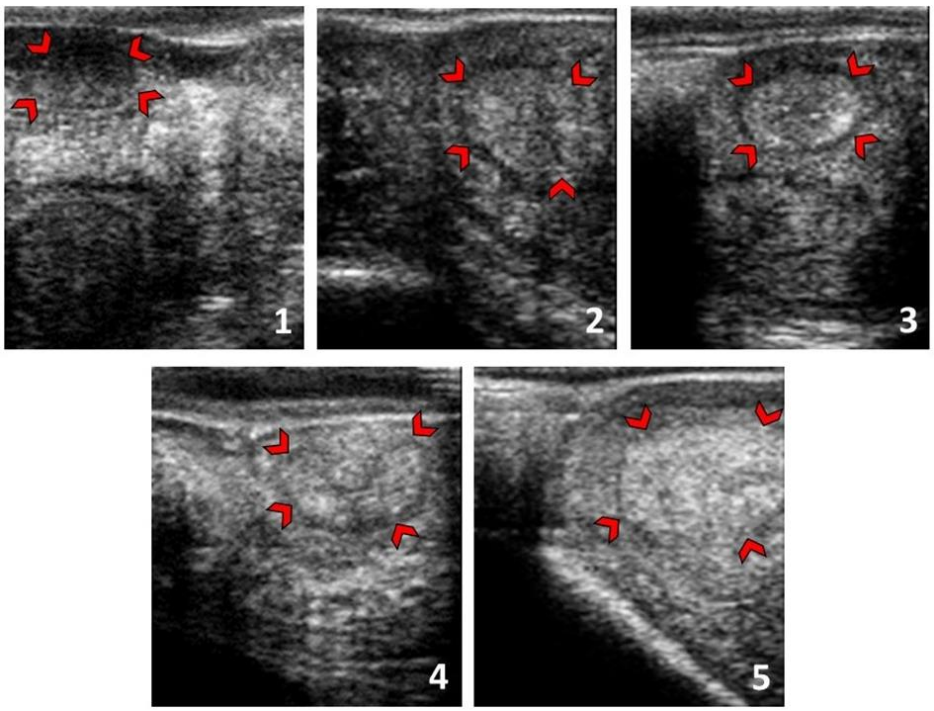

Figure 1: Ultrasonographic appearance of ovulation side uterine horn of heifers and cows. The red arrows heads in each image indicate the border of the uterus tissue that interested and scored by raters. The numbers in the lower corner of the right side of images indicate the Gray Tone Scores given for each sample. The gray tone of the uterus tissue in the images were scored according to white point accumulation in the interested area. Interested area was identified as including the entire endometrial tissue in the serosa layer for an image (same area with the ROI that was used in the MGV analysis). Score 1 represented almost black (0-20 % white point in interested area), Score 2 represented dark gray (20-40 % white point), Score 3 represented moderate gray (40-60 % white point), Score 4 represented light gray (60-80 % white point) and Score 5 represented almost white (80-100 % white point).

Interested area was the same area with the ROI that was used in the MGV analysis identified as including the entire endometrial tissue in the serosa layer for an image (Figure 2). Score 1 represented almost black (0-20 % white point in interested area), Score 2 represented dark gray (20-40 % white point), Score 3 represented moderate gray (40-60 % white point), Score 4 represented light gray (60-80 % white point) and Score 5 represented almost white (80-100 % white point). Scoring of the uterus was made by two independent raters (Rater 1 and Rater 2). Rater 1 was determined as an experienced rater when Rater 2 was enrolled as an inexperienced rater.

For MGV analysis ImageJ (ImageJ 1.53k; NIH, ABD) software was used. In the analysis, the whole region in each longitudinal view was evaluated. A single region of interest (ROI) was identified as including the entire endometrial tissue in the serosa layer for an image (Figure 2). The determined ROI's were analyzed objectively according to the MGV parameter (Scully vd., 2014).

Descriptive statistics for each variable were calculated and presented as "mean ± standard error of mean (SEM)". The variables were examined as parametric test assumptions. To test the effects of groups, pregnancy status, and sampling times, a linear mixed model for repeated measures was performed. Animals were included in the model as random effects, while group, pregnancy status, and sampling times were included as fixed effects. Besides, two-way and three-way interactions were also evaluated in the model. Variance components were used as the covariance structure in the established model since it resulted in the lowest Akaike information criterion (AIC). When a significant difference was obtained, all significant terms were compared using a simple effect analysis with Bonferroni correction. The model shown below was used for statistical analysis.

$$Y_{ijklmnoprs} = \mu + G_i + P_j + T_k + (G*P)_m + (G*T)_n + (P*T)_s + e_{ijklmnoprs}$$

Where, $Y_{ijklmnoprs}$, is the dependent variable; μ, is the overall mean; G_i, is the effect of the group (i= 2 classes; heifer and cow); P_j, is the effect of pregnancy status (j= 2 classes; pregnant and non-pregnant); T_k, is the effect of sampling time (k= 11 classes; Day 0 to 20); $(G*P)_m$, is the interaction of group and pregnancy status; $(G*T)_n$, is the interaction of group and sampling time; $(P*T)_s$, is the interaction of pregnancy status and sampling time; and $e_{ijklmnoprs}$, is the residual error.

For analysis of the gray tone scoring method descriptive statistics were calculated. The agreement level of the results obtained with the different raters was determined by calculating the inter-rater agreement (Cohen's kappa coefficient, $\varkappa$). Measure of agreement levels of $\varkappa < 0.20$, $0.20 < \varkappa \leq 0.40$, $0.40 <$

$\varkappa \leq 0.60$, $0.60 < \varkappa \leq 0.80$, and $\varkappa > 0.80$ indicated poor, fair, moderate, substantial and almost perfect agreement, respectively. Pearson correlation coefficient was performed to assess the correlation between MGV, Rater 1 scores, Rater 2 scores and median scores of raters. Differences with $p<0.05$ were considered statistically significant.

To test the differences of gray tone scores according to pregnancy status and sampling times, a linear mixed model for repeated measures was performed. Animals were included in the model as random effects, while pregnancy status, and sampling times were included as fixed effects. When a significant difference was obtained, all significant terms were compared using a simple effect analysis with Bonferroni correction. The model shown below was used for statistical analysis.

$$Y_{ijk} = \mu + P_i + S_j + (S*P)_k + e_{ijk}$$

Where; Y_{ijk}, is the dependent variable; μ, is the overall mean; P_i, is the effect of pregnancy status (j= 2 classes; pregnant and non-pregnant); S_j, is the effect of sampling time (k= 11 classes; Day 0 to 20); $(S*P)_k$, is the interaction of pregnancy status and sampling time ve e_{ijk}, is the residual error.

Statistical analyses were performed using IBM SPSS Statistics software Version 26.0.

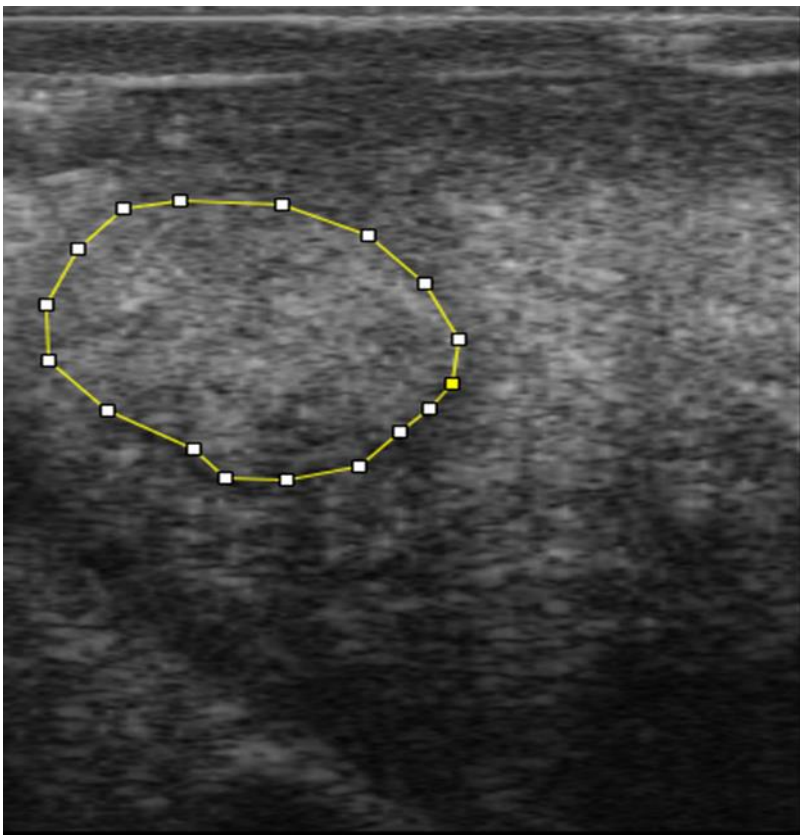

Figure 2: An example for determination of region of interest. A single ROI was identified as including the entire uterine tissue in the serosa layer for an image. The determined ROIs were analyzed according to the MGV.

RESULTS

Mean MGV values indicated statistically significant differences only between days of sampling ($p<0.05$). On the other hand, according to other main effects as Group and Pregnancy Status, statistical significance was not observed ($p>0.05$). Similarly, in the two- and three-way interactions were not concluded with statistically significant differences ($p>0.05$; Table 1).

In the analysis on scoring of the ultrasonographic images of uterus according to MGV values (Table 2), it has been determined that experienced (Rater 1) and inexperienced (Rater 2) raters have agreement ($p<0.001$) in poor level ($\varkappa=0.15$). Moreover, scores of the Rater 1 (0.720) were observed that had higher level correlations than Rater 2 (0.541; Table 3).

Pregnancy determination of cows and heifers by gray tone scoring was investigated by only according to the scores of Rater 1, because of poor agreement level ($\varkappa=0.15$; $p<0.001$) and high correlation coefficient (0.720; $p<0.001$). On the other hand, gray tone scoring analyses were conducted on according to Sampling Time and Pregnancy Status, independent from cow and heifer groups, in case there was no significant differences between cows and heifers according to MGV ($p>0.05$). It has been concluded that the mean of gray tone scoring were not differed significantly in terms of means and two-way interactions between sampling time and pregnancy status ($p>0.05$; Table 4).

Table 1. Means and interactions of MGV in pregnant (P) and non-pregnant (NP) cows and heifers evaluated from D0 to D20 (Mean ± SEM).

Sampling Time	Groups	
	Cow	Heifer
D0	94.25 ± 4.82	110.08 ± 6.13
D2	107.63 ± 4.64	100.72 ± 6.69
D4	96.01 ± 3.61	105.66 ± 4.5
D6	101.36 ± 3.22	95.32 ± 9.02
D8	103.45 ± 4.66	118.86 ± 5.23
D10	104.76 ± 4.57	118.11 ± 6.47
D12	107.43 ± 4.42	122.16 ± 10.47
D14	106.62 ± 3.47	111.51 ± 4.31
D16	106.64 ± 3.95	117.63 ± 4.81
D18	94.86 ± 3.62	96.02 ± 3.96
D20	98.43 ± 4.08	102.73 ± 6.5
Pregnancy Status		
Non-Pregnant	100.82 ± 1.41	107.72 ± 2.55
Pregnant	104.82 ± 2.63	111.74 ± 2.48
p		
Group (G)	0.138	
Sampling Time (S)	0.006	
Pregnancy Status (P)	0.342	
G*S	0.324	
G*P	0.982	
S*P	0.191	
G*S*P	0.941	

G: Group; **S:** Sampling Time; **P:** Pregnancy Status **G*S:** Interaction between G and S; **G*P:** Interaction between G and P; **S*P:** Interaction between S and P **G*S*P:** Interactions between G, S, and P

Table 2. Measurements of agreement level of two different and independent raters (Rater 1 and Rater 2) on gray tone scoring of ultrasonographic images of the ovulation side uterine horn.

			Rater 2 (Inexperienced)					Total
			Score 1	Score 2	Score 3	Score 4	Score 5	
Rater 1 (Experienced)	Score 1	N	29	24	5	0	0	58
		% Rater 1	50.0%	41.4%	8.6%	0.0%	0.0%	100.0%
		% Rater 2	19.6%	5.3%	0.9%	0.0%	0.0%	3.6%
	Score 2	N	77	214	178	69	8	546
		% Rater 1	14.1%	39.2%	32.6%	12.6%	1.5%	100.0%
		% Rater 2	52.0%	47.5%	33.8%	16.8%	10.3%	33.8%
	Score 3	N	35	175	297	239	32	778
		% Rater 1	4.5%	22.5%	38.2%	30.7%	4.1%	100.0%
		% Rater 2	23.6%	38.8%	56.4%	58.2%	41.0%	48.2%
	Score 4	N	7	38	47	102	38	232
		% Rater 1	3.0%	16.4%	20.3%	44.0%	16.4%	100.0%
		% Rater 2	4.7%	8.4%	8.9%	24.8%	48.7%	14.4%
	Score 5	N	0	0	0	1	0	1
		% Rater 1	0.0%	0.0%	0.0%	100.0%	0.0%	100.0%
		% Rater 2	0.0%	0.0%	0.0%	0.2%	0.0%	0.1%
Total		N	148	451	527	411	78	1615
		% Rater 1	9.2%	27.9%	32.6%	25.4%	4.8%	100.0%
		% Rater 2	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

$\varkappa$ = 0.15 ; p<0.001

Table 3. Correlation analysis of two different and independent raters (Rater 1 and Rater 2) on gray tone scoring of ultrasonographic images of the uterus according to MGV.

	MGV	Rater 1 (Experienced)	Rater 2 (Inexperienced)
MGV	1	0.720***	0.541***

***. Correlation is significant at the 0.001 level (2-tailed).

Table 4. Means and interactions of Gray Tone Scoring in pregnant (P) and non-pregnant (NP) cows and heifers evaluated from D0 to D20 (Mean ± SEM).

Sampling Time	Pregnancy Status	
	NP	P
D0	2.28 ± 0.12	2.48 ± 0.16
D2	2.25 ± 0.17	2.29 ± 0.19
D4	2.62 ± 0.17	2.19 ± 0.18
D6	2.18 ± 0.14	1.80 ± 0.20
D8	2.41 ± 0.16	2.44 ± 0.14
D10	2.38 ± 0.17	2.12 ± 0.18
D12	2.44 ± 0.17	2.15 ± 0.30
D14	2.31 ± 0.14	2.43 ± 0.18
D16	2.16 ± 0.14	2.17 ± 0.15
D18	1.89 ± 0.14	1.84 ± 0.22
D20	1.85 ± 0.15	2.00 ± 0.24
p		
Sampling Time (S)	0.065	
Pregnancy Status (P)	0.311	
S*P	0.623	

S: Sampling Time; **P:** Pregnancy Status; **S*P:** Interaction between S and P

DISCUSSION

Detailed observation of the reproductive organs and evaluation of the structural differences in cows have been possible since the ultrasonographic imaging was integrated into veterinary medicine (Pierson and Ginther, 1984). The variations in the structure of the sonographic images occur as a result of different reproductive events such as estrous cycle, pregnancy, etc. Therefore, these events are observed as different echogenicity because of different level of steroid hormones to which the endometrium is exposed (Schmauder et al., 2008; Vollmerhaus, 1958). Different echogenicities of a B-Mode ultrasonograpcih image could be defined as different gray tones by the human eye that could identify almost 20 different gray tones (Pierson and Adams, 1995). It has been hypothesized that variations in the MGV of the B-Mode ultrasonographic images of the ovulation side uterine horn can be detected by human eye in a 5 graded (1-5) scale and pregnancy can be diagnosed with this scoring scale in the first 20 days after artificial insemination. According to the hypotheses, the aim of the study conducted was to discriminate the gray tones of the B-Mode ultrasonographic images of the endometrial tissue of the Holstein cows and heifers in a 5 graded scoring scale, and to identify pregnancies in terms of the subjective scoring method in the first 20 days after artificial insemination.

Scully et al. (2014) examined the sonographic images of uterus in cows between the 18th and 21st days following artificial insemination, and they reported that the only uterine echogenicity could not be a pregnancy determining parameter. In a similar study performed in heifers (Scully et al., 2015), uterine echogenicity of pregnant, non-pregnant (inseminated) and cyclic (non-inseminated) heifers was evaluated on the 7th, 11th, 14th, 16th, 18th days following ovulation. Although there were significant differences between cyclic and inseminated heifers, it was concluded that there was no difference in uterine echogenicity between heifers that were pregnant and non-pregnant after insemination. Similarly, in a study in which the ultrasonographic image of the uterus was evaluated by the Optic Density Image (ODI) analysis method, which is based on the determination of the white spot density in the image (Cengiz et al., 2017),

any significant difference was not found between the ODI values in pregnant and non-pregnant animals. In the present study, the MGV analysis has been concluded with similar results to the previous studies. According to these results, MGV of the ultrasonographic appearance of the ovulation side uterine horn of cows and heifers did not differ in terms of parturition history or present pregnancy status, but only the day of the images were taken. It could be though that the exposure of steroid hormones caused the similar differences in endometrial echotexture in cows and heifers in the first 20 days after artificial insemination.

To the best of knowledge, in reproductive medicine for cows any subjective scoring method has not been developed for using in pregnancy determination, yet. On the contrary, in mares, ultrasonographic examinations of the changes in the uterus can be detected subjectively by unique cart wheel structure. Thanks to the structural properties, endometrial folds change under effects of steriod hormones, which vary in the estrus cycle. Also, there are 4 (0-3) graded (McCue et al., 2011) and 6 (0-5) graded (Samper, 2010) subjective scoring methods for mare uterus, based on various echotexture and appearance of the endometrial folds. Moreover, these scoring methods is used to predict pregnancy in mares. In the present study, it has been tried to reveal a novel subjective scoring approach to evaluate the ultrasonographic examination of the endometrial tissue of cows and heifers. Totally 1320 B-Mode ultrasonographic images have been evaluated in a 5 graded scoring scale that has been conducted on the different tones of gray, and compared to the MGV values. It has been concluded that the MGV values could be detected by this novel scoring method. Also, scores that have been rated by experienced practitioner have been found more consequent than the inexperienced practitioner. However, the gray tone scoring method has been resulted as insufficient on prediction or definition of the pregnancy in cows and heifers in the first 20 days after artificial insemination. The fact that the computer assisted MGV has not been succeed on the identifying the pregnancy could indicated the insufficient results obtained from 5 graded gray tone scoring method.

CONCLUSION

In conclusion, it has been observed that the 256 different shades of gray (MGV) which is analyzed by computer assisted image analysis could be identifed by human eye in a 5 graded scale. On the other hand, experienced practitioners might detected the different tones of gray of the endometrial tissue in cows and heifers more coefficiently than inexperienced ones. However, the pregnancy in cows and heifers in the first 20 days could not be predicted or diagnosed by neighter computer assissted nor subjective methods in terms of the echotexture of endometrial tissue. According to the results, the endometrium of the ovulation side uterine horn reflects the same differentiation in the presence or absence of pregnancy, in the first 20 days. Otherwise, the success of the gray tone scoring method in identifying MGV changes could be used to evaluate different physiological and pathological conditions such as estrus cycle, ovulations time, uterine soundness, subclinical endometritis, ovarian cysts, etc. in further studies.

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

Authors' Contributions: KTO contributed to the project idea, design and execution of the study, acquisition and analysed the data, drafted, wrote, edited and finalized the manuscript.

Ethical approval: All procedures applied on animals in the study were allowed by Ankara University Animal Experiments Local Ethics Committee (No: 2021-20-180).

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REFERENCES

Abdalla, H., Salem, S. E., Elsohaby, I., & Swelum, A. A. (2021). Likelihood of pregnancy in cows identified with different amounts of anechoic intrauterine fluid at the time of insemination. Animal Reproduction Science, 226, 106688.

Camela, E. S., Nociti, R. P., Santos, V. J., Macente, B. I., Murawski, M., Vicente, W. R., Bartlewski, P. M., & Oliveira, M. E. F. (2019). Changes in testicular size, echotexture, and arterial blood flow associated with the attainment of puberty in Dorper rams raised in a subtropical climate. Reproduction in Domestic Animals, 54, 131-137.

Cengiz, M., Çolak, A., Hayırlı, A., & Cannazik, O. (2017). Optical density changes in ultrasonographic images of the endometrium and corpus luteum in pregnant and cyclic cows. Turkish Journal of Veterinary and Animal Science, 41, 18-24.

Cengiz, M., Kanca, H., Salar, S., Bastan, A., Kucukaslan, I., Alkan, H., Karakas, K., Yuksel, O., & Hayırlı H. (2014). Endometrial echotexture parameters in Turkish Saanen Goats (Akkeci) during oestrus and early pregnancy. Animal Reproduction Science, 146, 27-33.

Dietrich, C. F., Wehrmann, T., Zeuzem, S., Braden, B., Caspary, W. F., & Lembcke, B. (1999). Analysis of hepatic echo patterns in chronic hepatitis C. Ultraschall in der Medizin 20(1), 9-14.

Giordano, J. O., Thomas, M. J., Catucuamba, G., Curler, M. D., Wijma, R., Stangaferro, M. L., & Masello, M. (2016). Effect of extending the interval from Presynch to initiation of Ovsynch in a Presynch-Ovsynch protocol on fertility of timed artificial insemination services in lactating dairy cows. Journal of Dairy Science, 99(1), 746-757.

Gordon, M. B., Dinn, N., & Rajamahendran, R. (2010). Effects of presynchronization and postinsemination treatments on pregnancy rates to a timed breeding Ovsynch protocol in dairy cows and heifers. Canadian Journal of Animal Science, 90(1), 35-44.

Holm, D. E., Nielen, M., Jorritsma, R., Irons, P. C., & Thompson, P. N. (2015). Evaluation of pre-breeding reproductive tract scoring as a predictor of long term reproductive performance in beef heifers. Preventive Veterinary Medicine, 118, 56–63.

Kauffold, J., von dem Bussche, B., Failing, K., Wehrend, A., & Wendt, M. (2010). Use of B-mode ultrasound and grey-scale analysis to study uterine echogenicity in the pig. Journal of Reproduction and Development, 56, 444-448.

Lee, S. H., Lee, S. H., Chan, V. W., Lee, J. O., & Kim, H. I. (2011). Echotexture and correlated histologic analysis of peripheral nerves important in regional anesthesia. Regional Anesthesia & Pain Medicine, 36(4), 382-386.

Martinoli, C., Derchi, L. E., Pastorino, C., Bertolotto, M., & Silvestri, E. (1993). Analysis of echotexture of tendons with US. Radiology, 186(3), 839-843.

McCue, P. M., Scoggin, C. F., & Lindhom, A. R. G. (2011). Estrus. In: Equine Reproduction. (McKinnon, A. O., E. L. Squires, W. E. Vaala, D. D. Varner, Eds.), WilleyBlackwell, Oxford, UK, pp. 1716-1727.

McKinnon, A. O., & Carnevale, E. M. (1993). Ultrasonography. In: Equine Reproduction. (McKinnon, A.O., J. L. Voss, Eds.), Lea & Febiger, Malvern, Pennsylvania, pp. 211-220.

Mee, J. F., Buckley, F., Ryan, D., & Dillon, P. (2009). Pre-breeding ovaro-uterine ultrasonography and its relationship with first service pregnancy rate in seasonal-calving dairy herds. Reproduction in Domestic Animals, 44, 331–337.

Pierson, R. A., & Adams, G. P. (1995). Computer-assisted image analysis, diagnostic ultrasonography and ovulation induction: strange bedfellows. Theriogenology, 43(1), 105-112.

Pierson, R. A., & Ginther, O. J. (1984). Ultrasonography for detection of pregnancy and study of embryonic development in heifers. Theriogenology, 22, 225–233.

Ribadu, A. Y., & Nakao, T. (1999). Bovine reproductive ultrasonography: a review. Journal of Reproduction and Development, 45(1), 13-28.

Samper, J. C. (2010). A review of a practitioner's perspective on endometrial edema. Pferdehelkunde 26, 14-18.

Sarwal, A., Parry, S. M., Berry, M. J., Hsu, F. C., Lewis, M. T., Justus, N. W., Morris, P. E., Denehy, L., Berney, S., Dhar, S., & Cartwright, M. S. (2015). Interobserver reliability of quantitative muscle sonographic analysis in the critically ill population. Journal of Ultrasound in Medicine, 34(7), 1191-1200.

Schmauder, S., Weber, F., Kiossis, E., & Bollwein H. (2008). Cyclic changes in endometrial echotexture of cows using a computer-assisted program for the analysis of first-and second-order grey level statistics of B-Mode ultrasound images. Animal Reproduction Science, 106, 153-161.

Scully, S., Butler, S. T., Kelly, A. K., Evans, A. C. O., Lonergan, P., & Crowe, M. A. (2014). Early pregnancy diagnosis on days 18 to 21 postinsemination using high-resolution imaging in lactating dairy cows. Journal of Dairy Science, 97, 3542-3557.

Scully, S., Evans, A., Carter, F., Duffy, P., Lonergan, P., & Crowe, M. (2015). Ultrasound monitoring of blood flow and echotexture of the corpus luteum and uterus during early pregnancy of beef heifers. Theriogenology, 83, 449-458.

Souza, A. H., Silva, E. P., Cunha, A. P., Gumen, A., Ayres, H., Brusveen, D. J., Guenther, J. N., & Wiltbank, M. C. (2011). Ultrasonographic evaluation of endometrial thickness near timed AI as a predictor of fertility in high-producing dairy cows. Theriogenology, 75, 722–733.

Stevenson, J. L., Rodrigues, J. A., Braga, F. A., Bitente, S., Dalton, J. C., Santos, J. E., & Chebel, R. C. (2008). Effect of breeding protocols and reproductive tract score on reproductive performance of dairy heifers and economic outcome of breeding programs. Journal of Dairy Science, 91, 3424–3438.

Themistokleous, K. S., Sakellariou, N., & Kiossis, E. (2022). A deep learning algorithm predicts milk yield and production stage of dairy cows utilizing ultrasound echotexture analysis of the mammary gland. Computers and Electronics in Agriculture, 198, 106992.

Vasconcelos, J. L. M., Sartori, R., Oliveira, H.N., Guenther, J. G., & Wiltbank, M. C. (2001). Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. Theriogenology, 56, 307-314.

Vollmerhaus, B. (1958). Die zyklischen Veranderungen des Endometriums beim Rind. Deutsche tierärztliche Wochenschrift, 65, 461–465.

Zagzebski, J. A. (1996). Essentials of ultrasound physics. Mosby, St. Louis.

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RESEARCH ARTICLE

Investigation on Antimicrobial Resistance Levels of *Escherichia coli* Strains Isolated from Bovine Fecal Samples and Comparison with Guidelines of the Clinical Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST)

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ABSTRACT

This study was carried out to investigate the antibiotic resistance levels of *Escherichia coli* isolates of bovine origin and to compare the results with CLSI and EUCAST guideline values. For this purpose, 97 *E. coli* strains isolated from fecal samples of cattle in 12 different farms were tested against 32 antibiotics by using the disk diffusion method. The zone diameters of 13 antibiotics examined within the scope of the study were compared according to the CLSI and EUCAST 2020 guidelines, and their consistency levels were evaluated statistically. The highest resistance rates in *E. coli* isolates were determined against tetracycline (68%), streptomycin (63.9%), ampicillin (58.8%), and doxycycline (50.5%) antibiotics. On the other hand, the isolates were found to be highly susceptible to amikacin and cephalosporin group antibiotics. When CLSI and EUCAST guidelines were compared, it was found that there were statistically significant differences between the resistance rates of nitrofurantoin, gentamicin, and amikacin. Only 10 (10.3%) of the isolates were detected to be susceptible to all the antibiotics tested, whereas 17.5% were resistant to 10 or more antibiotics. The results of this study showed that *E. coli* isolates of bovine origin were highly resistant against antibiotics used in the field for a long period, especially the number of isolates with multiple antibiotic resistance was striking. It was concluded that due to substantial inconsistencies between the CLSI and EUCAST guidelines for some antibiotics such as amikacin, nitrofurantoin, and gentamicin, there is an urgent need to execute necessary updates in both guidelines.

Keywords: Antibiotic resistance, Bovine, CLSI, *Escherichia coli*, EUCAST

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Sığır Dışkı Örneklerinden İzole Edilen *Escherichia coli* Suşlarının Antimikrobiyal Direnç Düzeylerinin Araştırılması ve Klinik Laboratuvar Standartları Enstitüsü (CLSI) ve Avrupa Antimikrobiyal Duyarlılık Testi Komitesi (EUCAST) Kılavuzlarıyla Karşılaştırılması

ÖZ

Bu çalışma, sığır orijinli *Escherichia coli* izolatlarının antibiyotik direnç düzeylerinin araştırılması ve sonuçların CLSI ve EUCAST kılavuz değerleri ile karşılaştırılması amacıyla yapılmıştır. Bu amaçla 12 farklı işletmedeki sığırların dışkı örneklerinden izole edilen 97 *E. coli* suşu, disk difüzyon yöntemi kullanılarak 32 antibiyotiğe karşı test edilmiştir. Çalışma kapsamında incelenen 13 antibiyotiğin zon çapları CLSI ve EUCAST 2020 kılavuzlarına göre karşılaştırıldı ve tutarlılık düzeyleri istatistiksel olarak değerlendirildi. *E. coli* izolatlarında en yüksek direnç oranları tetrasiklin (%68), streptomisin (%63,9), ampisilin (%58,8) ve doksisiklin (%50,5) antibiyotiklerine karşı saptanmıştır. Öte yandan, izolatların amikasin ve sefalosporin grubu antibiyotiklere karşı yüksek duyarlı olduğu saptanmıştır. CLSI ve EUCAST kılavuzları karşılaştırıldığında nitrofurantoin, gentamisin ve amikasin direnç oranları arasında istatistiksel olarak anlamlı farklar olduğu görüldü. İzolatların sadece 10'unun (%10,3) test edilen tüm antibiyotiklere duyarlı olduğu, %17,5'inin ise 10 ve daha fazla antibiyotiğe dirençli olduğu saptandı. Bu çalışmanın sonuçları sığır orijinli *E. coli* izolatlarının uzun süredir sahada kullanılan antibiyotiklere karşı oldukça dirençli olduğunu göstermiştir, özellikle çoklu antibiyotik direncine sahip izolatların sayısı dikkat çekicidir. Amikasin, nitrofurantoin ve gentamisin gibi bazı antibiyotikler için CLSI ve EUCAST kılavuzları arasındaki önemli tutarsızlıklar nedeniyle, her iki kılavuzda da gerekli güncellemelerin acilen yapılması gerektiği sonucuna varılmıştır.

Anahtar Kelimeler: Antibiyotik direnci, CLSI, *Escherichia coli*, EUCAST, Sığır

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INTRODUCTION

Increasing resistance to antibiotics used in the control and treatment of bacterial infections in humans and animals has become a global problem threatening public health. Unfortunately, the increase in antimicrobial resistance makes it difficult to control bacterial infections and leads many drugs available in the market to lose their effectiveness (Prestinaci et al. 2015). Antibiotics used widely for the control and treatment of diseases in food animals have an important role in the emergence of resistance as they can be transferred to humans through food chain (Vidovic and Vidovic 2020). Also, antibiotic resistance genes in the intestinal bacteria of animals can pass to humans by direct contact with animal or through animal products. The number and diversity of antibiotic resistant genes may be increased in parallel with the increase in the number of resistant bacteria (Liu et al. 2016). The shedding of bacteria carrying resistance genes with feces of animals and humans makes it possible to transfer these genes to non-resistant microorganisms in the environment. Fecal contamination of surface waters, rivers, wetlands and even drinking waters plays an important role in the spread of this resistance (Bengtsson-Palme et al. 2018).

Escherichia coli (*E. coli*), one of the most important members of the normal intestinal microbiota, plays a significant role in the contamination of environment with human and animal feces (Odonkor and Ampofo 2013). Although *E. coli* can be found as harmless and commensal microorganism in the gastrointestinal system of humans and animals, it may cause many important intestinal and extraintestinal infections (Stromberg et al. 2018). The World Health Organization (WHO) has described *E. coli* among nine pathogens that are recognized globally as the most worrying in terms of antibiotic resistance (WHO, 2014). *E. coli* is thought to be an excellent tool for monitoring antimicrobial resistance in food and environmental samples, due to the fact that it acquires antimicrobial resistance readily and has a very broad host spectrum. In addition, changes in antibiotic resistance in *E. coli* are considered as an early warning of the possibility of developing resistance in other pathogenic bacteria and therefore this agent has been used as an indicator (van den Bogaard and Stobberingh 2000). Although *E. coli* is naturally susceptible to many antibiotics used in the market, it can take resistance genes from other bacteria through horizontal gene transfer, as well as transfer its resistance genes to other bacteria in the environment (Blake et al. 2003). For this reason, antimicrobial resistance in *E. coli* is considered as one of the major concerns for both human and animal healthcare. The increase in the prevalence of multiple antibiotic resistant *E. coli*, especially in the last 20 years, has reached important levels which puts forward severity of the problem. Therefore, determination of antimicrobial resistance in *E. coli* is of great importance in terms of developing effective measures to tackle this problem.

Molecular detection of resistance genes and phenotypic methods such as antibiogram tests are mostly used for the examination of antimicrobial resistance levels. Disc-diffusion method, which allows to test many antibiotics at the same time, is one of the most frequently used phenotypic tests in clinical microbiology laboratories (Boyen et al. 2010). The two most popular guidelines used worldwide in evaluating disk diffusion test results are the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and The Clinical Laboratory Standards Institute (CLSI). However, studies have reported that there are inconsistencies between EUCAST and CLSI standards in evaluating antibiotic susceptibilities. (Kassim et al. 2016, Sánchez-Bautista et al. 2018). Therefore, revised guidelines have been published since 2014 to regulate the clinical limit values of both guidelines.

This study was carried out to investigate the levels of antimicrobial resistance in *E. coli* field strains isolated from fecal samples of clinically healthy cattle (ages were 1-5) located in Bingol province and the surrounding areas. The results obtained here were evaluated by comparing with the criteria announced in EUCAST and CLSI guidelines.

MATERIALS AND METHODS

Bacteria strains

A total of 97 *E. coli* strains isolated from fecal samples collected from 12 different cattle farms located in Bingol province and its surrounding were used in the study. Fecal samples (received directly from the rectum or after fresh defecation) were taken in sterile stool collection container and transported immediately to the laboratories under cool conditions (at 4 °C) for culture. The samples (1 g) were aseptically transferred to 9 ml of Tryptic Soy Broth and were then incubated at 37°C for 18-24 h under aerobic atmosphere for pre enrichment. Following pre-enrichment, a loopful of broth was inoculated onto MacConkey Agar (Merck, 105465) and Eosin Methylene Blue Agar (Merck, 101347) and, was incubated under the same conditions. Isolates identified as *E. coli* by conventional methods were also confirmed by Polymerase Chain Reaction (PCR) using a pair of primers specific to this species (Acik et al. 2004).

Antimicrobial susceptibility tests

Antimicrobial susceptibility tests were conducted by Kirby-Bauer disc diffusion method according to the 2020 guidelines published by the Clinical and Laboratory Standards Institute (CLSI, 2020). Before the antimicrobial susceptibility tests, all the isolates

were cultivated in Mueller-Hinton Agar (HIMEDIA, M173) and incubated at 37 °C for 16-18 h under aerobic conditions. *E. coli* isolates were suspended with physiological saline and adjusted to 0.5 McFarland standard by densitometer. Then, the suspension was poured onto Mueller-Hinton Agar and spread over the entire surface with the help of a sterile swab. All the isolates were tested by Disc diffusion method against 32 different antibiotics listed in Table 1 and Figures 2 and 3.

Since the zone diameter ranges for 13 antibiotics listed in Table 1 were included in both EUCAST and CLSI guidelines, only these antibiotics were evaluated comparatively (CLSI, 2020; EUCAST, 2020). Because the zone diameters of nine antibiotics presented in Figure 1 were only included in the CLSI guideline, the results of these antibiotics were evaluated according to CLSI criteria, but the comparison with EUCAST data was not possible. On the other hand, due to the absence of zone diameters in both CLSI and EUCAST guidelines for 10 antibiotics listed in Figure 2, no sensitivity assessment was made. Nevertheless, evaluation was carried out by dividing them into four groups according to the zone parameters as 10 mm and below, 11-15 mm, 16-20 mm and 21 mm and above.

Statistical Analyses

Data were analyzed with SPSS (Statistical Package for the Social Sciences) Version 22.0. The zone diameter breakpoints were determined according to both CLSI 2020 and EUCAST 2020 guidelines to categorize them as either susceptible, intermediate or resistant. Cohen's Kappa statistics were conducted to determine the proportion of agreement over and above chance between CLSI 2020 and EUCAST 2020 guidelines. The p-value less than 0.05 indicated that the agreement was significantly different from "0" and it was not due to chance.

RESULTS

The highest antibiotic resistance rate was obtained against tetracycline (68%), which was followed by streptomycin (63.9%), ampicillin (58.8%) and doxycycline (50.5%) in the evaluation of susceptibility test findings of 97 *E. coli* isolates to 22 antimicrobial drugs by taking the CLSI 2020 guideline values into consideration. On the other hand, the isolates were found to be highly susceptible to amikacin and cephalosprin group antibiotics (cefaperazone, ceftazidime, cefepime) (Table 1, Fig. 1). When CLSI and EUCAST guidelines were compared, statistically significant differences were found between the resistance rates against nitrofurantoin, gentamicin and amikacin antibiotics (Table 1). The kappa value for ampicillin was measured as 1, while the kappa values for cefepime, cefocyte, ciprofloxacin, levofloxacin and chloramphenicol were above 0.9 (Table 1). Only 10 (10.3%) of the isolates were determined to be susceptible to all the antibiotics tested, whereas 17.5% were found to be resistant to 10 or more antibiotics. Meanwhile, one isolate was noticed to show resistance to 17 of 22 antibiotics tested (Fig. 3).

Among 10 antibiotics, the zone diameters of which were not provided in the CLSI and EUCAST guidelines, the percentage of isolates producing a zone diameter of 21 mm and above against enrofloxacin was 77.3%. On the other hand, a zone diameter of 10 mm and below was obtained in 94.8% of the isolates against tilmicosin (Fig. 2).

Table 1. Resistance rates of *E. coli* isolates to various antibiotics, concordance and kappa statistical data between the CLSI and EUCAST guideline data.
Tablo 1. *E. coli* izolatlarının çeşitli antibiyotiklere direnç oranları, CLSI ve EUCAST kılavuz verileri arasındaki uyum ve kappa istatistiksel verileri

	CLSI (%) n = 97			EUCAST (%) n = 97			Concordance (%)	Kappa, (95 % CI)
	R	I	S	R	I	S		
Ampicillin	58.76	0.00	41.24	58.76	0.00	41.24	100.00	1
Amoxicillin Clav.	24.74	17.53	57.73	35.05	0.00	64.95	82.47	0.674 (0.566-0.762)
Cefepime	9.28	5.15	85.57	11.34	3.09	85.57	97.94	0.919 (0.759-1.000)
Ceftazidime	8.25	2.06	89.69	9.28	4.12	86.60	96.91	0.806 (0.628-0.906)
Cefoxitin	21.65	1.03	77.32	22.68	0.00	77.32	98.97	0.971 (0.902-1.000)
Ciprofloxacin	13.40	10.31	76.29	23.71	3.09	73.20	98.97	0.975 (0.910-1.000)
Levofloxacin	17.53	5.15	77.32	22.68	6.19	71.13	96.91	0.926 (0.827-1.000)
Norfloxacin	16.49	4.12	79.38	25.77	0.00	74.23	90.72	0.748 (0.624-0.900)
Amikacin	6.19	8.25	85.57	28.87	0.00	71.13	77.32	0.393 (0.247-0.523)
Gentamicin	16.49	1.03	82.47	47.42	0.00	52.58	69.07	0.366 (0.224-0.487)
Chloramphenicol	44.33	0.00	55.67	45.36	0.00	54.64	98.97	0.979 (0.930-1.000)
Nitrofurantoin	14.43	16.49	69.07	8.25	0.00	91.75	77.32	0.360 (0.140-0.584)
Trimethoprim-Sulfa.	42.27	2.06	55.67	42.27	0.00	57.73	97.94	0.959 (0.913-1.000)

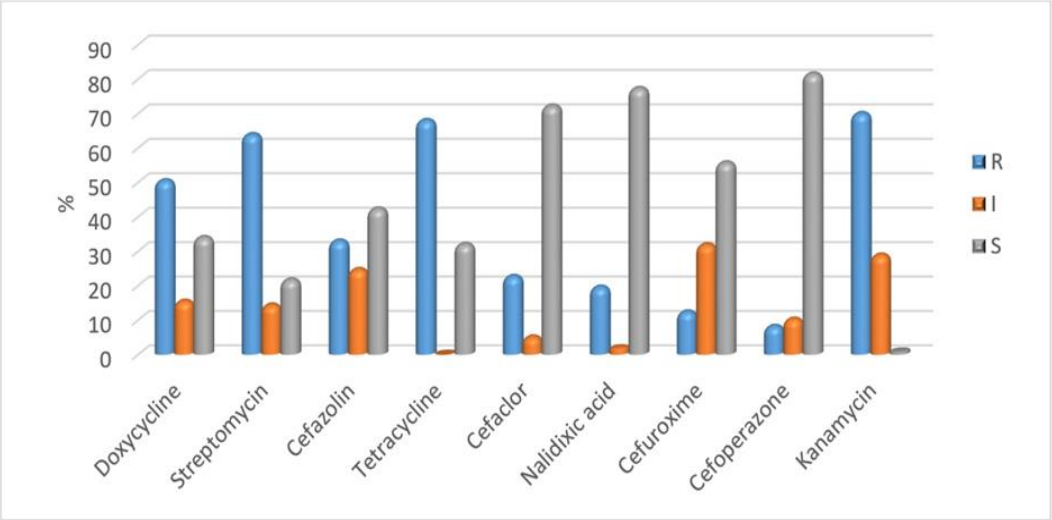

Figure 1: Resistance rates of *E. coli* isolates to various antibiotics according to CLSI 2020 guidelines
Şekil 1: CLSI 2020 kılavuzuna göre *E. coli* izolatlarının çeşitli antibiyotiklere direnç oranları

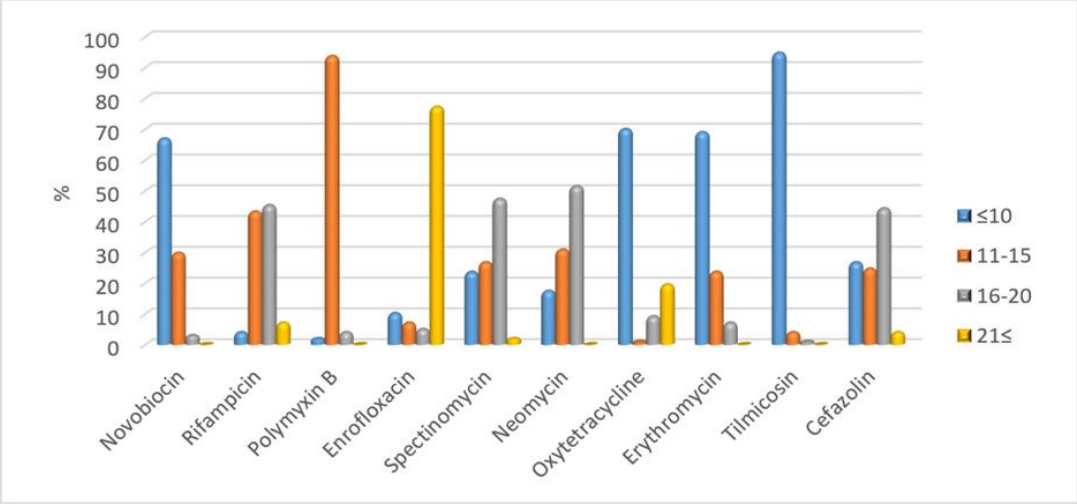

Figure 2: Zone diameter ranges of antibiotics that were not available in the CLSI and EUCAST 2020 guidelines
Şekil 2: CLSI ve EUCAST 2020 kılavuzlarında bulunmayan antibiyotiklerin zon çapı aralıkları

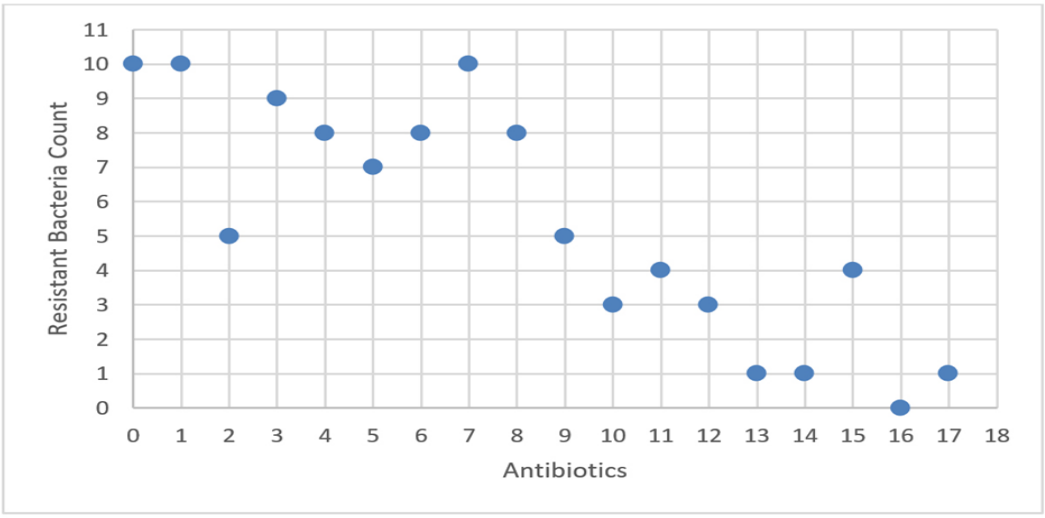

Figure 3: Number of *E. coli* isolates showing multiple antibiotic resistance
Şekil 3: Çoklu antibiyotik direnci gösteren *E. coli* izolatlarının sayısı

DISCUSSION

In this study, the most common antibiotic resistance in *E. coli* isolates was obtained against tetracycline, streptomycin and ampicillin, respectively. It should be underlined that these three antibiotics were the most preferably used antibiotics for the treatment of animal diseases in the field by both veterinarians and animal owners. Similar results concerning these antibiotics have also been reported in studies carried out in different parts of the world (Yassin et al. 2017, Aasmäe et al. 2019). High resistance rates against these antibiotics are not surprising thank to the common and long term use in animals for either treatment of prophylactic purposes in many countries. Due to the widespread and long-term use of these antibiotics for prophylactic purposes in animals in many countries, high resistance is expected. Tetracycline, which was approved for use in 1948 and used extensively for treatment and/or as feed additive, has an important place among these antibiotics. In many studies conducted in different countries, the resistance rate against tetracyclines has been reported to be rather high (Boireau et al. 2018, Aasmäe et al. 2019). In a previous study conducted to compare resistance levels in *E. coli* isolates between 2002 and 2015, an average resistance rate of 73% has been reported against tetracycline among the isolates of bovine origin and it was noted that this resistance showed a horizontal course over the years (Boireau et al. 2018). Also, it is possible that *E. coli* isolates resistant to tetracycline can generate resistance against other antimicrobial agents over time (Tadesse et al. 2012, Shin et al. 2015). A previous study has reported that 62.5% of tetracycline resistant isolates also showed multiple antibiotic resistance (Shin et al. 2015). Also, the presence of a mutual resistance has been showed between tetracycline and several antibiotics such as nalidixic acid, trimethoprim-sulfate, ampicillin and streptomycin (Tadesse et al. 2012). When the isolates resistant against 10 or more antibiotics were evaluated in this study; it was determined that all of the isolates resistant to tetracycline were also resistant to streptomycin and 94% showed resistance to ampicillin.

The resistance rate of *E. coli* to aminoglycosides shows variability according to animal species and the frequency of field use. While streptomycin resistance was reported in 68% of poultry isolates, it was detected in 18% of cattle isolates (Yassin et al. 2017). In another study carried out by Lim et al. (2010), the percentage of streptomycin resistance was found as 80.7% in isolates obtained from diarrheic cattle feces, whereas only 1.7% resistance was noted against amikacin. On the other hand, White et al. (2000) could not detect a resistance against amikacin in *E. coli* strains isolated from cattle with diarrhea. In this study, while a high resistance rate (64%) was detected against streptomycin, it was found to be as low as 6% against amikacin. The reasons for low level of resistance against amikacin might be that this antibiotic is not preferred in the treatment of animals and also it is a new generation antibiotic.

In this study, a relatively low rate of resistance was detected against cephalosporins, which also are not used frequently in animals. A total of six antibiotics from first to fourth generation of cephalosporin were evaluated in the study, and the resistance rates against first and second generation antibiotics were found to be higher than those in the third and fourth generation antibiotics. These findings showed the presence of higher resistance to antibiotics which were being used long before in the treatment of diseases. It is believed that the third and fourth generation cephalosporins were relatively new compared to others and the low rate of use in animal treatment may have prevented the development of resistance. However, it should not be neglected that the transfer of isolates resistant to cephalosporins with both environmental samples and food may lead to increased resistance against these antibiotics in humans. Therefore, observing and monitoring cephalosporin resistance in the field is thought to be important for human health.

A considerable resistance rate (44%) was obtained against chloramphenicol in the present study. The resistance rate against this antibiotic has been reported to range from 8% to 90% in *E. coli* isolates of bovine origin in previous studies conducted elsewhere in the world (White et al. 2000, Lim et al. 2010, Yassin et al. 2017). Remarkably high resistance rates against chloramphenicol were surprising due to the fact that its use in animals has been prohibited in Turkey, as well as many other countries. This suggests that the drug in question continues to be used by animal owners despite the ban. However, some studies have revealed that florphenicol, which is in the same group as chloramphenicol and used in the treatment of respiratory diseases of cattle, may play a role in chloramphenicol resistance (White et al. 2000). It has been reported that florphenicol resistance mediated by Flo gene provided enzymatic cross-resistance to chloramphenicol and has recently played an important role in the increased resistance (Tadesse et al. 2012). Although the above mentioned factors might have led to chloramphenicol resistance, it is thought that resistance mechanisms developed against other antibiotics could also have contributed to the occurrence of resistance against chloramphenicol in nearly half of the isolates tested in this study. Nevertheless, future studies are needed to reveal the factors likely to play a role in chloramphenicol resistance.

Quinolone group antibiotics are frequently used by animal owners and veterinarians in the treatment of gastrointestinal and respiratory diseases in animals. In recent years, a significant increase in resistance to quinolone group antibiotics has been observed in *E. coli* isolates of both human and animal origin (Moniri and Dastehgoli 2005, Tchesnokova et al. 2019). The fact that the resistance occurring against one of the quinolone group antibiotics causes emergence of resistance to other members of the group might accelerate this increase. The highest resistance among the five quinolone group antibiotics investigated in this study was found against enrofloxacin. Enrofloxacin is the only quinolone group antibiotic used in the treatment of gastrointestinal diseases in animals. In a previous questionnaire survey in the study area, it was reported that 5% of the farmers used enrofloxacin unconsciously and with no prescription (unpublished data). Therefore, high resistance to this drug was not considered as an unexpected finding. However, resistance rates ranging from 13% to 19% were determined against other quinolones (ciprofloxacin, norfloxacin, lovofloxacin and nalidixic acid), which are not used for therapeutic purposes in animals, in this study. This can be explained by that resistance to enrofloxacin might have generated development of resistance against other quinolone antibiotics. Indeed, the findings of a study that resistance against enrofloxacin caused resistance to ciprofloxacin and nalidixic acid supported this view (Moniri and Dastehgoli 2005). In the present study, the highest resistance subsequent to enrofloxacin was obtained against nalidixic acid, which was never used in the veterinary field. Similar to our findings, Yassin et al. (2017) reported 21.3% resistance against nalidixic acid in cattle isolates. It is thought that the transfer of plasmid-derived genes, which play a role in the resistance, from resistant bacteria to susceptible bacteria by horizontal route can have a significant effect on the occurrence of resistance to nalidixic acid (Mammeri et al. 2005). It has also been reported that the use of enrofloxacin in animals has significantly decreased susceptibility to nalidixic acid in *E. coli* isolates (Dheilly et al. 2012).

The CLSI and EUCAST guidelines recommended by the World Health Organization's Global Antimicrobial Resistance Surveillance System are used to evaluate and interpret antimicrobial susceptibility tests. The comparison of these guidelines, which are updated annually, in terms of reference ranges revealed that although moderate to high level of consistencies were present for some antibiotics, significant inconsistencies for various antibiotics were noticeable. Kassim et al. (2016) compared EUCAST and CLSI 2015 guidelines for the interpretation of antibiotic susceptibilities in *E. coli* isolates, and reported that though there were medium and/or high consistencies in the vast majority of antibiotics they tested, a mark inconsistency was noted between the two guidelines in terms of amikacin and nitrofurantoin antibiotics. In addition, in a study using commercial micro-dilution method, it has been reported that the kappa value for amikacin in *E. coli* isolates was as low as 0.27 and there was a significant difference between EUCAST and CLSI guidelines (Sánchez-Bautista et al. 2018). The comparison of zone diameters of 13 antibiotics used in the present study with the reference ranges in the two guidelines showed that there was a perfect agreement with a kappa value of over 0.8 for nine antibiotics. However, in parallel with other studies, there was a serious inconsistency in nitrofurantoin and amikacin values in this study. The resistance rate of 29% was obtained against amikacin according to the EUCAST guideline, where 18 mm and above values were evaluated as sensitive, whereas this rate was found to be 6.2% according to the CLSI guideline, where 17 mm and above values were considered as sensitive. The fact that the zone diameter of 14 *E. coli* isolates was detected as 17 mm in this study was responsible for this difference. On the other hand, the significant inconsistency regarding nitrofurantoin arises from highly different zone diameters in the EUCAST (11 mm and above sensitive) and CLSI (17 mm and above sensitive) guidelines and, also the absence of intermediate values in the former. Although previous studies have reported a high consistency between the two guidelines for gentamicin, a relatively low consistency was found in this study. The reference ranges for sensitivity to gentamycin were acknowledged as 15 mm and above in the CLSI guide, and as 17 mm and above in the EUCAST guide (CLSI, 2020, EUCAST, 2020). In the current study, the gentamicin zone diameter was detected as 16 mm in 26 of *E. coli* isolates. Since this value was between the upper and lower limits of the EUCAST and CLSI guide values, a very low kappa value was calculated. Furthermore, it should not be ignored that an evaluation was made according to the 2015 and 2017 guidelines in previous studies and the reference ranges for gentamicin were updated in 2020. In the EUCAST guidelines published earlier years, the resistance limit for gentamicin was considered as 14 mm and below, while in the 2020 manual this limit was updated to 17 mm and below. It should be underlined that a significant inconsistency emerged between the two guidelines for gentamicin with the last update. In the EUCAST directives, some stricter limits have been set for some antibiotics in order to prevent inappropriate use of antibiotics and control increased antibiotic resistance, which contributed to the increase in this inconsistency.

CONCLUSION

This study revealed that *E. coli* isolates of bovine origin showed a high rate of resistance against antibiotics used in the field for a long time. Particularly, the increase in the number of multiple antibiotic resistant isolates was striking. In addition,

it was thought that high resistance against antibiotics such as nalidixic acid, chloramphenicol, first and second generation cephalosporins, which were not used in the field for the treatment and prophylactic purposes, may pose a serious problem for human and animal health. This should therefore be considered in the development of strategies to combat resistance problem. Finally, it is believed that due to substantial inconsistencies between the CLSI and EUCAST guidelines for some antibiotics such as amikacin, nitrofurantoin and gentamicin, there is an urgent need to execute necessary updates in both guidelines.

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

Authors' Contributions: RK, MNA and BÇ contributed to the research idea, design and execution of the study. RK, BK, MNA, MK and YÖ contributed to the acquisition of data. RK, BK, MNA, MK and YÖ analysed the data. RK and MNA drafted and wrote the manuscript. MNA, RK and BÇ reviewed the manuscript critically. All authors have read and approved the finalized manuscript.

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

REFERENCES

Aasmäe B, Häkkinen L, Kaart T, Kalmus, P. Antimicrobial resistance of *Escherichia coli* and *Enterococcus* spp. isolated from Estonian cattle and swine from 2010 to 2015. Acta Vet Scand. 2019; 61(1):5.

Acik, MN, Yurdakul NE, Cakici L, Onat N, Dogan O, Cetinkaya B. traT and CNF2 genes of *Escherichia coli* isolated from milk of healthy cows and sheep. Res Vet Sci. 2004; 77(1):17-21.

Bengtsson-Palme J, Kristiansson E, Larsson, DJ. Environmental factors influencing the development and spread of antibiotic resistance. FEMS Microbiol Rev. 2018;.42(1):fux053.

Blake DP, Hillman K, Fenlon DR, Low JC. Transfer of antibiotic resistance between commensal and pathogenic members of the Enterobacteriaceae under ileal conditions. J Appl Microbiol. 2003;. 95(3):428-436.

Boireau C, Morignat É, Cazeau G, Jarrige N, Jouy É, Haenni M, Madec, JY, Leblond A, Gay É. Antimicrobial resistance trends in *Escherichia coli* isolated from diseased food-producing animals in France: A 14-year period time-series study. Zoonoses Public Health. 2018; 65(1):e86-e94.

Boyen F, Vangroenweghe F, Butaye P, De Graef E, Castryck F, Heylen P, Vanrobaeys M, Haesebrouck F. Disk prediffusion is a reliable method for testing colistin susceptibility in porcine *E. coli* strains. Vet Microbiol (2010). 144(3-4):359-362.

Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI supplement M100. 2020.

Dheilly A, Le Devendec L, Mourand G, Bouder A, Jouy E, Kempf I. Resistance gene transfer during treatments for experimental avian colibacillosis. Antimicrob Agents Chemother. 2012; 56(1), 189-196.

Kassim A, Omuse G, Premji Z, Revathi G. Comparison of clinical laboratory standards institute and European committee on antimicrobial susceptibility testing guidelines for the interpretation of antibiotic susceptibility at a university teaching hospital in Nairobi, Kenya: a cross-sectional study. Ann Clin Microbiol Antimicrob. 2016; 15(1): 21.

Lim SK, Lim KG, Lee HS, Jung SC, Kang MI, Nam HM. Prevalence and molecular characterization of fluoroquinolone-resistant *Escherichia coli* isolated from diarrheic cattle in Korea. J Vet Med Sci. 2010; 72(5):611-614.

Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu LF, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu JH, Shen J. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: A microbiological and molecular biological study. Lancet Infect Dis. 2016; 16(2):161-168.

Mammeri H, Van De Loo M, Poirel L, Martinez-Martinez L, Nordmann P. Emergence of plasmid-mediated quinolone resistance in *Escherichia coli* in Europe. Antimicrob Agents Chemother. 2005; 49(1):71-76.

Moniri R, Dastehgoli K. Fluoroquinolone-resistant *Escherichia coli* isolated from healthy broilers with previous exposure to fluoroquinolones: Is there a link? Microb Ecol Health Dis. 2005; 17(2):69-74.

Odonkor ST, Ampofo JK. *Escherichia coli* as an indicator of bacteriological quality of water: an overview. Microbiol Res. 2013; 4(1):e2-e2.

Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: A global multifaceted phenomenon. Pathog Glob Health. 2015; 109:309-318.

Sánchez-Bautista A, Coy J, García-Shimizu P, Rodríguez JC. From CLSI to EUCAST guidelines in the interpretation of antimicrobial susceptibility: What is the effect in our setting? Enferm Infecc Microbiol Clin (English ed.) 2018; 36(4):229-232.

Shin SW, Shin MK, Jung M, Belaynehe KM, Yoo HS. Prevalence of antimicrobial resistance and transfer of tetracycline resistance genes in *Escherichia coli* isolates from beef cattle. Appl Environ Microbiol. 2015; 81(16):5560-5566.

Stromberg ZR, Van Goor A, Redweik GA., Wymore Brand MJ, Wannemuehler MJ, Mellata M. Pathogenic and non-pathogenic *Escherichia coli* colonization and host inflammatory response in a defined microbiota mouse model. Dis Model Mech. 2018;11(11):dmm035063.

Tadesse DA, Zhao S, Tong E, Ayers S, Singh A, Bartholomew MJ, McDermott PF. Antimicrobial drug resistance in *Escherichia coli* from humans and food animals, United States, 1950-2002. Emerging infectious diseases. 2012; 18(5), 741-749.

Tchesnokova V, Radey M, Chattopadhyay S, Larson L, Weaver JL, Kisiela D, Sokurenko EV. Pandemic fluoroquinolone resistant *Escherichia coli* clone ST1193 emerged via simultaneous homologous recombinations in 11 gene loci. Proc Natl Acad Sci. 2019; 116(29):14740-14748.

The European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoint tables for interpretation of MICs and zone diameters. Version 10.0, 2020.

van den Bogaard AE, Stobberingh EE. Epidemiology of resistance to antibiotics: Links between animals and humans. Inter J Antimicrob Agents. 2000; 14(4):327-335.

Vidovic N, Vidovic S. Antimicrobial resistance and food animals: Influence of livestock environment on the emergence and dissemination of antimicrobial resistance. Antibiotics 2020; 9(2):52.

White DG, Hudson C, Maurer JJ, Ayers S, Zhao S, Lee MD, Bolton L, Foley T, Sherwood J. Characterization of chloramphenicol and florfenicol resistance in *Escherichia coli* associated with bovine diarrhea. Journal of clinical microbiology. 2000; 38(12), 4593–4598.

World Health Organization (WHO) 2014. Antimicrobial resistance: global report on surveillance.https://apps.who.int/iris/bitstream/handle/10665/112642/9789241564748 eng.pdf.

Yassin AK, Gong J, Kelly P, Lu G, Guardabassi L, Wei L, Han X, Qiu H, Price S, Cheng D, Wang C. Antimicrobial resistance in clinical *Escherichia coli* isolates from poultry and livestock, China. PLoS One. 2017; 21;12(9):e0185326.

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RESEARCH ARTICLE

Technical and Economic Analysis of Sheep Breeding Enterprises in Selcuklu District of Konya Province

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ABSTRACT

In this study; It is aimed to determine the problems faced by breeders along with the structural and economic analysis of sheep farming enterprises located in Selcuklu district of Konya province. The main material of the study consisted of the data obtained through a survey from 62 enterprises engaged in sheep breeding activities in Selcuklu district in 2021. Random sampling method was used as the sampling method. According to the results of the research, feed expenses had the largest share with 46,1% among the cost factors that make up the cost in the enterprises, followed by labor expenses with 40,9%. As enterprises grew, the ratio of fixed costs to total costs decreased. The biggest income element in the enterprises was lamb income with a rate of 49,8%. Sales revenues per sheep, production expenses and operating gross profits were calculated as $144,2; $77,6 and $66,6, respectively. As a result of the study, it is suggested to decrease the costs, increase the sales income and profit, professionalize the enterprises and enlarge the scale of the enterprises in the sheep farms in Konya.

Key Words: Cost, Economic analysis, Profitability, Selcuklu district, Sheep breeding

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Konya İli Selçuklu İlçesi Koyunculuk İşletmelerinin Teknik ve Ekonomik Analizi

ÖZ

Bu çalışmada; Konya ili Selçuklu ilçesinde yer alan koyunculuk işletmelerinin yapısal ve ekonomik analiziyle birlikte yetiştiricilerin karşılaştıkları sorunların belirlenmesi amaçlanmıştır. Araştırmanın ana materyalini 2021 yılında Selçuklu ilçesinde koyunculuk faaliyeti yapan 62 adet işletmeden anket yolu ile temin edilen veriler oluşturmuştur. Örnekleme metodu olarak rastgele örnekleme yöntemi kullanılmıştır. Araştırmanın sonuçlarına göre işletmelerde maliyeti oluşturan masraf unsurları arasında %46,1 ile yem giderleri en büyük paya sahip olurken, bunu %40,9 ile işçilik giderleri izlemiştir. İşletmeler büyüdükçe, sabit maliyetlerin toplam maliyetlere oranı azalmıştır. İşletmelerde en büyük gelir unsuru ise %49,8 oranla kuzu geliri olmuştur. İşletmelerde koyun başına düşen satış gelirleri, üretim giderleri ve işletme brüt kârları sırasıyla 1.282 TL; 690 TL ve 592 TL olarak hesaplanmıştır. Çalışmada sonuç olarak Konya ilinde koyunculuk işletmelerinde maliyetlerin düşürülmesi, satış geliri ve kârın artması, işletmelerin profesyonelleşmesi ve işletme ölçeklerinin büyütülmesi önerilmektedir.

Anahtar Kelimeler: Ekonomik analiz, Kârlılık, Koyunculuk, Maliyet, Selçuklu ilçesi

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*Koyun başına düşen satış gelirleri, üretim giderleri ve işletme brüt kârları makalenin İngilizce özet kısmında 2021 yılına ait dolar kuru ortalamasına göre incelenmektedir. Bu yıla ait dolar kuru ortalaması 8,89 TL'dir. (TCMB, 2023).

GİRİŞ

Koyun yetiştiriciliği; koyunların farklı koşullara uyum sağlayabilmesi, az bir emekle besleme ve bakımının gerçekleştirilmesi, düşük sermayeyle yapılabilmesi gibi özelliklere sahiptir (Semerci ve Çelik 2016). Ayrıca, koyun yetiştiriciliğinde diğer hayvancılık faaliyetlerine kıyasla ürün çeşitliliği daha zengindir ve sektör için önemli bir avantajdır (Skapetas ve Kalaitzidou 2013). Koyun yetiştiriciliğinin bir diğer avantajı olarak ise sığır yetiştiriciliğine uygun olmayan düşük kaliteli meralar ve dağlık alanların koyun yetiştiriciliğinde kullanılmasıyla birlikte ekonomik yapıya katkı sağlanması sayılabilir (Günaydın 2009).

Koyun yetiştiriciliği, hayvansal üretimde önemli ve öncelikli bir yere sahiptir. Koyunlardan et, süt, yapağı, deri, gübre gibi ürünlerin elde edilmesinin yanında cerrahi müdahalelerde kullanılan dikiş iplerinde, düğme, tarak, tutkal, boya üretimi, ilaç ve kozmetik sanayinde faydalanılmaktadır (Şahin 2001). Türkiye'deki ekonomik ve coğrafi koşullar, kırsal nüfus ve istihdam oranı, bilgi birikimi ve geleneksel yapı her türlü hayvansal üretime oldukça uygun olmakla birlikte özellikle koyun ve keçi yetiştiriciliği bu alanda özel bir konumdadır (Günlü ve Mat 2021). Türkiye'de koyun yetiştiriciliğinin büyük bir bölümü köylerde ve mezralarda yapılırken köylerdeki koyunlar çoğunlukla küçük aile işletmelerinde, düşük maliyeti ve düşük sermaye gereksinimi ile yıl boyunca istihdam olanağı sağlamaktadır (Nalan ve Bingöl 2020).

Türkiye'de 2020 verilerine göre, koyun varlığının en yoğun olduğu bölgeler %18 oran ile Güneydoğu Anadolu Bölgesi ve %16 oran ile Ortadoğu Anadolu Bölgesi olmuştur. Güneydoğu Anadolu Bölgesi, toplamda 7.607.878 baş koyun ile koyun sayısında Türkiye coğrafi bölgeleri için birinci sırada yer almaktadır (TÜİK 2020). Ancak genelde koyun mevcudiyetinde 1980'lere kıyasla 1/3 oranı civarında azalma yaşanmıştır (FAOSTAT 2019). 1980'li yılların son çeyreğinden başlayarak 2010 yılına kadar Türkiye'de devlet destek ve teşviklerinin ağırlıklı olarak büyükbaş hayvancılığa verilmesi bu azalmanın bir sebebidir. Ayrıca şehir hayatının cazibesi, kırsal yaşamın sosyoekonomik dezavantajları, özellikle küçükbaş hayvan yetiştiriciliğinde beklenen üretim artışlarında yetersizlik oluşturarak üretiminin azalmasındaki bir diğer neden olarak incelenebilir (Günlü ve Mat 2021). Öte yandan çoban bulamama sorunun yaşanması, koyunculukta elde edilen yan ürünlerin (yapağı, deri, gübre vs)düşük değerlerde alıcı bulması koyunculuk faaliyetlerinden vazgeçişlerin diğer nedenleri olarak sayılabilir.

Konya ili; etrafında yer alan dağların arasında çok geniş ovalara sahip olması, Orta Anadolu'da yer alması ve coğrafi konumuyla tarihsel hayvancılık kültüründen dolayı küçükbaş hayvan yetiştiriciliği için oldukça önemli bir ildir (Ermetin 2014). Ayrıca, Konya ili ve çevresinin sahip olduğu bitki örtüsü ve iklim de ili koyun yetiştiriciliği açısından önemli hale getirmektedir. Kurak iklim koşullarıyla birlikte geniş yaylalar ve meralar bölgeyi koyun yetiştiriciliği için uygun bir ortam haline getirmektedir (Şahinli 2011; Özsayın ve Everest 2019).

2011-2020 yıllarına ait Türkiye geneli, Konya ili ve Selçuklu ilçesi özelinde koyun sayılarına ilişkin bilgiler verilmiştir (Tablo 1).

Tablo 1 incelendiğinde; Türkiye'deki koyun sayısının 2011 yılında 25.031.565 baş olduğu görülürken 2020 yılına gelindiğinde %68,29 artışla koyun mevcudiyetinin 42.126.781'e yükseldiği görülmektedir. Ayrıca, aynı yıllar arasında Konya ilindeki koyun sayısı 1.475.300 baş koyundan %73,29 artışla 2.556.610 başa çıkarken; Selçuklu ilçesindeki artış oranı 89.492 baş koyundan 104.884 baş koyuna %17,20 oranla artış göstermiştir (TÜİK 2020).

Bu çalışmanın amacı, Konya ili Selçuklu ilçesinde yer alan koyunculuk işletmelerinin yapısal ve ekonomik analizlerinin gerçekleştirilerek işletmelerin üretimde karşılaştıkları sorunların belirlenmesidir.

MATERYAL ve METOT

Çalışmanın materyalini, Konya ili Selçuklu ilçesi ve köylerinde bulunan tabakalı tesadüfi örnekleme yöntemiyle seçilen ve yüz yüze anket uygulanan koyunculuk işletmelerinin 2021 yılı üretim faaliyetlerine ilişkin verileri oluşturmuştur. Çalışmada ayrıca Konya İl Tarım ve Orman Müdürlüğü ve Selçuklu İlçe Tarım ve Orman Müdürlüğüne ait rapor ve kaynaklarla birlikte konu ile ilgili bilimsel çalışmalar, birtakım raporlardan da ikincil veri olarak yararlanılmıştır.

Araştırma evreni 589; güven düzeyi %90 olduğunda kabul edilebilir örneklem sayısı 62 olarak hesaplanmıştır. Hesaplamada elde edilen örneklem büyüklüğü minimum örneklem büyüklüğünü ifade etmektedir ve hesaplamada kullanılan formül aşağıda verilmiştir (Scheaffer ve ark. 2011).

$$n_0 = \frac{z^2 \times p \times (1-p)}{d^2} = \frac{1{,}64^2 \times 0{,}5 \times 0{,}5}{(0{,}1)^2} = 68$$

$$n = \frac{n_0}{1 + \frac{n_0}{N}} = \frac{68}{1 + \frac{68}{589}} \approx 62$$

[Güven düzeyine karşılık gelen tablo değeri (z=1,64); evrende gözlenme oranı (p=0,5)(Bu oran bilinmediği durumlarda en yüksek değeri veren 0,5 olarak alınmıştır), kabul edilebilir sapma toleransı (d=0,1), N: Evren büyüklüğü, n: Örneklem büyüklüğü (Israel 2009)]

İşletmelerde toplam maliyet hesabı için gerekli olan yem giderleri, işçilik giderleri, veteriner hekim, aşı ve ilaç giderleri, diğer giderler, genel idare giderleri, amortismanlar, bakım-onarım giderleri gibi çeşitli masraf unsurları tespit edilmiştir (Açıl ve Köylü 1971).

İncelenmesi planlanan işletmelerde aile iş gücüne ilişkin maliyetlerin hesaplanmasında yetişkin iş gücü birimi/erişkin iş gücü birimi ve ilgili dönüşüm katsayılarından yararlanılmıştır. Şöyle ki; 65 yaş ve üzeri için katsayı 0,5; 19-64 yaşlar arası katsayı 1 olarak hesaplanmışken; 16-18 yaşlar arası için katsayı 0,7 ve 0-15 yaş arası için katsayı 0 olarak hesaplanmıştır (Erkuş ve ark. 1995).

Çalışmada genel idare gelirleri, masraflar toplamının %3'ü olarak hesaplamaya dâhil edilmiştir (Mat 2020). Bakım-Onarım giderleri hesaplanırken ise üreticinin beyanı dikkate alınarak; alet ve ekipmanın, bina onarım ve bakım giderleri hesaplamaları yapılmıştır. Ancak hesaplamada bu durumun mümkün olmadığı durumlarda bina elde ediliş bedelinin %2'si onarım gideri, %1'i ise bakım gideri şeklinde hesaplamaya dâhil edilmiştir (Aydın, 2011). Ayrıca, büyüme çağındaki genç hayvanların belirli bir yaşa kadar değer artışı mevcut olduğundan genç hayvanlara yönelik amortisman hesaplanması yapılmamıştır (Aktaş 2009).

Produktif Demirbaş Kıymet Artışı (PDKA) hesaplanırken; (Sene sonunda sürünün kıymeti + Satılmış olan hayvanların değerleri + Kesilmiş olan hayvanların değerleri) – (Senenin başında sürünün kıymeti + Satın alınmış olan hayvanların değerleri) formülü kullanılmıştır. (Kaymak 2015). Formül sonucuna göre elde edilen sonuç negatif değerse maliyetlere eklenirken; elde edilen sonuç pozitifse tali gelire ilave edilmiştir.

BULGULAR

Anketler yoluyla sağlanan verilerden yola çıkarak Konya ili Selçuklu ilçesindeki koyunculuk işletmelerine yönelik bulgular elde edilmiştir.

Sosyoekonomik Bulgular

Konya ili Selçuklu ilçesinde anket yapılan koyunculuk işletmelerinin %50'si 1991-2010 yılları kurulurken;

2010 yılından sonra kurulan işletmeler %29 oranındadır. Geriye kalan %21'lik dilim ise 1990 yılı ve öncesinde kurulan işletmelerdir. Yapılan bu çalışmaya göre, işletme sahiplerine ait sosyoekonomik bulgular sunulmuştur (Tablo 2).

Tablo 2 incelendiğinde; işletme sahiplerinin %45,2 oranının 26 ile 44 yaşları arasında olduğu tespit edilirken; en düşük oran %6,5 oranıyla 18-25 yaş arası işletme sahipleridir. Aynı tabloda işletme sahiplerinin %51,6'sının ilkokul mezunu olduğu tespit edilirken; ilkokul mezunlarını takiben yetiştiricilerin %21,0'ının ortaokul mezunu, %16,1'inin lise mezunu, %8,1'inin üniversite mezunu, %1,6'sının yüksekokul mezun olduğu ve yine %1,6'sının da okuryazar olmadığı sonucu elde edilmiştir. İşletme sahiplerinin eğitim durumu incelendiğinde ilkokul mezunu olanların işletme sahiplerinin tamamı için yarıdan fazla olması dikkat çekmektedir. Bu durum işletme sahiplerinin belirli bir eğitim aldıktan sonra atadan kalma bir şekilde mesleğe devam ettiklerini düşündürebilir. İncelenen işletmelerde işletme sahiplerinin %93,5'i 40 ve altı yıl koyunculuk faaliyeti deneyimine sahipken bunun %50'si 1-20 yıl, %43,5'i 21-40 yıl arasında deneyim sahibidir. Geriye kalan %6,5 oran ise 41 yıl ve üzeri yıl koyunculuk faaliyeti yapan işletmelere aittir. İşletme sahiplerine koyunculuk yapma nedenleri sorulduğunda ise; incelenen işletmelerin neredeyse yarısı (%45,2) asıl işinin koyunculuk olduğunu ifade ederken; ek gelir elde etmek amacıyla ikincil iş olarak yapanların %17,7 oranında olduğu tespit edilmiştir. Ayrıca; işletme sahiplerinin %25,8'i koyunculuğu aileden miras kalması sebebiyle yaparken; %11,3'ü başka imkânları olmadığı için bu işi yaptıklarını ifade etmişlerdir.

İncelenen bu işletmelerde, işletme sahiplerinin 43 tanesi (%69,4) koyunculuk konusunda herhangi bir eğitim almamışken; 19 tanesi (%30,6) koyunculuk konusunda eğitim aldığını bildirmiştir. Koyunculuk faaliyetinin yapılış şekli olarak; işletmelerin %68,3'ü yerleşik, %30,6'sı yayla ve %1,1'i ise koyunculuğu göçer şekilde yapmaktadır.

Yapılan çalışmanın sonuçlarına göre, incelenen işletmelerin 37 tanesinde (%59,7) çobanlık görevini aile bireyleri yürütürken; işletmelerin 25 tanesinde (%40,3) ise çobanlık görevi için dışardan çoban temin edilmektedir.

İşletmelerin 47 tanesi %75,8 gibi büyük bir oranla herhangi bir kooperatife üye değilken; incelenen işletmelerin 50 tanesi kooperatifler tarafından hiçbir hizmetin sunulmadığını ifade etmektedir. Bu durum dikkat çekicidir.

Sürü ve Sürü Yönetimi Bulguları

Konya ili Selçuklu ilçesinde işletmelerin %74,2'sinde yetiştirilen ağırlıklı ırk Akkaraman olarak belirlenmiştir. Akkaraman ırkını takip eden ırklar ise %11,3 oranıyla Merinos ve %6,5 oranıyla Kıvırcık ırkıdır. Geri kalan %8'lik kısım ise %1,6'şar oranla Asaf, Karaman-Merinos melezi, Kangal Akkaraman, Morkaraman ve Tahirova-Şarole melezidir.

İşletmelerdeki koyunlarda infertilite ve abort oranları ortalaması sırasıyla %4,8 ve %3,0 olarak tespit edilmiş olup; kuzulardaki ikizlik ve ölüm oranları ortalaması sırasıyla %7,6 ve %10,4 olarak tespit edilmiştir.

İncelenen işletmelerde %69,4 gibi büyük bir oranla koçlar sürüye koç katım zamanında katılmaktadır. Koçlar bu dönemin dışındaki dönemlerde sürüden çıkartılmıştır. Ancak işletmelerin %30,6'sında koçlar sürüde tüm yıl boyunca kalmaktadır. Bu doğrultuda koçlar, ağırlıklı olarak yaz sonu ve sonbahar aylarında sürüye katılmıştır. İşletmelerin 20'sinde (%32,3) koç katımı Ağustos ayında başlarken; işletmelerin 18'inde (%29,1) koç katımı Eylül ayında başlamaktadır. Eylül ve Ekim ayına kadar sürüde kalan koçlar daha sonra belirli işletmelerde sürüden çıkartılmaktadır.

Yetiştiricilerin 28'i (%61,3) koç katımını serbest usulle yaparken; 23'ü (%37,1) grup sınıf usulüyle yapmaktadır. İşletmelerden sadece 1'i (%1,6) ise koç katımında elde sıfat yöntemini kullanmaktadır. Yetiştiricilerin sadece 18'i (%29) koç katım döneminde ek yemleme yaparken; 44 (%71) adet işletme ek yemleme yapmamıştır.

İncelenen işletmelerde yetiştiricilerin 44 tanesinin sağım yapmadığı tespit edilirken; bölgede incelenen işletmelerin 18'i koyunlarını sağdıklarını ifade etmişlerdir. Yetiştiricilerin %88,7'si gibi büyük çoğunluğu sütü yoğurt yapımı, tereyağı işlenmesi, köy içerisinde satışının yapılması gibi usullerle değerlendirirken; üreticilerinin çok az bir kısmı %4,8 oranında mandıraya satış yapanları ifade etmektedir. İşletmelerde damızlık dışı kalan hayvanlar ise; %64,5 oranıyla 40 işletmede celepler aracılığıyla elden çıkartılırken; 12 işletmede de kasaplar aracılığıyla değerlendirilmektedir. Bunlara ek olarak işletme sahiplerinin 6'sı damızlık dışı kalan hayvanlarını il/ilçe pazarlarında sattıklarını, 4'ü ise diğer şekillerde (tandıklar, birlikler vasıtasıyla vb.) değerlendirdiklerini beyan etmiştir.

Yapılan görüşmelerde, işletme sahiplerine koyunlarını sürüde kaç yıl damızlık olarak tuttukları sorusuna üreticilerin tamamı (%100) cevap vermiştir. İşletme sahipleri, dişi koyunlarını en fazla 2 yaşına kadar damızlık olarak kullandığını beyan etmiştirler. Ancak işletme sahiplerinin %8,1'i erkek koyunlarını 3 yaşa kadar damızlık olarak kullanmaktadır. Buna göre, bazı üreticilerin koçlarını dişi koyunlarına oranla daha fazla sürüde tuttuğu söylenebilir.

İşletmelerde en sık görülen hastalıklar sunulmuştur (Şekil 1). Şekil 1 incelendiğinde, üreticilerin en sık karşılaştıkları hastalıkların %38,7 ve %29,0 oranla sırasıyla babesiosis ve enterotoksemi olduğu dikkat çekerken; incelenen işletmelerde pnömoninin karşılaşılma oranı da %12,9 olarak tespit edilmiştir. İşletmelerde kuzu kayıplarının sebeplerinin başında ishal %39 oranla gelirken devam eden sebepler soğuk şoku, anasını bulamama, açlık ve diğer sebepler olarak kaydedilmiştir. Ayrıca, yapılan çalışma sonucunda koyunlardaki ölümlerin sebebi olarak küçükbaş vebası, bruselloz, topallık gibi hastalıklar diğer başlığı altında incelenerek %36,5 oranıyla en önde gelen hastalıklar olarak sayılabilirken babesiosis %33,2 oranıyla ikinci sırada kendine yer edinmiştir. İncelenen 62 işletmede, işletme sahipleri çeşitli sorunlarla karşılaşmaktadır. Buna ilişkin veriler sunulmuştur (Şekil 2).

Şekil 2 incelendiğinde, koyunculuk faaliyetiyle uğraşan işletme sahiplerinin başlıca karşılaştığı en büyük sorun fiyatlar başlığı altında incelenmiştir. Bu doğrultuda üreticiler, artan maliyetlerin koyunculuktan elde edilen ürün gelirleriyle karşılanmadığını ve bunun sonucunda dar boğaza girdiklerini ifade etmiştirler. Bunun yanında üreticiler için bir diğer sorun mera yetersizliğidir. Bölgedeki meraların yetersiz olması üreticilerin yeme ilave harcama yapmasına sebep olmaktadır. Üreticilerin %15,8'i için bu durumu sorun olarak bildirmiştir. Bunların yanında üreticilerin karşılaştığı diğer sorunlar Şekil 2'de de incelendiği gibi hastalıklar, finansman yetersizliği, pazarlama, çoban bulma sorunu gibi çeşitli sorunlarda tespit edilen diğer sorunlardır.

Ekonomik Bulgular

İncelenen işletmelerin üretim masraflarına ait oransal dağılım ve koyun başına düşen masraf miktarı sunulmuştur (Tablo 3).

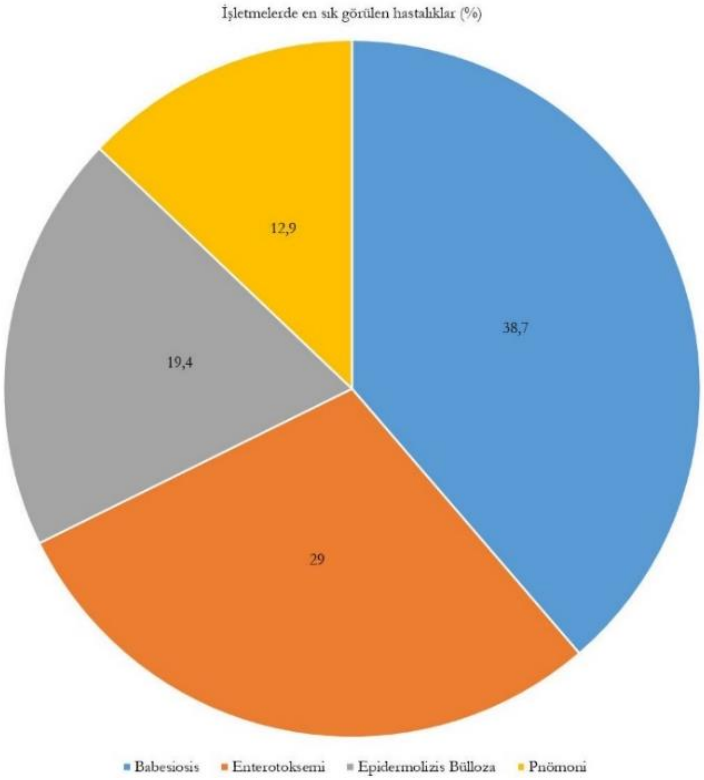
Tablo 3 incelendiğinde, işletmelerdeki masraflar genel toplamının %95'ini değişken masraflar toplamının oluşturduğu ve masraflar genel toplamının %5'ini ise sabit masrafların oluşturduğu tespit edilmiştir. Aynı tabloda üretim masrafları içindeki en masraflı unsurun %46,1 oranıyla yem giderleri olduğu dikkat çekmektedir. Yem masrafını takip eden ikinci büyük masraf ise %40,9 oranıyla işçilik giderleridir. Sabit masraflar içinde bulunan amortisman masrafları ise işletmelerin en az masraf yaptığı masraf unsuru

olarak saptanmıştır. İşletmeler çerçevesinde toplam geliri oluşturan unsurlar ise; kuzu satışlarından elde edilen gelirler, et gelirleri, süt gelirleri, ıskarta hayvanların satışından sağlanan gelirlerden oluşmaktadır. Sütün kuzulara verildiği, koyunların sağılmadığı işletmeler çerçevesinde süt satışından elde edilen gelirin hesaplanması bu hesaplamaya dâhil edilmemiştir. Hesaplamaya ilişkin gelirler ortalamalarının oransal dağılımı sunulmuştur (Tablo 4). Tablo 4 incelendiğinde; işletmelerin toplam gelir içinde %49,8 oranıyla kuzu geliri elde ettiği tespit edilmiştir. Et geliri ve PDKA, gelir unsurları içinde birbirine yakın oranlara sahiptir ve sırasıyla %22,4 ile %22,2 oranlarıyla çalışmada tespit edilmiştir.

İşletme sahiplerinden alınan bilgiler doğrultusunda; koyun başına düşen gelir, koyun başına düşen maliyet ve koyun başına net kâr/zarar durumu incelenmiştir. Sonuç olarak, koyun başına düşen toplam gelir 1.282 TL; koyun başına düşen toplam maliyet 690 TL olarak hesaplanmıştır. İşletmelerdeki koyun başına düşen net kâr/zarar durumu incelendiğinde ise; işletmelerin koyun başına 592 TL kâr elde ettiği tespit edilmiştir. Koyun başına düşen toplam gelir, toplam maliyet ve net kâr/zarar durumu çalışmanın yapıldığı 2021 yılına ait dolar ($) kuru ortalamasına (8,89 TL) göre incelendiğinde ise, koyun başına düşen toplam gelir, toplam maliyet ve net kâr/zarar durumu sırasıyla $144,2; $77,6 ve $66,6 olarak tespit edilmiştir (TCMB, 2023).

Tablo 1. Türkiye geneli, Konya ili ve Selçuklu ilçesine ait canlı koyun sayıları (Baş)
Table 1. Number of live sheep in Türkiye in general, Konya province and Selçuklu district (Head)

Yıllar	Selçuklu Koyun Sayısı (Baş)	% Endeks	Konya Koyun Sayısı (Baş)	% Endeks	Türkiye Koyun Sayısı (Baş)	% Endeks
2011	89 492	100,00	1 475 300	100,00	25 031 565	100,00
2012	107 370	119,98	1 733 655	117,51	27 425 233	109,56
2013	98 560	110,13	1 928 807	130,74	29 284 247	116,99
2014	142 900	159,68	1 895 986	128,52	31 140 244	124,40
2015	82 370	92,04	1 862 022	126,21	31 507 934	125,87
2016	81 300	90,85	1 826 773	123,82	30 983 933	123,78
2017	99 314	110,98	1 894 530	128,42	33 677 636	134,54
2018	89 728	100,26	2 001 010	135,63	35 194 972	140,60
2019	93 285	104,24	2 191 228	148,53	37 276 050	148,92
2020	104 884	117,20	2 556 610	173,29	42 126 781	168,29



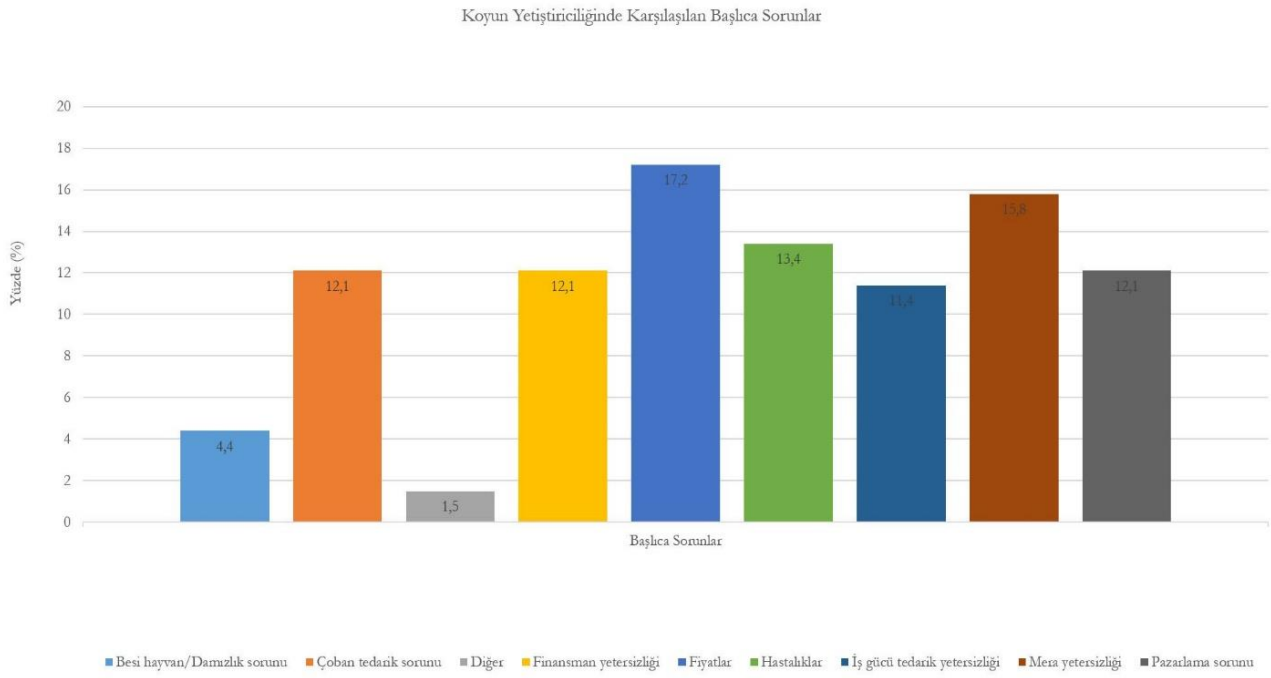
Şekil 1. İşletmelerde en sık karşılaşılan hastalıklar

Figure 1. The mort common diseases in enterprises

Tablo 2. İncelenen işletmelerde işletme sahiplerinin sosyoekonomik yapısı

Table 2. Socio-economic structure of enterprises owners in the examined enterprises

Özellik	Dağılım	Sayı	Oran (%)
Yaş (Yıl)	18-25	4	6,5
	26-44	28	45,2
	45-59	25	40,2
	60 ve üzeri	5	8,1
Eğitim Durumu	Okuryazar değil	1	1,6
	İlkokul	32	51,6
	Ortaokul	13	21,0
	Lise	10	16,1
	Yüksekokul	1	1,6
	Üniversite	5	8,1
Tecrübe (Yıl)	1-20	31	50,0
	21-40	27	43,5
	41 ve üzeri	4	6,5
Koyunculuk Yapma Nedeni	Asıl işi olduğu için	28	45,2
	Aileden miras kaldığı için	16	25,8
	Ek gelir elde etmek için	11	17,7
	Başka imkân olmadığı için	7	11,3



Şekil 2. Koyunculuk işletmelerinde işletme sahiplerinin karşılaştığı başlıca sorunlar
Figure 2. The main problems faced by enterprise owners in sheep breeding enterprises

Tablo 3. Koyunculuk işletmelerindeki yıllık üretim masrafları ortalaması oransal dağılımı
Table 3. Proportional distribution of the average annual production costs in sheep breeding enterprises

Masraf Unsurları	**Oransal Dağılım (%)**
1. Toplam Yem Masrafı	46,1
a. Kaba Yem Masrafı	14,0
b. Kesif Yem Masrafı	32,1
2. İşçilik	40,9
a. Yabancı İş Gücü	14,7
b. Aile İş Gücü	26,2
3. Veteriner Sağlık Masrafları	4,6
4. Diğer Giderler	3,4
Değişken Masraflar Toplamı	95,0
5. Genel İdare- Bakım Onarım Giderleri	2,9
6. Amortismanlar	2,1
Sabit Masraflar Toplamı	5,0
Masraflar Genel Toplamı	100,0

Tablo 4. Koyunculuk işletmelerine ait yıllık işletme gelirleri ortalaması oransal dağılımı

Table 4. Proportional distribution of the average annual operating income of sheep breeding enterprises

İşletme Gelirleri (TL/Yıl)	Oransal Dağılım (%)
Kuzu Geliri	49,8
Et Geliri	22,4
PDKA*	22,2
Süt Geliri	2,1
Iskarta Hayvan Bedeli	3,2
Diğer	0,3

***** PDKA: Produktif Demirbaş Kıymet Artışı

TARTIŞMA

Yapılan bu çalışmada işletme sahipleri içinde yaşları 18-59 arasında olan işletme sahipleri %92 oranla çoğunluktadır. Çanakkale ili Gökçeada ilçesinde yapılan bir çalışmada 20-59 yaş aralığında işletme sahiplerinin oranı ise %76,8'dir ve bu çalışmaya kıyasla düşük bir orana sahiptir. İller arasındaki fark 60 yaş ve üzeri işletme sahiplerinden kaynaklanabilmektedir. Yapılan bu çalışmada 60 yaş ve üzeri işletme sahiplerinin oranı %8,1 oranındayken; Gökçeada ilçesindeki 60 yaş ve üzeri işletme sahibinin oranı %23,2 olarak saptanmıştır (Özsayın ve Everest 2019). Konya ilinde yapılan bir çalışmada ise 60 yaş ve üzeri üreticilerin oranı %42,1 olarak tespit edilerek her iki çalışmadan da bir hayli yüksek bulunmuştur (Arıtunca ve Karabacak 2020).

İncelenen işletme sahipleri içerisinde üreticilerin büyük çoğunluğu %51,6 oranla ilkokul mezunuyken; %1,6'sı okuma-yazma bilmiyor ve %9,7'si ise yüksekokul-üniversite mezunudur. Ardahan ilinde yapılan çalışmada ise; işletme sahiplerinin %53 oranla büyük çoğunluğu ilkokul mezunu, %7,6'sı yüksekokul mezunudur (Ayvazoğlu Demir ve ark. 2015). Yine Erzincan ilinde yapılan bir çalışmada ise; yetiştiricilerin büyük bir çoğunluğu %63,1 oranla ilkokul mezunu, %4,8'i yüksekokul-lisans mezunudur (Özyürek ve ark. 2018). Çanakkale ili Gökçeada ilçesinde yapılan çalışmada ise yine yetiştiricilerin büyük çoğunluğunun %47,2 oranla ilkokul mezunu olduğu ve yüksekokul-üniversite mezunu oranının toplamının ise 8,8 oranında olduğu tespit edilmiştir (Özsayın ve Everest 2019). Yapılan bu çalışmalar incelendiğinde; Konya ili Selçuklu ilçesi, Ardahan ili, Çanakkale ili Gökçeada ilçesinde yapılan çalışmalar sonuçlar birbirine oldukça yakındır. Ancak, Erzincan ilinde ilkokul mezunlarının oranının diğer çalışmalara göre nispeten yüksek ve yüksekokul, üniversite mezunlarının da diğerlerine nazaran daha düşük olduğu sonucuna ulaşılmıştır. Yetiştiricilik açısından eğitim düzeyinin yükselmesi ile bütün gelişmelere ve yeniliklere adaptasyon bakımından avantaj sağlayacağından işletmeler açısından mevcut tablo olumsuz olarak görülmelidir.

İşletme sahiplerinin %69,4'ü küçükbaş hayvancılık konusunda herhangi bir eğitim almadığını ve %30,6'sı ise eğitim aldığı bildirmişlerdir. Bu çalışma Konya ve Ardahan ilinde daha önce yapılan çalışmaları destekler niteliktedir. Konya ilinde yapılan bir çalışmada küçükbaş hayvan yetiştiriciliği konusunda eğitim almayan işletme sahiplerinin oranı %65,38; bu konuda eğitim almış işletme sahiplerinin oranı %34,62 olarak tespit edilmiştir (Şahinli 2011). Ardahan ilinde yapılan bir çalışmada ise; işletme sahiplerinin %37,9'u koyunculukla ilgili seminer veya kursa katıldıklarını ifade ederken; işletme sahiplerinin %62,1'i koyunculukla ilgili seminer veya kursa katılmadıklarını ifade etmişlerdir (Ayvazoğlu Demir

ve ark. 2015). Bu çalışmalarda eğitim alan işletme sahiplerinin oranı, Muş ili Korkut ilçesinde yapılan çalışmaya göre oldukça yüksek bulunmuştur. Muş ili Korkut ilçesinde yapılan bir çalışmaya göre, işletme sahiplerinin sadece %4,9'u küçükbaş hayvancılık konusunda eğitim aldıklarını bildirmişken; işletme sahiplerinin %95,1 gibi oldukça büyük kısmı küçükbaş hayvancılık konusunda herhangi bir eğitim almadıklarını ifade etmiştir (Kaymak ve Sarıözkan 2016). Bu noktada; işletme sahiplerinin büyük bir çoğunluğunun küçükbaş hayvancılık konusunda eğitim almadığı sonucuna ulaşılmıştır. Bu doğrultuda yetiştiriciye konu hakkında verilecek eğitimler ve yetiştiricinin eğitimlere katılmasının teşvik edilmesi büyük önem arz etmektedir. Böylece bir yandan işletmelerinin kârlılığını yükseltmek mümkün olabilirken diğer yandan geleneksel yetiştiricilik usulünden entansif yetiştiricilik sistemine geçiş daha kolay olabilecektir.

İncelenmekte olan işletmelerde koyunlarda kısırlık ve yavru atma oranlarının sırasıyla %4,8 ve %3,0 olduğu sonucuna ulaşılmıştır. Yozgat ilinde yapılan bir çalışmaya göre ise; kısırlık ve yavru atma oranı %3,7 olarak tespit edilmiştir (Tamer ve Sarıözkan 2017). Genel olarak birbiriyle örtüşen sonuçlar elde edilmiştir. Doğuran koyun oranının yüksek olmasını isteyen işletme sahipleri için bu oranların iyi olduğunu söylemek mümkündür. Yapılan bu çalışmaların aksine; Burdur ilinde yapılan bir çalışmada kısır hayvan sayısının %11,7 oranıyla diğer iki ile nazaran oldukça yüksek olması dikkat çekmektedir (Bilginturan ve Ayhan 2009). Kuzulardaki ölüm oranı yapılan bu çalışmada işletme başına %10,4 olarak bulunmuştur ve Burdur, Muş ve Yozgat illerinde yapılan çalışmalara göre yüksek bulunmuştur. Burdur, Muş, Yozgat illerinde yapılan çalışmalara göre kuzu ölüm oranları sırasıyla %7,6; %8,3; 9,5 olarak bildirilmiştir (Bilginturan ve Ayhan 2009; Kaymak 2015; Tamer ve Sarıözkan 2017). Kuzu ölümlerinin sebebi olarak ise; başta ishal yapan çeşitli hastalıklar, bakım-besleme yetersizliği veya hataları, soğuk şoku gibi sebepleri saymak mümkündür.

Yapılan bu çalışmada, işletme sahiplerinin %69,4'ü büyük bir oranda koçlarını koç katım zamanında sürüye katarken; koçlarını tüm yıl boyunca sürüde tutanların oranı %30,6'dır. Çalışma bulguları, Bursa ilinde ve Burdur ilinde yapılan çalışmaların bulgularından farklı bulunmuştur. Bursa ilinde işletmelerin %29,8'i koçlarını koç katım zamanı sürüye katıyorken; işletme sahiplerinin büyük bir çoğunluğu %70,2 oranla koçlarını tüm yıl boyunca sürüde tuttuklarını ifade etmişlerdir (Dönmez 2008). Yine, Burdur ilinde yapılan çalışmada oldukça yüksek bir oranla işletme sahiplerinin %97'sinde koçun tüm yıl boyunca sürü içinde kaldığını ifade edilmiştir (Bilginturan ve Ayhan 2009). Yapılan bu çalışmanın bulgularına benzer bir bulgu Niğde ilinde yapılan çalışmada karşımıza çıkmaktadır. Niğde ilinde yapılan bir çalışmada, işletme sahiplerinin %30,2'si koçu yıl boyunca sürüde tuttuğunu, %69,8'i koçlarını koç katım zamanında sürüye dâhil ettiklerini ifade etmişlerdir (Ceyhan ve ark. 2015).

Yetiştiricilerin %61,3'ü koç katım yöntemi olarak serbest aşımı kullanırken; %37,1'i sınıf usulü aşım yöntemini kullanmaktadır. Burdur ilindeki, işletme sahiplerinin sadece %0,5'i koç katımında sınıf usulü aşımı kullanırken; işletme sahiplerinin neredeyse tamamı %99,5 oranla serbest aşımı kullanmaktadır (Bilginturan ve Ayhan 2009). Bursa ilindeki yapılan çalışmalarda ise koç katımında tek bir yöntemin kullanıldığını ve bunun sadece serbest aşımla sağlandığı tespit edilmiştir (Dönmez 2008; Öziş Altınçekiç 2014). Çanakkale ili Gökçeada ilçesinde, sınıf usulü aşımın %13,6, serbest aşımın %77,6 oranında olduğu tespit edilmiştir (Özsayın ve Everest 2019). Çalışmalar, bu çalışmayla kıyaslandığında; bu çalışmada serbest aşım diğerlerine nazaran düşük bulunurken; sınıf usulü aşımın diğerlerinden yüksek olduğu sonucuna ulaşılmıştır. İşletmeler tarafından

kullanılan serbest aşımın kızgınlık gösteren koyunların herhangi bir koç tarafından aşılması sebebiyle olumsuzlukları vardır. Ayrıca, koyunlarda döl tutmama problemi ve diğer yöntemlere göre daha fazla koç gerektirmesi de serbest aşımın olumsuzlukları olarak sayılabilecek diğer özellikleridir (Köseman ve ark. 2022).

Yapılan bu çalışmada, işletme sahiplerinin %71'i koç katım döneminde ek yemlemeye başvurmamaktadır. Ancak, %29 oranında bir kesimi koç katım döneminde ek yemlemeye başvurmaktadır. Bursa'da yapılan bir çalışmada ise, işletmelerin %23,4'ün de koç katım döneminde ek yemleme yapıldığı sonucu elde edilirken; işletmelerin büyük bir çoğunluğunda %76,6 oranla ek yemleme yapılmadığı sonucuna ulaşılmıştır (Dönmez 2008). Bursa ilindeki yapılan çalışmanın bulguları, bu çalışmayla uyumlu bulunmuştur. Çanakkale ili Gökçeada ilçesinde ise, ek yemleme yapılmayan işletmelerin oranı bu çalışmaya kıyasla yüksek bulunmuştur. İlçede ek yemleme yapılmayan işletmelerin oranı ortalaması %91 olarak tespit edilmiştir (Özsayın ve Everest 2019). Ek yemleme yapılmayan işletmelerin oranının ek yemleme yapılan işletmelere göre yüksek olmasında iki sebepten bahsedilebilir. Bunlardan birincisi, yetiştiricilerin ek yem masrafına katlanmak istememesidir. Sebeplerden bir diğeri ise, yetiştiricilerin ek yemlemenin avantajları konusunda eksik bilgi sahibi olmasıdır (Dönmez 2008).

Çalışmada işletmelerin %29'unda koyunların sağıldığı ancak %71 oranla koyunların sağılmadığı sonucuna ulaşılmıştır. Yozgat ilinde yapılan çalışmanın bulgularıyla kıyaslandığında sonuçların farklı olduğu dikkat çekmektedir. Yozgat ilinde yapılan çalışmada, işletmelerin %60,3'ünde sütün sağıldığı; %39,7'sinde sütün sağılmayıp kuzulara içirildiği sonucuna ulaşılmıştır (Tamer ve Sarıözkan 2017). Bursa ilinde yapılan bu çalışmanın bulguları ise bu çalışmayla uyumludur. Bursa ilinde yapılan çalışmada da tıpkı bu çalışmadaki gibi işletmelerin büyük bir kısmında %63,8 oranla sağım yapılmadığı; işletmelerin %36,2'sinde sağım yapıldığı sonucuna ulaşılmıştır (Dönmez 2008). Bursa'da yapılan ve çalışmada çeşitli sebeplerden dolayı sağım oranı düşük bulunmuştur. Sağımcı temin etmedeki güçlükler, ekstra iş gücünün gereksinimi ve süt fiyatlarının yetersiz olması gibi sebepler bu duruma neden olan faktörler arasındadır (Dönmez 2008).

İşletmeler damızlık dışı kalan hayvanları genel olarak celepler, kasaplar, il ve ilçe pazarlarında değerlendirmektedir. Bu yöntemlerin oranı işletmeler özelinde sırasıyla %64,5; %19,3; %9,7'dir. İşletmelerin geri kalanı ise %6,5 oranla damızlık dışı kalan hayvanlarını tanıdıklar, birlikler vasıtasıyla gibi şekillerde değerlendirmektedir. Yine Konya ilinde yapılan başka bir çalışmaya göre; işletmelerin %21,7'si yapılan çalışmayla uygun bir şekilde kasaplar aracığıyla damızlık dışı hayvanlarını değerlendirirken; celepler aracılığıyla değerlendirenlerin oranı %24,1'dir ve yapılan çalışmaya göre oldukça düşük bulunmuştur. İldeki yapılan çalışmada %54,2 gibi büyük bir oran ise damızlık dışı hayvanlarını kurbanlık olarak değerlendiren yetiştiricileri göstermektedir (Arıtunca ve Karabacak 2020).

İncelenen işletmelerde, yetiştiricilerin %39'u çoğunlukla babesiosis, %29'u enterotoksemi, %19'u epidermolizis bülloza, %13'ü pnömoni ile karşılaştıklarını ifade etmişlerdir. Burdur ilinde yapılan bir çalışmada, yapılan bu çalışmaya da yakın bir şekilde işletmelerin %26'sında enterotoksemiyle karşılaşıldığı ifade edilmektedir. Bunun dışında, ildeki işletmelerin %26,4'ünde çiçek hastalığı, %15,9'unda dış parazitlere bağlı hastalıklar, %11,5'inde solunum yolu hastalıkları, %20,2'sinde ise agalaksi, mavi dil, ektima, boyun urları, ayak hastalıklar, şap, brucelladan en az birisine rastlandığı bildirilmiştir (Bilginturan ve Ayhan, 2009). Bursa ilinde yapılan çalışmada ise; yetiştiricilerin %17,0'ı veba, %10,6'sı brucella, %8,5'u piyeten ile karşılaştıklarını ifade ederken; yetiştiricilerin %59,6'sı bu hastalıkların tümüyle

karşılaştıklarını ifade etmişlerdir (Dönmez 2008). Adıyaman ilinde yapılan bir çalışmaya göre ise sürüde aşılamaya rağmen çiçek başta olmak üzere şap, solunum yolu hastalıkları ve entretoksemi gibi bulaşıcı hastalıklara rastlandığı tespit edilmiştir (Ayvazoğlu Demir ve ark. 2015). Çeşitli illerde çeşitli hastalıklar gözlenmektedir. Bu hastalıkların bazıları iller arası ortaklık taşırken; bazı illerde farklı hastalıklar da görülmektedir. Bu farklılığın sebebinin bölgeler arası coğrafya ve iklim koşulları farklılıkları, işletme sahiplerinin hastalıklar hakkında bilgi ve tecrübe düzeyi farklılıkları, yetiştirilen hayvanların ırkı gibi özellikler kaynaklı olduğu söylenebilir.

Araştırmada incelenen işletmeler kapsamında toplam üretim masraflarının %95,0 oranıyla değişken masraflardan oluştuğu ve geri kalan %5 oranın ise sabit masraflara ait olduğu tespit edilmiştir. İşletmelerde üretim masrafları içerisinde yer alan birinci masraf kalemi %46,1 oranla yem masraflarına aitken; bunu %40,9 oranla işçilik masrafları, %4,6 oranla veteriner-sağlık harcamaları takip etmektedir. Bu çalışma sonucundan oldukça farklı olarak; Antalya ilinde yapılan bir çalışmada değişken masrafların tüm masrafların %49,6'sını oluşturduğu ve sabit masrafların ise tüm masrafların %50,3'ünü oluşturduğu tespit edilmiştir. Antalya ilinde yapılan çalışmaya göre; %50,3 oranında olan sabit masrafların %31,4'ü aile iş gücü masraflarından kaynaklanmaktadır (Özalp ve Sayın 2018). Antalya ilinde yapılan çalışma bulgularının bu çalışma bulgularıyla farklılık göstermesinde bir sebep olarak, Antalya ilinde işçilik masrafları içerisinde aile iş gücünün sabit masraflar içerisinde gösterilmesidir. Yozgat ilinde yapılan bir çalışmada ise; değişken masrafların oranı %91,3; sabit masrafların oranı %8,7 olarak bulunarak bu çalışmayla benzerlik göstermiştir. İldeki yem masrafları oranı %59,5 oranla bu çalışmadan yüksek; ancak iş gücü masrafları %23,2 oranıyla bu çalışmadan düşük bulunmuştur (Tamer ve Sarıözkan 2017). Yine Muş ili Korkut ilçesinde yapılan bir çalışmada, değişken masrafların oranı %95,3; sabit masrafların oranı %4,7 olarak tespit edilirken sonuç bu çalışmayla uyumludur. Ancak ildeki yem masrafı oranı %61,9 oranıyla bu çalışmadan yüksek ve %30,5 oranıyla işçilik masrafı bu çalışmadan düşük bulunmuştur (Kaymak ve Sarıözkan 2016). Ardahan ilinde yapılan bir çalışmada da, yem masrafları %48,9 oranla bu çalışmadan yüksek ve işçilik masrafları %16,9 oranla bu çalışmadan düşük bulunmuştur (Ayvazoğlu Demir ve ark. 2015). Yapılan başka bir çalışmada ise bölgeler arası farklılık göstermesine rağmen yem masrafları ortalama olarak %40,2 oranında bulunarak en büyük gider kalemini oluşturmaktayken bunu %26,8 oranla iş gücü masrafları takip etmiştir (Satar ve Sakarya. 2021). Çalışmalar genel olarak incelendiğinde; en büyük maliyet unsurunun yem maliyetleri olduğu ve bunu takip eden bir diğer maliyet unsurunun ise iş gücü masrafı olduğu dikkat çekmektedir.

Toplam gelirin %49,8'ini kuzu satışından gelen gelirler, %22,2'sini PDKA' dan gelen gelirler, %22,4'ünü et satışından elde edilen gelir ve %3,2'si ise ıskarta hayvan bedelinden elde edilen gelirleri ifade etmektedir. Ayrıca, süt satış geliri %2,1 oranda gelir kaynağıdır. Geri kalan %0,3 ise yoğurt, yapağı, peynir, yün, gübre gibi yan ürünlerin satışından elde edilen geliri ifade etmektedir. Yozgat ilinde yapılan çalışmada gelirin dağılımı ise; %56,4 oranıyla PDKA, %32,4 oranıyla kuzu/toklu satışı başta olmak üzere %3,9 oranla devlet desteği, %3,5 oranla damızlık satışı, %3,0 süt satış geliri, %0,9 oranla et satış geliridir (Tamer ve Sarıözkan 2017). Bu çalışma, belirtilen çalışmayla kıyaslandığında; PDKA oranının Yozgat ilinde yapılan çalışmada yüksek olduğu; diğer gelir kaynaklarının ise genel olarak bu çalışmadan yüksek olduğu dikkat çekmektedir. Muş ili Korkut ilçesinde yapılan çalışmada ise; gelirin %63,5'u kuzu/toklu satışından elde edilmiştir. Ayrıca, bunu takip eden diğer gelir kaynakları %17,9 oranla PDKA, %11,4 oranla süt geliri, %4,9'u devlet desteği ve %2,3'ü et

geliri kaynaklıdır. Süt geliri ve kuzu/toklu satışından elde edilen gelir oranı, bu çalışmadan yüksek bulunurken; diğerleri genellikle bu çalışmadan düşük bulunmuştur (Kaymak ve Sarıözkan 2016).

İncelenen işletmelerde, sonuç itibariyle işletme başına yıllık 279 445 TL kâr olarak tespit edilirken; koyun başına düşen toplam kâr yıllık 592 TL'dir. Muş ili Korkut ilçesinde ise yapılan çalışmada; küçük işletmeler haricinde işletmeler yıl sonunda net kâr elde etmiştir. Elde edilen kâr ortalaması genel olarak işletme başına yılda 11.092,6 TL olarak tespit edilmiştir. İlçedeki net kâr/zarar durumu hayvan başına incelendiğinde ise; genel ortalama yılda 45,8 TL olarak tespit edilmiştir (Kaymak ve Sarıözkan 2016). Yozgat ilinde yapılan çalışmada da; işletmeler küçük, orta, büyük ölçekli işletmeler özelinde net kâr elde etmiştir. İşletme başına elde edilen genel net kâr/zarar durumu incelendiğinde; yılda 28.497,1 TL'lik kâr elde edildiği tespit edilmiştir. İldeki hayvan başına net kâr/zarar durumunun genel ortalaması ise; yılda 185,3 TL olarak tespit edilmiştir (Tamer ve Sarıözkan 2017). Çalışmalardaki bulgular arasında farklar mevcuttur. Bu farkların sebebinin başta çalışma yapılan yıllara göre enflasyon oranları olmak üzere diğer makro parametrelerle ilgili olduğu düşünülmektedir. Ayrıca döviz kuru üzerinden bir değerlendirme yapılmak istendiğinde döviz bazında koyunculuk işletmelerinin karlılığının genel olarak azaldığı geçmişten günümüze yapılan tüm çalışmalarda ortak olarak dikkat çeken bir husustur.

SONUÇ

Çalışmanın gerçekleştirildiği bölgede hayvanların birincil gereksinimi olan kaba yem ihtiyaçlarının giderilebilmesi açısından meraların yetersiz özellikte olduğu anlaşılmıştır. Ekilebilir arazilerin içerisinde bulunan yem bitkileri ekilme sahalarının yetersiz kaldığı, bundan dolayı hayvancılık faaliyetlerinin en mühim girdisi olan kaba yem gereksiniminin istenilen düzeyde karşılanmadığı anlaşılmıştır. Kaba yem eksikliğinin giderilebilmesi açısından arazisi bulunan çiftçilerin yem bitkisi ekimine yöneltilmesi gerekmektedir. Kesif yem fiyatlarında görülen artış göz önünde bulundurularak yağlı tohum, yem ve yem hammaddeleri üretimine verilmekte olan destek ve teşvikler artırılarak üretiminin teşviki sağlanmalıdır. Tarım Kredi Kooperatifleri ve Toprak Mahsulleri Ofisi tarafından hayvancılık yapan işletmelere küspe, mısır, arpa, buğday benzeri yem hammaddelerinin uygun fiyatlar ile tedarik edilmesi için gerekli önlemlerin alınması büyük öneme sahiptir.

Çalışma bölgesinde gerçekleştirilen koyunculuk faaliyetlerinin başta gelen problemlerinden birisi de tecrübeli çobanların olmayışıdır. Bu sorun koyunculuk faaliyetlerinde tüm Türkiye'de ortak problemdir ve güncelliğini korumaktadır. Kırsal bölgelerde genç nüfus köyden kente göç ile paralel bir şekilde azalmakta olup köylerde kalan nüfus koyunculuk faaliyetlerini zahmetli ve zor gördüklerinden dolayı yavaş yavaş terk etmektedirler. Kırsal bölgelerde refah düzeyini iyileştirmek suretiyle üretim faaliyetlerine gençlerin daha fazla katılabilmesi ve çobanlık mesleğinin cazibesinin artırılması gerekmektedir.

İşletmelerin önemli bir bölümü, pazarlama ile ilgili sorunlarının bulunduğunu ve Türkiye'de koyunculuk sektörüne canlı kasaplık hayvan ithalatının zarar vermekte olduğunu ifade etmektedirler. Üreticilerin sorunlarından birisi de kesime götürülen hayvanların satış bedellerinin artmakta olan maliyetleri karşılayamamasıdır. Geçmiş yıllarda yapılan çalışmalar incelendiğinde günümüzde koyun başına elde edilen gelirlerin nominal olarak artmasına karşın reel olarak gerilediği görülmüştür. Üreticilerin ihtiyaç zamanında ve yeterli şekilde desteklenememesi üretim süreçlerinde sürdürülebilirliğin önünde engel teşkil etmektedir. Bu nedenlerle üreticilere yem ve damızlık materyal başta olmak üzere üretim maliyetlerini düşürecek şekilde yan ürünlerin üretilmesinin teşvik edilmesi ve pazarlama imkânlarının geliştirilmesine yönelik

küçükbaş hayvancılık sektörüne daha fazla destek ve hibe verilmesi gerekmektedir.

Yetiştiricilerin yarısından fazlası yeni yaklaşımlara ve uygulamalara mesafeli olduklarını, faaliyet alanları ile ilgili yenilikleri takip etmediklerini ve teknik bilgi almadıklarını ifade etmişlerdir. Geleneksel tipte yapılan küçükbaş hayvan yetiştiriciliğinin geliştirilebilmesi, bu faaliyetlerin daha modern yöntemler ile gerçekleştirilmesine bağlıdır. Özel sektör, kamu ve üniversiteler bu amaca yönelik sivil toplum kuruluşları ile ortaklaşa eğitim seminerleri/projeler düzenlemek suretiyle yetiştiricilerin bilgilendirilmesini sağlanmalı, eğitim düzeyi yüksek ve genç üreticilerin sektöre kazandırılması gerekmektedir.

Türkiye genelinde koyunculuk işletmelerinin dağınık yapıda ve küçük ölçekte olması, üretici örgütlerinin yetersiz kalması gibi durumlarla birleşince pazarlama zincirlerinin uzamasına ve pazarlama ile ilgili maliyetlerin artmasına yol açmaktadır. Üretici örgütlerinin yetki sorumluluk ve görevlerinin yeniden tespit edilerek etkinliklerinin artırılması sağlanmalıdır. Koyunculuk alt sektöründe sıklıkla karşılaşılmakta olan sorunların başında yer alan pazar probleminin çözümlenmesi bu sektörde kârlılığın artmasına imkân sağlayacaktır. Yem fiyatlarının düşürülmesi, ithalat politikasının canlı ve kasaplık hayvan için sınırlandırılması gibi benzeri önlemler bir an önce alınmalıdır. Bununla birlikte hayvansal üretimi verilmekte olan hibe ve desteklerin doğru kanallar ile aktarılması, meraların vasıf değişikliklerine karşı korunması, aşırı otlamaya engel olunması ve mera ıslahının yapılması, yem bitkileri üretiminde mevcut olan sorunların çözülmesi gibi ek önlemler ile koyunculuk faaliyetlerinin daha cazip hale getirilmesi sağlanabilir.

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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REFERENCES

Açıl, A., & Köylü, K. (1971). Zirai ekonomi ve işletmecilik dersleri. Ankara Üniversitesi Ziraat Fakültesi, Yayın No: 465, Ders Kitabı: 168. Ankara. p, 234.tez

Aktaş, A. (2009). Konya ili Karapınar ilçesi koyunculuk işletmelerinin ekonomik analizi. Selçuk Ünv. Fen Bilimleri Enstitüsü – Yüksek Lisans Tezi.

Arıtunca, D., & Karabacak, A. (2020). Konya merkez ilçelerinde koyunculuk işletmelerinin durumu. Bahri Dağdaş Hayvancılık Araştırma Dergisi, 9(1), 13-24.

Aydın, E. (2011). Kars ve Erzurum illeri sığır besi işletmelerinin ekonomik analizi. Ankara Ünv. Sağlık Bilimleri Enstitüsü – Doktora Tezi.

Ayvazoğlu Demir, P., Adıgüzel Işık, A., Aydın, E., Yazıcı, K., & Ayvazoğlu, C. (2015). Ardahan ilinde koyun yetiştiriciliğinin sosyo-ekonomik önemi. Van Vet J, 26(3), 141-146.

Bilginturan, S., & Ayhan,V. (2009). Burdur ili damızlık koyun ve keçi yetiştiriciler birliği üyesi koyunculuk işletmelerinin yapısal özellikleri ve sorunları üzerine bir araştırma. Hayvansal Üretim, 50 (1), 1-8.

Ceyhan, A., Şekeroğlu, A., Ünalan, A., Çınar, M., Serbester, U., Akyol, E., & Yılmaz, E. (2015). Niğde ili koyunculuk işletmelerinin yapısal özellikleri ve sorunları üzerine bir araştırma. KSÜ Doğa Bilimleri Dergisi, 18(2), 60-68. https://doi.org/10.18016/ksujns.10904.

Dönmez, O. (2008). Bursa ili koyunculuk işletmelerinin yetiştiricilik açısından yapısı. Namık Kemal Ünv. Sağlık Bilimleri Enstitüsü – Yüksek Lisans Tezi.

Erkuş, A., Bülbül, M., Kıral, T., Açıl, A., & Demirci, R. (1995). Tarım ekonomisi. Ankara Üniversitesi Ziraat Fakültesi Eğitim, Araştırma ve Geliştirme Vakfı, 5-29.

FAOSTAT, (2019). Food and Agriculture Organization of the United Nations-Statistic. https://www.fao.org/faostat/en/#data. . [Erişim tarihi: 04.03.2019]

Günaydın, G. (2009). Koyun yetiştiriciliğinin ekonomi politiği. Uludağ Üniversitesi Ziraat Fakültesi Dergisi, 23, 15-32.

Günlü, A., & Mat, B. (2021). Türkiye ekonomisinde koyun keçi yetiştiriciliğinin yeri ve önemi. Kuzu ve Oğlak

Kayıplarının Önlenmesinde Koyun Keçi Sağlığı ve Yetiştiriciliği (1. Baskı, s.3-14). Ankara, Akademisyen Yayınevi.

Israel, G.D. (2009). Determining sample size. University of Florida, IFAS Extension.

Kaymak, K. (2015). Muş ili Korkut ilçesinde koyunculuk işletmelerinin sosyo-ekonomik yapısı ve üretim maliyetleri. Erciyes Ünv. Sağlık Bilimleri Enstitüsü – Yüksek Lisans Tezi**.**

Kaymak, K.,& Sarıözkan, S. (2016): Muş ili Korkut ilçesinde koyunculuk işletmelerinin sosyo-ekonomik yapısı ve üretim maliyetleri. Van Vet J, 27, 141-146.

Köseman, A., Şeker, İ., Selim, K., & Karaca, M. (2022). Elazığ ilindeki koyunculuk işletmelerinde sürü yapısının ve yetiştiricilik uygulamalarının araştırılması. KSÜ Tarım ve Doğa Dergisi, 25, 555-565. https://doi.org/10.18016/ksutarimdoga.vi.1086849**.**

Mat, B. (2020). Balıkesir ilinde süt sığırcılığı yapan işletmelerin teknik ve sosyo ekonomik analizi ile rekabet güçlerine etki eden faktörlerin araştırılması. Ankara Ünv. Sağlık Bilimleri Enstitüsü – Doktora Tezi.

Nalan, A., & Bingöl, M. (2020). Diyarbakır ili koyunculuğun mevcut durumu. OKU Fen Bilimleri Enstitüsü Dergisi, 3, 213-218. https://doi.org/10.47495/okufbed.784713**.**

Özalp, M., & Sayın, C. (2018). Antalya'da küçükbaş hayvancılıkta sürdürülebilirliğe etki eden ekonomik faktörlerin değerlendirilmesi. KSÜ Tarım ve Doğa Dergisi, 21, 1-11. https://doi.org/10.18016/ksutarimdoga.vi.472776.

Öziş Altınçekiç, Ş. (2014). Bursa ili koyunculuk işletmelerinin yapısal özellikleri ve refah ölçütleri açısından değerlendirilmesi. Doktora Tezi, Uludağ Üniversitesi Fen Bilimleri Enstitüsü. Bursa.

Özsayın, D., & Everest, B. (2019). Koyun yetiştiriciliği yapan üreticilerin sosyo-ekonomik yapısı ve koyunculuk faaliyetiyle ilgili uygulamaları. KSÜ Tarım ve Doğa Dergisi, 22, 440-448. https://doi.org/10.18016/ksutarimdoga.vi.589725**.**

Özyürek, S., Türkyılmaz, D., Dağdelen, Ü., Esenbuğa, N., & Yaprak, M. (2018). Erzincan ili koyunculuk işletmelerinin yapısal özellikleri ve sorunlarının işletme büyüklüğüne göre incelenmesi. Akademik Ziraat Dergisi, 7, 219-226. https://doi.org/10.29278/azd.476651**.**

Satar, M., & Sakarya, E. (2021). Kırsal kalkınma kapsamında, genç çiftçi projeleri ile desteklenen küçükbaş hayvancılık işletmelerinin sosyo-ekonomik analizi. Eurasian J Vet Sci, 37, 274-285. https://doi.org/ 10.15312/EurasianJVetSci.2021.353**.**

Scheaffer, R.L., Mendenhall, W., & Gerow, K.G. (2011). Elementary survey sampling. Cengage Learning.

Semerci, A., & Çelik, A. (2016). Türkiye'de küçükbaş hayvan yetiştiriciliğinin genel durumu. MKÜ Ziraat Fakültesi Dergisi, 21(2).

Skapetas, B., & Kalaitzidou, M. (2017). Current status and perspectives of sheep sector in the world. Livestock Research for Rural Development, 29 (2).

Şahin, A. (2001). Van ili Merkez ilçede koyunculuk yapan işletmelerin ekonomik analizi. Yüzüncü Yıl Ünv. Fen Bilimleri Enstitüsü – Yüksek Lisans Tezi.

Şahinli, M. A. (2011). Konya ilinde koyunculuk faaliyetine yer veren tarım işletmelerinin ekonomik analizi ve koyunculuk faaliyetinde etkili olan unsurların saptanması. Doktora Tezi, Ankara Ünv. Fen Bilimleri Enstitüsü – Doktora Tezi.

Tamer, B., & Sarıözkan, S. (2017). Yozgat merkez ilçede koyunculuk yapan işletmelerin sosyo-ekonomik yapısı ve üretim maliyetleri. Erciyes Üniv Vet Fak Derg, 14(1), 39-47.

TÜİK, (2020). Türkiye İstatistik Kurumu. Hayvancılık istatistikleri. https://biruni.tuik.gov.tr. [Erişim tarihi: 14.03.2020)

TCMB, (2023). Türkiye Cumhuriyeti Merkez Bankası. Türkiye Cumhuriyeti Merkez Bankası. https://www.tcmb.gov.tr/. [Erişim tarihi: 21.03.2023)

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RESEARCH ARTICLE

The Effect of Granulocyte Colony Stimulating Factor Use in addition to Classical Treatment on Prognosis and Blood Values in Patients with Feline Panleukopenia

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ABSTRACT

Feline panleukopenia virus is a notable disease in cats and has a very contagious progression especially in young and not vaccinated individuals. Worse still, conventional treatments have a below 50 % success rate in treating this disease. This study investigated the prognostic success of administering a granulocyte colony-stimulating factor, filgrastim, with the conventional treatment for cats with feline infectious enteritis. The study had 48 sick individuals, 31 of whom had conventional + filgrastim treatment, whereas 17 had only conventional treatment (control group). A ratio of 93.61 % of all individuals showed leukopenia and 82.97 % of all individuals had neutropenia. The ratio of not vaccinated individuals in the study sample was 92.3 %. The recovery ratio in the study group in which filgrastim was administered was 72.41 %, whereas this ratio was 58.82 % in the control group, yet the difference was not statistically significant ($p>0.9999$). In the blood count values of the control group, there was no statistically significant difference between pre-treatment and post-treatment measurements. On the other hand, WBC, LYM, and NEU values were significantly different in the study group with additional filgrastim treatment ($p<0.001$). The study results indicated that vaccination is critical in protection against feline parvovirus, diarrhea, and vomiting symptoms in the first diagnosis are noteworthy, and using filgrastim in addition to the conventional treatment did not have a considerable impact on prognosis, although it did ameliorate blood values.
Keywords: Colony-stimulating factor, Feline panleukopenia Virus, Filgrastim, Leukopenia, Treatment

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Feline Panlökopeni Hastalarında Tedaviye Ek Koloni Stimüle Edici Faktör Kullanımının Kan Değerleri ve Prognostik Açıdan Önemi

ÖZ

Feline panlökopemi virüs özellikle genç ve aşısız bireylerde çok bulaşıcı seyreden, klasik tedaviler ile iyileşme oranları %50'nin altında olan, kedilerin önemli bir hastalığıdır. Bu çalışmada feline parvovirüs hastalarında klasik tedaviye ek olarak granülosit koloni sitümile edici faktör olarak filgrastim kullanımının prognostik başarısının incelenmesi amaçlanmıştır. Çalışmaya alınan 48 hastanın 31'inde filgrastim uygulaması yapılırken 17'si sadece klasik tedavi (kontrol grubu) yapılmıştır. Hastaların %93,61'inde lökopeni, %82,97'sinde ise nötropeni tespit edilmiştir. Hastaların %92,3'ünün aşısız olduğu görülmüştür. Filgrastim kullanılan grupta sağkalım %72,41 iken kontrol grubunda %58,82 dir ve aradaki fark istatistik yönden anlamsız bulunmuştur ($p>0,9999$). Kontrol grubunun tedavi öncesi ve sonrası kan değerlerindeki değişim anlamsız bulunurken filgrastim grubunda tedavi öncesi WBC, LYM, NEU değerlerinin tedavi sonrası değişimi istatistik olarak önemli bulunmuştur ($p<0,001$). Sonuç olarak feline parvovirüs hastalığından korunmada aşılamanın çok önemli olduğu, ilk muayenede ishal ve kusma gibi semptomların yanında lökopeninin dikkat çekici olduğu, tedavide klasik tedaviye ek olarak filgrastim kullanımının kan değerlerini yükselttiği ancak prognoza etkisinin olmadığı görülmüştür.
Anahtar kelimeler: Feline panlökopeni virus, Filgrastim, Lökopeni, Koloni sitümile edici faktör, Tedavi

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INTRODUCTION

Feline parvoviral enteritis, feline distemper, or more generally feline panleukopenia is a highly contagious and fatal disease for cats (Barker et al., 1983; Scott, 1987; Steinel et al., 2001). The factor is also known as Feline Panleukopenia Virus (FPV). This virus is in the Parvoviridae family, parvovirinae subfamily, protoparvovirus genus, and Carnivore protoparvovirus 1 species (Truyen et al., 1994). FPV has a single-stranded DNA without an envelope. Its genome is 18-22 nm in diameter, and 5 kb length. FPV is a small DNA virus with icosahedral symmetry (Miranda et al., 2017). FPV is highly resistant to environmental conditions and not vulnerable to a variety of chemical agents (e.g. alcohol, iodine, phenolic compounds, and chloroform). Nevertheless, sodium hypochlorite can quickly inactivate the virus (Rehme et al., 2022; Schultz and Scott, 1973). FPV is not sensitive to high or low environmental temperatures, and it can survive up to one year in room temperature conditions (Poole, 1972). The purpose of the treatment is to stimulate the immune system and facilitate active immunity. Generally, symptomatic treatments are preferred and applied. Parenteral liquid therapy is critical to re-establish the hydration, electrolyte, and acid-base equilibriums. Usually, the intravenous route is recommended (Rice, 2017). As long as vomiting continues, antiemetics should be administered, and oral feeding should be halted (Awad et al., 2019). Broad-spectrum antibiotics and the vitamin-B complex can be used (Hartmann and Hein, 2022; Rice, 2017). The most effective method to treat the disease and prevent it is vaccination and biosecurity precautions (Jacobson, 2021).

In recent years, granulocyte-colony stimulating factors (G-CSF), such as filgrastim, have been used in treating FPV disease. Thanks to these agents, higher treatment successes were reported (Rice, 2017). CSFs permit the generation of the primary cells from bone marrow and stimulate the blood cell processes. They are among the cytokines as growth factors (Akan, 1991). Nowadays, colony-stimulating factors can be genetically designed and administered as drugs. Filgrastim is the first known human recombinant G-CSF (Akan, 1991; Groblewska et al., 2004; Bolis et al., 2013). Moreover, in addition to its use in chemotherapy and Feline panleukopenia, veterinary medicine also utilizes filgrastim in lentivirus infections, e.g. FIV, and parvoviral enteritis in dogs (Bolis et al., 2013).

FPV disease is particularly prevalent in not vaccinated juvenile cats, or kittens. White blood cells decrease in number and immunity collapses in this disease. Hence, stimulating immunity is crucial in obtaining a successful treatment result. At this point, CSF agents, such as filgrastim, can be beneficial. This study aimed to uncover the impact of using filgrastim in addition to the conventional treatment applied to cats suffering from FPV disease.

MATERIALS AND METHOD

The study sample included 48 cats of both sexes and diverse races and 2-24 months old. Detailed general diagnosis of the cats brought to the clinics with depression, anorexia, diarrhea, vomiting, and fever symptoms and when the sick individuals were suspected of FPV disease, FPV quick diagnosis kit (Asan Easy Test® FPV Ag, ASAN PHARM. CO. LTD., Gyeonggi-do, Republic of Korea) were used. Individuals with positive test results for the disease were in the study sample.

The treatment for the disease included total parenteral nutrition, antibiotics, antiemetics, and stomach-protector medicines. Patients with FPV who were treated only with conventional treatment constituted the control group of this study. FPV patients who used filgrastim (FRAVEN® 30MIU/0.5 mL, ARVEN İlaç, Kırklareli, Turkey/Turkey) in their treatment in addition to conventional treatment formed the filgrastim group. Owner consent forms were obtained for the patients included in the study. Additionally, a retrospective (both retrospective and prospective data) scan using the disease monitoring program took place, and the data meeting the relevant criteria were in this study.

Filgrastim administration was in 6 μg/kg in the first, second, and third days subcutaneously. On the fourth day, a blood count test with the automatic tester device (Abacus Junior Vet 5, Hungary) was conducted, and for the individuals whose blood values rose back to the reference values, the treatment ended; otherwise, an additional two doses, in fifth and sixth days were also administered. Except for the one cat with agony and advanced dehydration, all cats had a blood count analysis in their first examination. The exceptional individual's treatment started immediately without a blood count test. Similarly, all individuals in the study (both study and control groups) had a blood count test on the fourth day. After the treatments, the study compared survival rates and pre and post-treatment blood values.

The evaluation of the results was done comparatively with percentage calculations for the symptoms observed from the individuals with FPV in the clinical examination and the impact of filgrastim use on survival. The check for the statistically significant difference between the survivals of the filgrastim and control group was done with chi-square tests. An independent t-test checked the difference in blood values before and after treatment.

RESULTS

Twenty-six of the 48 individuals in the study sample had information on vaccination: only two (7.7 %) were vaccinated. While forty-two individuals had racial information: twenty-two of them (52.38 %) were a mix, eleven (26.19 %) were tabby, three (7.14 %) were orange tabby, three (7.14 %) were British, one (2.38 %) was Bombay, one (2.38 %) was sphinx, and one (2.38 %) was Scottish. Finally, thirty-nine individuals had sex information, of which 21 (53.84 %) were female and eighteen (46.15 %) were male.

The first examination of the FPV cases with available clinical examination information had the following frequency of symptoms: 72.72 % vomiting, 45.45 % diarrhea, 93.93 % weakness and anorexia, and 3.03 % conjunctivitis. Twenty-six individuals had available information on their body temperature, and half had a value over 39.2°C . Blood count test results of the 47 FPV cases were available. The ratio of cases with leukopenia was 93.61 %, while it was 82.96 % for neutropenia.(Table 1).

According to the three age group divisions of the filgrastim group as younger than three months, between 3-12 months, and older than 12 months, the number of survivals to the total number in that group are as follows: 2/3 in younger than three months, 16/23 between 3-12 months, and 5/5 older than twelve months. The same division in the control group had the following survival outcomes: 2/4 in younger than three months, 7/11 between 3-12 months, and 1/2 older than twelve months. Considering the survival ratio according to the filgrastim use, the cases with filgrastim use had a survival ratio of 74.19 %, while the control group had 58.82 % (Table 2).

Table 1. Clinical symptoms observed in the first examination of the individuals with FPV diagnosis

Clinical symptom	Total Number of Sick Individuals	The Number of Individuals Showing the Clinical Symptom	The Frequency of the Clinical Symptom
Vomiting	33	24	72.72 %
Diarrhea	33	15	45.45 %
Fever	26	13	50.00 %
Weakness	33	31	93.93 %
Anorexia	33	31	93.93 %
Dehydration	33	12	36.36 %
Conjunctivitis	33	1	3.03 %
Leukopenia	47	44	93.61 %
Neutropenia	47	39	82.97 %

Table 2. The survival ratio of cats in different age groups according to the use of filgrastim

Age	Total	Survival	Death	Filgrastim
<3 months	3	2	1	Yes
3-12 months	23	16	7	Yes
>12 months	5	5	0	Yes
<3 months	4	2	2	No
3-12 months	11	7	4	No
>12 months	2	1	1	No
Total number of cats	48	33	15	-
Survived with filgrastim/Total filgrastim use	23/31	74.49 %	25.81 %	Yes
Survived without filgrastim/ Total of individuals without filgrastim	10/17	58.82 %	41.18 %	No

The comparison of the filgrastim group and control group according to the survival ratio did not yield statistically significant results (p=0.3364) (Figure 1).

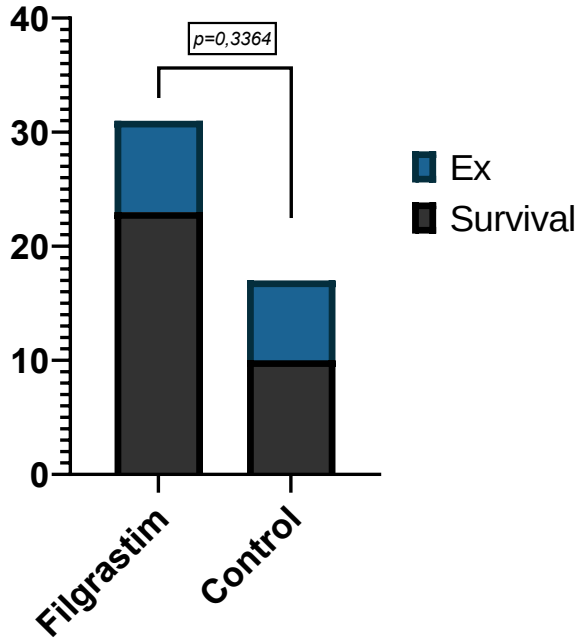

Figure 1. The evaluation of the filgrastim and control group in terms of prognosis

Comparing the filgrastim and control group's blood values in pre- and post-treatment periods; WBC, LYM, and NEU values were statistically significantly higher in the filgrastim group (p<0.001), while the differences between RBC and HCT values were not statistically significant (p>0.001). Whereas the difference in the corresponding values before and after the treatment in the control group was not statistically significant (p>0.001) (Table 3).

Table 3. The comparison of the filgrastim group and control group in terms of pre and post-treatment blood count values

	Filgrastim								
	Pre-treatment				Post-treatment				*p*
	n	Median	Mean	Std. Dev.	*n*	Median	Mean	Std. Dev.	
WBC	31	1.43	1.766129	1.696781	25	13.56	24.4284	24.72811	***p*>0.001**
LYM		0.59	0.666129	0.539065		2.82	3.8588	4.502287	***p*>0.001**
NEU		0.35	0.963548	1.421751		9.64	15.2796	15.194	***p*>0.001**
RBC		8.96	8.716129	3.192261		8.66	8.024	2.663145	*p*=0.389
HCT		33.9	34.19226	10.8136		32.08	31.754	9.892831	*p*=0.387
	Control								
	Pre-treatment				Post-treatment				*p*
	n	Median	Mean	Std. Dev.	*n*	Median	Mean	Std. Dev.	
WBC	16	1.665	6.69875	13.70496	10	11.165	11.327	7.518401	*p*=0.339
LYM		0.575	1.43125	2.306067		2.04	2.721	2.59193	*p*=0.198
NEU		0.525	4.517125	10.34071		6.045	6.9271	6.437099	*p*=0.516
RBC		7.955	7.628125	3.934362		8.13	7.859	1.602577	*p*=0.862
HCT		28.8	23.06125	20.35124		31.75	30.1881	12.39642	*p*=0.330

DISCUSSION

Generally, FPV is transmitted orally, and within 18-24 hours, it infests nasopharyngeal lymph nodes. Two-to-seven days later, it advances into the viremia state, and through the circulatory system, spreads all tissues and organs (Csiza et al., 1971). In the tissues with high mitotic activity, it infects the cells during the mitotic division (Garigliany et al., 2016; Parrish, 1995). It causes the destruction of the leukocytes in the bone marrow, spleen, and thymus. Thus, the infected individuals experience panleukopenia. Since the intestinal epithelial cells and crypts go through necrosis, malabsorption manifests. Disorders in absorption and ingestion result in diarrhea. Then, severe diarrhea leads to dehydration (Fei-Fei et al., 2017; Parrish, 1995).

FPV disease has been known since the beginning of the 20th century and has mostly been in cats without vaccination (Miranda et al., 2017). The effective vaccination procedure against FPV is as follows: first dose at the eighth-ninth weeks' old age, second dose after three to four weeks later, and third dose at 16-20 weeks old if the juvenile is in a high-risk environment. One year later, this procedure will be repeated, and the following vaccination periods will take place at three-year intervals (Truyen et al., 2009). Kruse et al. (2010) conducted a study including 244 cats with FPV. They reported that even if 39.7 % had a vaccination, none of them met the sample inclusion criteria of Truyen et al. (2009). Similar to that study, this study has mostly not vaccinated individuals (92.3 %). Considering the relevant literature and the results of this study, almost all individuals suffering from FPV are either not vaccinated or improperly vaccinated, which translates into the critical importance of vaccination in protection against FPV.

This study included mostly mixed and domestic short-hair races, with only approximately 15 % exotic ones. In the study of Kruse et al. (2010), over 90 % of the sick individuals were from domestic short-hair and mixed races. Citravoa et al. (2022) studied nine cats from domestic short-hair races. Among them, six had contact with the external environment, while three were shelter animals. At that point, their results were compatible with this study. Nevertheless, the fact that this study included a lower number of exotic race cases does not mean that such individuals are more resistant to FPV, but it is because there is a low number of exotic races in the cats brought to our clinics. Still more, exotic cats' street contact is generally limited, while a considerable fraction of the cats with FPV have extensive contact with the streets. In other words, a sampling bias might explain the race-related frequencies.

Juma (2023) reported that 40 % of the individuals with FPV were male and 60 % female. While Kruse et al. (2010) reported that 59.5 % of the sick individuals were male, while the remaining 40.5 % were female. This study sample had a similar sex distribution to other studies.

The clinical symptoms are particularly evident in this disease which is widespread globally and can impact a diverse set of races are intense gastroenteritis and leukopenia (Barrs, 2019). The first finding in the infected animals is leukopenia. The source of this problem is generally severe neutropenia. Moreover, fever, anorexia, weakness, and depression are present. Vomiting manifests most of the time. However, most cases may not show diarrhea (Addie et al., 1996; Greene, 2012; Litster and Benjanirut, 2013). This study observed the frequency of leukopenia in the sick individuals as 93.61 %, and 82.97 of all individuals had neutropenia. Approximately 93 % of the cases had anorexia and weakness. The prevalence of vomiting was 72.72 %, while diarrhea was present in 45.55 % of the cases. Half of the cases with body temperature measurements (13/36) had a fever. In the study of Citarova et al. (2022), which included nine cats, five had leukopenia, seven had mild apathy, eight had anorexia and intermittent vomiting, and all cases had a fever. However, no cases showed diarrhea in their early stage. Different from the literature, one cat had a different symptom, conjunctivitis. Nevertheless, this finding might not be directly related to FPV and may be a coexisting condition with another source.

The survival ratio in individuals with FPV older than six-to-eight weeks can vary according to the following: virus load, immunity, age, and infectious comorbidities developed with the FPV (Foley et al., 1999). Generally, recovering cases show hints of recovery in the first seven days of the treatment (Avad et al., 2019; Greene, 2012). Death generally develops after dehydration, septicemia, and disseminated intravascular coagulation (Litster and Benjanirus, 2013). The acute form of FPV has a varying fatality between 25-90 %, while the peracute form may have up to 100 % fatality (Addie et al., 1998; Cave et al., 2002). FPV, which progresses quite fatally in cats, had a varying survival ratio after the conventional treatment, changing between 11.2 % and 57.1 % (Kruse et al., 2011; Litster and Benjanirut, 2014; Porporato et al., 2018; Barrs, 2019; Isaya et al., 2021; Citarova et al., 2022). One study added Neupogen (filgrastim) to the treatment, and the recovery rate was almost three times higher compared to the control group (33 % vs. 91 %) (Rice, 2017). Rice (2017) attributed this very high success rate to the early diagnosis and additional filgrastim treatment to the aggressive symptomatic treatment. This study conducted a comparative case for that of Rice's study (2017) but did not observe a considerable difference in prognosis after using an additional filgrastim in the treatment schedule. While the survival ratio was 72.41 % in the filgrastim group, it was 58.82 % in the control. The literature values on the survival ratio of the control

group vary between 11.2-57.1 % and this study has a similar result. Even though the survival ratio in the filgrastim group is notably higher than the control group, the difference was low compared to Rice's (2017) study. One reason is that Rice (2017) excluded cases with agony from the calculations. In this study, even the cases that died right after 24 hours from the onset of the treatment, i.e. without successfully concluding the treatment, were present in the study. Without modifying the control group, excluding these cases from the filgrastim group, and then, making the calculations widens the gap in favor of the filgrastim use and changes the treatment efficiency statistics considerably.

The survival rate in individuals in the filgrastim group younger than three months and older than 12 months was the same, 50 %. However, this ratio was 70 % in cases in the filgrastim group between 3-12 months old. Rice (2017) reported a 100 % recovery in FPV cases younger than three months after filgrastim-included treatment. Whereas, only one case in the four older than three months responded negatively to the filgrastim treatment. This lost case brought in agony and treatment started in that condition (Rice, 2017). Previous studies did not uncover a statistically significant relationship between the severity of the clinical symptoms, and consequence of the disease, and the age of the case (Kruse et al., 2010). In short, both studies reported similar survival ratios in different age groups, and the results were in line with the previous studies.

Considering the blood count values, Kuffer and Frank (1999) reported a very positive impact of filgrastim use in cats with FPV on WBC count. While there was no statistically significant difference in prognosis ($p=0.3364$), pre and post-treatment blood count parameters WBC, LYM, and NEU values were significantly different ($p<0.001$).

CONCLUSION

As a result, vaccination is critical in protecting from FPV. Indeed, only a fraction of the cats with FPV were vaccinated. Anorexia, weakness, vomiting, diarrhea, fever, and dehydration are remarkable clinical outcomes in cats with FPV, and a very high prevalence of leukopenia is noteworthy. While the extra filgrastim administration did not significantly affect the survival ratio between the filgrastim and control groups, WBC, LYM, and NEU values from blood count tests were higher in the filgrastim group and this difference was statistically significant. Considering all the data, future studies including larger study samples on filgrastim use to treat FPV disease are promising to reach the targeted success ratio in FPV treatment.

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Authors' Contributions: Authors contributed equally in the design, data collection, and manuscript preparation phases of this study.

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REFERENCES

Akan, Ö.A. (1991). Koloni Stimüle Edici Faktörler. Mikrobiyoloji Bülteni 25, 285-293.

Awad, R.A., Hassan, S.A., Marets, B. (2019). Treatment of Feline panleukopenia virus infection in naturally infected cats and its assessment. J Biol. Sci., 19(2), 155-160. https://doi.org/10.3923/jbs.2019.155.160

Addie, D.D., Jarrett, O., Simpson, J., Thompson, H. (1996). Feline Parvovirus in Pedigree Kittens. Veterinary Record 138, 119.

Addie, D.D., Toth, S., Thompson, H., Greenwood, N., Jarrett, J.O. (1998). Detection of Feline Parvovirus in Dying Pedigree Kittens. Veterinary Record 142, 353-356.

Barker, I.K., Povey, R.C., Voigt, D.R. (1983). Response of Mink, Skunk, Red fox and Raccoon to İnoculation with Mink Virus Enteritis, Feline Panleukopenia and Canine Parvovirus and Prevalence of Antibody to Parvovirus in Wild Carnivores in Ontario. Canadian Journal of Comparative Medicine, 47, 188-197.

Barrs, V.R. (2019). Feline panleukopenia: a re-emergent disease. Veterinary Clinics: Small Animal Practice, 49(4), 651-670. https://doi.org/10.1016/j.cvsm.2019.02.006

Bolis, S., Cocorocchio, E., Corti, C., Ferreri, A.J.M., Frungillo, N., Grillo, G., Omodeo, E.S., Tedeschi, L., Zilioli, V.R. (2013). Clinical implications, safety, efficacy of recombinant human Granulocyte Colony-Stimulating Factors and pegylated equivalent. Epidemiology Biostatistics and Public Health, Volume 10, Number 4. https://doi.org/10.2427/8845

Cave, T.A., Thompson, H., Reid, S.W., Hodgson, D.R., Addie, D.D. (2002). Kitten Mortality in the United Kingdom: A Retrospective Analysis of 274 Histopathological Examinations (1986 to 2000). Veterinary Record 151, 497-501. https://doi.org/10.1136/vr.151.17.497

Citarova, A., Mojzisova, J., Vojtek, B., Zakutna, L., Drazovska, M. (2022). Nachweis von FPV, CPV-2a, CPV-2b und FCoV bei Katzen mit Symptomen der felinen Panleukopenie. Berliner und Münchener Tierärztliche Wochenschrift 135, 1–6. https://doi.org/10.2376/1439-0299-2022-13

Csiza, C.K., Scott, F.W., De Lahunta, A., Gillespie, J.H. (1971a). İmmune Carrier State of Feline Panleukopenia Virus-Infected Cats. American Journal of Veterinary Research 32, 419-426.

Csiza, C.K., Scott, F.W., De Lahunta, A., Gillespie, J.H. (1971). Pathogenesis of Feline Panleukopenia Virus in Susceptible Newborn Kittens. II. Pathology and Immunofluorescence. Infection and Immunity 3, 838-846.

Digangi, B.A., Gray, L.K., Levy, J.K., Dubovi, E.J., Tucker, S.J. (2011). Detection Protective Antibody Titers Against

Feline Panleukopenia Virus, Feline Herpesvirus-1 and Feline Calicivirus in Shelter Cats Using a Point-of-care an ELISA. Journal Feline Medicine and Surgery 13, 912- 918. https://doi.org/10.1016/j.jfms.2011.07.009

Digangi, B.A., Levy, J.K., Griffin, B., McGorray, S.P., Dubovi, E.J., Dingmann, P.A., Tucker, S.J. (2012). Prevalence of serum antibody titers against feline panleukopenia virus, feline herpesvirus 1, and feline calicivirus in cats entering a Florida animal shelter. JAVMA, 241(10), 1320-1325. **https://doi.org/10.2460/javma.241.10.1320**

Esfendiari, J., Klingeborn, B. (2000). A Comparative Study of a New Rapid and One-Step Test for The Detection of Parvovirus in Faeces from Dogs, Cats, and Mink. Journal of Veterinary Medicine. B, Infectious Diseases and Veterinary Public Health 47, 145-153.

Fei-Fei, D., Yong-Feng, Z., Jian-Li, W., Xue-Hua, W., Kai, C., Chuan-Yi, L., Zhi-Jing, X. (2017). Molecular characterization of feline panleukopenia virus isolated from mink and its pathogenesis in mink. Vet. Microbiol, 205, 92-98. **https://doi.org/10.1016/j.vetmic.2017.05.017**

Foley, J.E., Orgad, U., Hirsh, DC., Poland, A., Pedersen, N.C. (1999). Outbreak of Fatal Salmonellosis in Cats Following Use of A High-Titer Modified-live Panleucopenia Virus Vaccine. Journal of the American Veterinary Medical Association 214; 67-70, 43-64.

Garigliany, M., Gilliaux, G., Jolly, S., Casanova, T., Bayrou, C., Gommeren, K., Fett, T., Mauroy, A., Levy, E., Cassart, D., Peeters, D., Poncelet, L., Desmecht, D. (2016). Feline panleukopenia virus in cerebral neurons of young and adult cats. BMC Vet Res, 12(1), 1-9. https://doi.org/10.1186/s12917-016-0657-0

Greene, C.E. (2012). Feline enteric viral infections. In: Infectious diseases of the dog and cat, p. 80-91. 4th ed. St. Louis, Mo., Elsevier/Saunders.

Groblewska, M., Mroczko, B., Szmitkowski, M. (2004). Czynnik stymuluj1cy tworze- ni kolonii makrofagowych (M-CSF) i jego zastosowanie w praktyce klinicznej. Pol. Arch. Med. Wewn 111, 355-365.

Gür, S., Avdatek, K. (2016). A Serological İnvestigation for Feline Panleucopenia Virus in Cats in Afyonkarahisar. Kocatepe Veterinary Journal 9(3), 165-170. https://doi.org/10.5578/kvj.26547

Isaya, R., Ciccarelli, S., Enache, D., Specchi, S., Pesaresi, M., Ferri, F., Porporato. F., Auriemma, EB., Coppola, LM., Zini, E. (2021). Correction to: Gastrointestinal ultrasonographic findings in cats with Feline panleukopenia: a case series. BMC Vet Res 17(1), 143. https://doi.org/10.1186/s12917-020-02720-w

Jacobson, L.S., Janke, K.J., Giancinti, J., Weese J.S. (2021). Diagnostic testing for feline panleukopenia in a shelter setting: a prospective, observational study. J Feline Med Surg, 23(12), 1192-1199. https://doi.org/110.1177/1098612X211005301

Jumaa, MA. (2023). Feline Panlökopenide Tiyol Disülfit Dengesi Ve İskemi Modifiye Albümin (İma) Seviyelerinin Araştırılması. Yüksek Lisans Tezi, Ankara Üniversitesi Sağlık Bilimleri Enstitüsü, Ankara.

Kuffer-Frank, M., Jung, H., Kraft, W. (1999). Use of recombinant human granulocyte colony-stimulating factor (r-metHuG-CSF) in neutropenic cats. Tierarztliche Praxis Ausgabe K: Kleintiere – Heimtiere 27, 136-143.

Kruse, B.D., Unterer, S., Horlacher, K., Sauter-Louis, C., Hartmann, K. (2010). Prognostic Factors in Cats with Feline Panleucopenia. J Vet İntern Med 24, 1271-1276. https://doi.org/10.1111/j.1939-1676.2010.0604.x

Kruse, BD., Unterer, S., Horlacher, K., Sauter-Louis, C., Hartmann, K. (2011). Feline Panleukopenie – differierender Krankheitsverlauf bei Katzen im Alter von unter bzw. über 6 Monaten. Tierarztl Prax Kleintiere 39(04), 237–242. **https://doi.org/10.1055/s-0038-1623584**

Litster, A., Benjanirut, C. (2013). Case Series of Feline Panleucopenia Virus in An Animal Shelter. Journal of Feline Medicine and Surgery 16(4), 346-353. https://doi.org/10.1177/1098612X13497738

Meurs, K.M., Fox, P.R., Magnon, A.L., Liu, S., Towbin, J.A. (2000). Molecular Screening by Polymerase Chain Reaction Detects Panleukopenia Virus DNA in Formalin-Fixed Hearts From Cats with İdiopathic Cardiomyopathy and Myocarditis. Cardiovascular Pathology, 9, 119-126. https://doi.org/10.1016/S1054-8807(00)00031-4

Mende, K., Stuetzer, B., Mueller, R.S., Sauter Louis, C., Homeier Bachmann, T., Truyen, U., Hartmann, K. (2013). Prevalenz von Antikörpern Gegen Feline Parvoviren bei Katzen in Süddeutschland. Tierarztliche Praxis 2, A27.

Mende, K., Stuetzer, B., Truyen, U., Hartmann, K. (2014). Evaluation of An in-House Dot Enzyme Linked İmmunosorbent Assay to Detect Antibodies Against Feline Panleukopenia Virus. Journal of Feline Medicine and Surgery, 16(10), 805-811. doi: 10.1177/1098612X14520812

Mende, K., Stuetzer, B., Sauter-Louis, C., Homeier, T., Truyen, U., Hartmann, K. (2014). Prevalence of antibodies against feline panleukopenia virus in client-owned cats in Southern Germany. Vet J, 199(3), 419-423. https://doi.org/10.1016/j.tvjl.2013.12.023

Miranda, C., Viera, M.J., Silva, E., Carvalheira, J., Parrish, C.R., Thompson, G. (2017). Genetic Analysis of Feline Panleukopenia Virus Full-length VP 2 Gene in Domestic Cats Between 2006-2008 and 2012-2014, Portugal. Transboundary and Emerging Diseases, 64(4), 1178-1183. https://doi.org/10.1111/tbed.12483

Parrish, C.R. (1995). Pathogenesis of feline panleukopenia virus and canine parvovirus. Bailliere's Clinical Haematology, 8(1), 57-71.

Poole, G.M (1972). Stability of A Modified, Live Panleukopenia Virus Stored in Liquid Phase. Applied Microbiology 24, 663-664.

Porporato, F., Horzinek, MC., Hofmann-Lehmann, R., Ferri, F., Gerardi, G., Contiero, B., Vezzosi, T., Rocchi, P., Auriemma, E., Lutz, H., Zini, E. (2018). Survival estimates and outcome predictors for shelter cats with feline panleukopenia virus infection. J Am Vet Med Assoc 253(2), 188-195. https://doi.org/10.2460/javma.253.2.188

Proksch, A.L., Unterer, S., Truyen, U., Mueller, R.S., Hartmann, K. (2015). İnfluence of Clinical and Laboratory Variables on Fecal Antigen ELISA Results in Dog with Parvovirus infection. Journal of Veterinary İnternal Medicine 27, 721. https://doi.org/10.1016/j.tvjl.2015.03.009

Rehme, T., Hartmann, K., Truyen, U., Zablotski, Y., Bergmann, M. (2022). Feline Panleukopenia Outbreaks and Risk Factors in Cats in Animal Shelters. Viruses, 14(6), 1248. https://doi.org/10.3390/v14061248

Rice J.K., 2017. Successful Treatment of Feline Panleukopenia: A guideline for rescuers and veterinarians, Part I. J Vet Sci Med Diagn, 6, 2. https://doi.org/10.4172/2325-9590.1000223

Schatzberg, S.J., Haley, N.J., Barr, S.C., Parrish, C., Steingold, S., Summers, B.A., Delahunta, A., Kornegay, J.N., Sharp, N.J. (2003). Polymerase chain reactions (PCR) Amplication of parvoviral DNA from the

brains of dogs and cats with cerebellar hypoplasia. Journal of Veterinary İnternal Medicine 17, 538-544.

Schmitz, S., Coenen, C., Konig, M., Thiel, H.J., Neiger, R., (2009). Comparison of Three Rapid Commercial Canine Parvovirus Antigen Detection Tests with Electron Microscopy and Polymerase Chain Reaction. Journal of Veterinary Diagnostic İnvestigation 21, 344-345.

Schultz, R.D., Scott, F.W., (1973). Absence of an immune response after oral administration of attenuated feline panleukopenia virus. Infection and Immunity, 7(4), 547-549.

Schultz, R.D., Ford, R.B., Olsen, J. (2002). Titer Testing and Vaccinations: A New Look At Traditional Practices. Veterinary Medicine 1-13.

Scott, F.W., 1987. Viral Diseases: Panleukopenia. In: Holzworth, J. (Ed.), Diseases of the Cat: Medicine and Surgery, WB Saunders Co, Philadelphia, PA, USA, pp. 182-193.

Steinel, A., Parrish, C.R., Bloom, M.E., Truyen, U. (2001). Parvovirus Infections in Wild Carnivores. Journal of Wildlife Diseases 37, 594-607.

Truyen, U., Agbanddje, M., Parrish, C.R. (1994). Characterization of the feline host range and a specific epitope of feline panleukopenia virus. Virology, 200(2), 494-503.

Truyen, U., Addie, D., Belak, S., Boucraut-Baralon, C., Egberink, H., Frymus, T., Gruffydd-Jones, T., Hartman, K., Hosie, MJ., Lloret A., Lutz H., Marsilio, F., Pennisi, MG., Rodford,, AD., Thiry, E., Horzinek MC. (2009) Feline panleukopenia. ABCD guidelines on prevention and management. Journal of Feline Medicine &Surgery, 11(7), 538-546. https://doi.org/10.1016/j.jfms.2009.05.002

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RESEARCH ARTICLE

The Investigation of the Relationship of Three Different Agents Causing Scabies in Cats and Dogs with Gender, Age and Some Behavioral Changes

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ABSTRACT

Scabies is a common parasitic disease in cats and dogs. This disease can easily spread between animals. It is also important in terms of zoonosis. Behavioral problems such as itching, fear, restlessness, aggression, growling, and loss of appetite may occur in animals infected with mange. These behavioral changes can cause communication problems between animals and their owners. In this case, both the health and welfare of the animal are adversely affected. Detection of behavioral changes associated with the disease allows the elimination of these behavioral problems. In this study, the relationship between the causative agents of scabies in cats and dogs and the behavioural changes that occur during scabies, the relationship between the causative agents of scabies and the sex of the animals, as well as the relationship between the causative agents of scabies and the age of the animals were investigated. As a result, a significant correlation was found between the causative agents of scabies in dogs and head shaking, fear, anorexia, and pinnal-pedal reflex. However, no significant correlation was found between aggression and growling behaviors and scabies causative agents in dogs. In cats, it was determined that there was a significant relationship between aggression and anorexia with the factors causing scabies, but there was no significant relationship found between fear, growling, and pinnal-pedal reflex. Finally, the research found no relationship between the age and sex of the animals and scabies agents.

Key words: Behavioral change, Pinnal-pedal reflex, Scabies, Skin disease

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Kedi ve Köpeklerde Uyuza Neden Olan Üç Farklı Etkenin Cinsiyet, Yaş ve Bazı Davranış Değişiklikleri ile İlişkisinin Araştırılması

ÖZ

Uyuz, kedi ve köpeklerde sıklıkla gözlenen paraziter bir hastalıktır. Bu hastalık hayvanlar arasında kolaylıkla yayılabilmektedir. Ayrıca zoonoz olması açısından da önem arz etmektedir. Uyuz hastalığına yakalanan hayvanlarda kaşıntı, korku, huzursuzluk, saldırganlık, hırlama ve iştahsızlık gibi davranış problemleri meydana gelebilmektedir. Bu davranış değişiklikleri hayvanlar ve sahipleri arasında iletişim problemlerine neden olabilmektedir. Bu durumda hayvanın hem sağlığı hem de refahı kötü etkilenmektedir. Hastalık ile ilişkili davranış değişikliklerinin tespit edilmesi bu davranış problemlerinin giderilmesine olanak tanımaktadır. Çalışmada kedi ve köpeklerde uyuz hastalığını meydana getiren etkenler ile uyuz hastalığı sırasında meydana gelen davranışsal değişiklikler, hayvanların cinsiyetleri ve hayvanların yaşı arasındaki ilişki varlığı araştırılmıştır. Sonuç olarak köpeklerde uyuza neden olan etkenler ile kafa sallama, korkma, iştahsızlık ve kulak-ayak refleksi arasında anlamlı bir ilişki belirlenirken, saldırganlık ve hırlama davranışları ile uyuz etkenleri arasında anlamlı bir ilişki olmadığı tespit edilmiştir. Kedilerde ise uyuz hastalığını meydana getiren etkenler ile saldırganlık ve iştahsızlık arasında anlamlı bir ilişkinin olduğu ancak korkma, hırlama ve kulak-ayak refleksi arasında anlamlı bir ilişkinin olmadığı görülmüştür. Hayvanların yaşı ve cinsiyeti ile uyuz etkenleri arasında bir ilişki olmadığı tespit edilmiştir.

Anahtar kelimeler: Davranış değişikliği, Deri hastalıkları, Kulak-ayak refleksi, Uyuz

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GİRİŞ

Kedi ve köpekler dünyada en çok yetiştirilen pet hayvanlarıdır (Oğuz, 2016). Bu hayvanlarda görülen hastalıkların bir kısmını deri hastalıkları oluşturmaktadır. Bazı deri hastalıkları çok ciddi bir problem oluşturmasa da bazıları hem hastanın genel durumunu bozmakta hem de zoonotik olması nedeniyle insanlara bulaşması yönünden daha fazla önem taşımaktadır (Arlian ve ark., 1984; Perego ve ark., 2019; Şimşek ve ark., 2019). Deri hastalıklarından biri olan uyuz hastalığı, tarihi eskiye dayanan ektoparaziter bir hastalıktır. Bu hastalığın etkeni artrapodlar şubesi içerisindeki Arachnoid sınıfında bulunan astigmatik ve prostigmatik akarlardır (Çakmak ve Vatansever, 1997; Saygın, 2022). Bu akarlar, kedi ve köpeklerin derisindeki üst tabakada tünel açmakta ve deri altında yaşayıp üremektedirler (Scott ve ark., 2001; Hill, 2006; Canpolat ve ark., 2018). Bu tüneller ve parazitin dışkıları, hayvanın derisinde yoğun kaşıntı, kızarıklık, kabuklanma, kıl dökülmesi ve hayvanda kilo kaybı gibi belirtilere neden olmaktadır (Curtis, 2004). Yaşamak ve üremek için ihtiyacı olan besini hayvanın cildindeki ölü deri hücreleri, yağ, ter ve kan ile sağlayan bu parazitlerin neden olduğu uyuz hastalığı, kedi ve köpeklerde farklı türler tarafından oluşturulmakta ve tüm deri yüzeyine yayılabilmektedir (Taylor ve ark., 2007). Köpeklerde uyuz hastalığı genellikle Sarcoptes scabiei, *Demodex canis* ve *Otodectes cynotis* gibi etkenlerin neden olduğu enfestasyon ile ortaya çıkmaktadır (Aydın, 2017). Bu etkenler köpeklerde özellikle kulakların içinde, dirseklerde ve karın bölgesinde lokalize olmaktadır. Bu etkenler bulaşıcıdır ve diğer köpeklere de kolaylıkla yayılabilmektedir (Ghubash, 2006). Kedilerde gözlenen uyuz hastalığına *Demodex cati*, *Notoedres cati, Otodectes cynotis* parazitlerinden kaynaklanan enfestasyonlar yol açmaktadır (Ghubash, 2006). *Demodex cati* kedilerin özellikle baş, yüz ve boyun bölgesinde kızarıklığa, kabuklanmaya ve tüy dökülmesine neden olmaktadır (Malik ve ark., 2006). *Notoedres cati* ise özellikle kulakların içinde bulunmakta, aynı zamanda baş ve boyun bölgesindeki deriyi etkilemektedir. Bu etken de diğer uyuz etkenleri gibi kaşıntı, kızarıklık ve kabuklanma gibi semptomlara yol açmaktadır. Kedilerde meydana gelen otodektik uyuz tüm dünyada prevalansı yüksek önemli bir paraziter enfestasyondur. Kedilerde meydana gelen otodektik uyuz enfestasyonunun erken dönemlerinde klinik belirtiler hemen oluşmayabilmektedir. Ancak bu etken köpeklerde ciddi klinik belirtilere sebep olabilmektedir (Aydın, 2017). Kedilerde de uyuz hastalığı bulaşıcıdır ve diğer canlılara bulaş söz konusu olabilmektedir (Sivajothi ve ark., 2015). Uyuz hastalığının görülme sıklığı mevsime, hayvanın ırkına veya cinsiyetine bağlı değildir (Thomas ve ark., 2020). Ancak deri hastalıklarından korunmak için koruyucu kıl örtüsü açısından iyi bir bakım ve besleme çok önemlidir (Cebecioğlu ve ark., 2012).

Hastalığın prevalansı ülkelerin sosyal ve ekonomik düzeylerine göre değişebilmektedir (Akgöl ve ark., 2022). Genetik faktörler, yaş, kötü bakım ve besleme, barınma ve travma bu hastalık için predispozisyon oluşturan sebepler arasındadır (Canpolat ve ark., 2018). Uyuz enfestasyonunun tanısında deri kazıntısı örnekleri alınmaktadır (Scott ve ark., 2001; Basavashree ve ark., 2022). Bir hayvana uyuz teşhisi konulması halinde, temas halindeki diğer hayvanların da enfestasyon varlığı açısından muayene edilmesi ve temasın hemen kesilmesi gerekmektedir. Ayrıca uyuz teşhisi konulan hayvanların kullandığı malzemeler atılmalı veya steril edilmelidir (Anonim, 2020). Bu hastalıktan hijyen dışında spesifik bir korunma şekli yoktur çünkü uyuz hastalığının aşısı henüz bulunmamıştır (Bhunu ve ark., 2013). Uyuz hastalığı nedeniyle meydana gelen şiddetli kaşıntı ve ağrı sebebiyle hayvanlarda çeşitli davranış değişiklikleri gözlenmektedir (Taylor ve ark., 2007; Aydın, 2017). Uyuzlu bir hayvanın davranışları, genellikle kaşıntının şiddetine bağlı olarak değişmektedir. Meydana gelen kaşıntı, hayvanların huzursuz ve agresif davranışlar sergilemesine neden olabilmekte ve bu durum hasta hayvanın diğer hayvanlar ve sahibi ile ilişkilerini etkileyebilmektedir (Miller ve ark., 2012). Uyuz etkenleri ile enfeste hayvanlarda kaşınma davranışı başta olmak üzere kafa sallama, huzursuzluk, saldırganlık, hırlama, iştahsızlık, kulak-ayak refleksi gibi davranışlar görülebilmektedir (Mueller ve ark., 2001). Kedi ve köpeklerde saldırganlık ve korku gibi davranışları anlamak için davranışların dikkatli bir şekilde gözlemlenmesi gerekmektedir. Saldırganlık davranışı sergileyen kedi ve köpeklerde kulaklarda dikleşme, kuyrukta gerilme ve dikleşme, göz bebeklerinde büyüme, diş gösterme, tıslama ve havlama, öne doğru atılma gibi tepkiler gözlenebilmektedir (Bennett ve ark., 2012). Literatürde kedilerde ve köpeklerde gözlenen "huzursuzluk" davranışı normalde ilgilendiği aktiviteleri yapmaktan kaçınma ve aktivite seviyesinin azalması, sevdiği yemekleri yemekten kaçınma, uykusuzluk, esneme, normalden daha az ilgili olma ve sosyal geri çekilme olarak tanımlanmaktadır (Beerda ve ark., 1998). Kedi ve köpeklerde gözlenen "korku" davranışı ise titreme, alçaltılmış duruş, kuyruğunu altına sokma veya geriye doğru kıvırma, sırtı kamburlaştırma, saklanmaya, kaçmaya ve geri kaçmaya çalışma, kulaklarını geride tutma, göz temasından kaçınma, titreyen sesler çıkarma ve agresif tepkiler ile kendisini göstermesiyle tanımlanmaktadır (Flint ve ark., 2018). Kulak-ayak refleksi, uyuzlu hayvanlarda sıklıkla görülen refleksif bir davranıştır. Bu refleks, kulağın arkasındaki deriye dokunulduğunda veya o bölgedeki deriye sürtünme uygulandığında meydana gelmektedir. Bu durum sonucunda hayvanın ipsilateral arka bacağı hareket etmekte ve hayvan kaşınma benzeri bir hareket yapmaktadır (Mueller ve ark., 2001). Bu refleks,

hayvanın deri duyusunun ve kas hareketlerinin normal çalıştığını göstermektedir. Bu nedenle uyuzlu bir hayvanın tedavisi sırasında, kulak-ayak refleksi gibi çeşitli refleksler test edilerek hayvanın tedaviye yanıt verip vermediği değerlendirilebilmektedir (Muir ve ark., 2009; Hayden ve ark., 2016). Bu çalışmada kedi ve köpeklerde meydana gelen davranış değişiklikleri, hayvanların cinsiyetleri ve yaşları ile hastalık etkenleri arasında bir ilişki olup olmadığının araştırılması amaçlanmaktadır.

MATERYAL ve METOT

Araştırmanın materyalini 01.01.2022 – 31.12.2022 tarihleri arasında özel bir veteriner kliniğine deri hastalığı şüphesi ile getirilip uyuz teşhisi konulan 68 köpek ve 31 kedi olmak üzere toplam 99 tane uyuz etkenleri ile enfeste hayvan oluşturmuştur. Kliniğe getirilen hayvanların muayenesi veteriner hekim tarafından gerçekleştirilmiştir. Kliniğe kaşıntı ve deri döküntüsü şikayeti ile gelen hastaların öncelikle anamnezi alınmış daha sonra fiziksel muayeneleri yapılmıştır. Ektoparazitten şüphelenilen hayvanların lezyonlu bölgelerinden deri kazıntısı örnekleri toplanılmış ve lam üzerine alınarak %10'luk KOH ile muamele edilmiştir. 30 dakika bekletildikten sonra binoküler ışık mikroskobunda (XSP-107BN, Çin) 10X, 40X ve 100X objektifler aracılığı ile hazırlanan preparatlar incelenmiştir (Chee ve ark., 2008; Chadwick ve ark., 2016; Kandi, 2017). Bu şekilde hastalığın tanısı konulmuş ve aynı zamanda etken identifikasyonu yapılmıştır. Kulak kaşıntısı ve kafa sallama şikayeti ile getirilen hastaların fiziksel ve otoskopik muayeneleri yapılmış ve kulak uyuzu süpheli hayvanlardan kulak sekresyonu örnekleri toplanmıştır. Yoğun kıvamda olan kulak sekresyonu, pamuklu swap yardımıyla lam üzerine alınarak %10'luk KOH ile muamele edilerek binoküler ışık mikroskobunda (XSP-107BN, Çin) 10X, 40X ve 100X objektifler aracılığıyla incelenmiştir. Hastalığın tanısı bu şekilde konulmuş ve etken identifikasyonu yapılmıştır (Rataj ve ark., 2004; Chadwick ve ark., 2016). Hastaların türü, ırkı, yaşı, cinsiyeti, identifiye edilen etken ve uyuz hastalığı sırasında hayvanın sergilemiş olduğu davranış değişiklikleri protokol defterine kaydedilmiştir (Ellis, 2018). Meydana gelen davranış değişiklikleri, ilgili davranışın literatürdeki tanımlarına sadık kalınarak 5 hafta boyunca haftada 1 kere olacak şekilde hayvanlar tedavi için kliniğe getirildiğinde veteriner hekim tarafından gözlemlenmiştir.

İstatistiksel Analiz

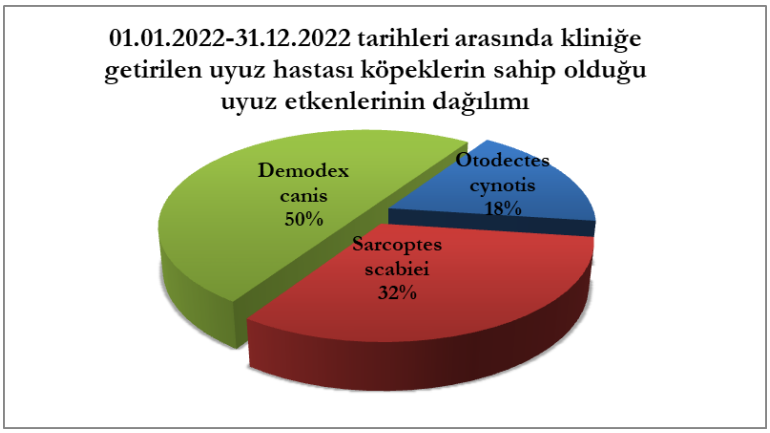
Elde edilen veriler, gözlenen davranış değişiklikleri ile uyuz etkenleri arasında bir ilişki olup olmadığının tespit edilmesi amacıyla Jamovi istatistik programı kullanılarak Ki-kare testi ile analiz edilmiştir. Ayrıca hayvanların yaşı ve cinsiyeti ile uyuz etkenleri arasında bir ilişki olup olmadığı yine aynı test kullanılarak analiz edilmiştir. Bu bağlamda kediler 0-4 ay yavru, 4-12 ay genç ve 12 ay + erişkin kabul edilerek 3 gruba ayrılmıştır. İlk grupta 3, ikinci grupta 18 ve üçüncü grupta 10 hayvan bulunmaktadır. Köpekler de aynı şekilde 0-4 ay yavru, 4-12 ay genç ve 12 ay+ erişkin kabul edilerek 3 gruba ayrılmıştır. Köpeklerin oluşturduğu ilk grupta 8, ikinci grupta 33, üçüncü grupta ise 27 hayvan bulunmaktadır.

BULGULAR

Bu çalışmada, dermotolojik hastalıkların köpeklerde kedilerden daha sık görüldüğünü bildiren bir çalışmayı (Saygın, 2022) destekler nitelikte kliniğe 1 yıl içerisinde getirilen uyuz etkenleri ile enfeste hayvanların %69'unu köpeklerin, %31'ini ise kedilerin oluşturduğu gözlemlenmiştir (Şekil 1.).

Şekil 1: Kliniğe getirilen uyuz etkenleri ile enfeste kedi ve köpeklerin dağılımı
Figure 1: Distribution of cats and dogs infected with scabies agents brought to the clinic

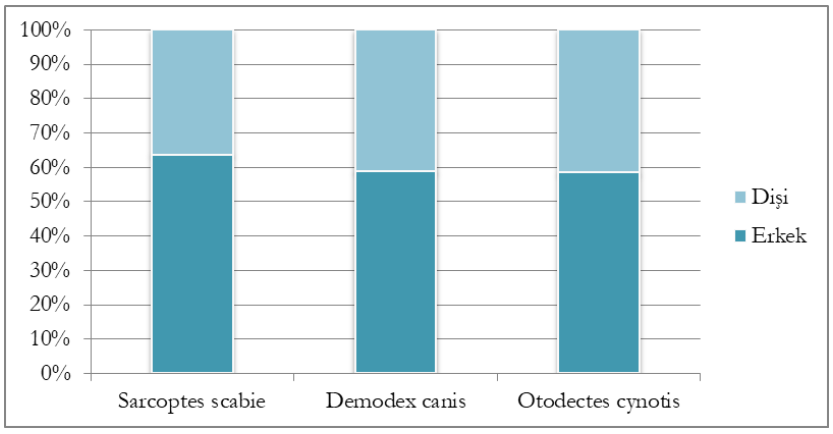
Ayrıca kliniğe getirilen köpeklerin %32'sinin *Sarcoptes scabiei*, %50'sinin *Demodex canis*, %18'inin ise *Otodectes cynotis* ile enfeste (Şekil 2.), kedilerin ise %16'sının *Demodex cati*, %42'sinin *Notoedres cati* ve yine %42'sinin *Otodectes cynotis* ile enfeste olduğunu tespit edilmiştir (Şekil 3.).



Şekil 2: Kliniğe getirilen uyuz etkenleri ile enfeste köpeklerin etkenler bazında dağılımı
Figure 2: Distribution of dogs infected with scabies agents brought to the clinic on the basis of causative agents

Şekil 3: Kliniğe getirilen uyuz etkenleri ile enfeste kedilerin etkenler bazında dağılımı
Figure 3: Distribution of cats infected with scabies agents brought to the clinic in terms of causative agents

Yapılan çalışmada sarcoptes scabiei ile enfeste köpeklerin %36'sının dişi, %64'ünün erkek, *Demodex canis* ile enfeste köpeklerin %41'inin dişi, %59'unun erkek ve *Otodectes cynotis* ile enfeste köpeklerin ise %42'sinin dişi, %58'inin erkek olduğu tespit edilmiştir (Şekil 4.). Yapılan ki-kare analizi sonucu, uyuz etkenleri ile cinsiyet arasında ilişki bulunmadığını bildiren bir çalışmayı (Thomas ve ark., 2020) destekler nitelikte uyuz etkenleri ve cinsiyet arasında istatistiksel açıdan anlamlı bir ilişki olmadığı tespit edilmiştir (Tablo 1.).



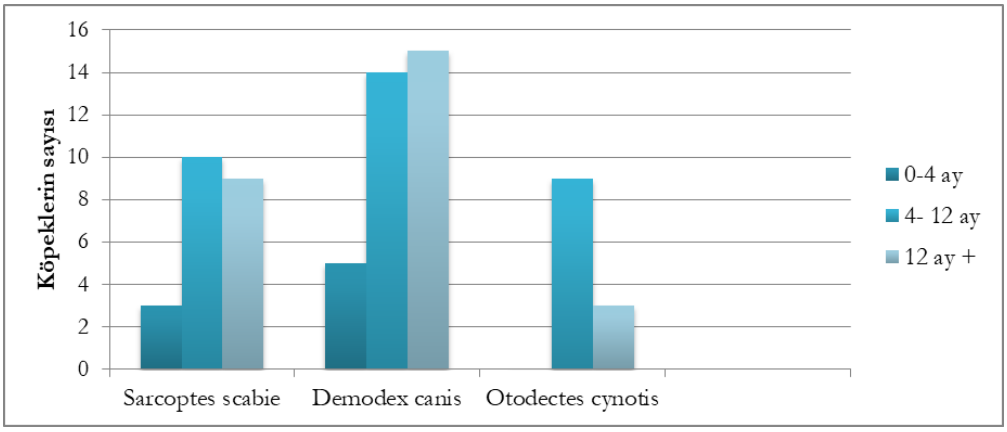
Şekil 4: Farklı uyuz etkenleri ile enfeste erkek ve dişi köpeklerin dağılımı
Figure 4: Distribution of male and female dogs infected with different scabies agents

Tablo 1. Farklı uyuz etkenleri ve köpeklerin cinsiyeti arasındaki ilişkinin ki-kare testi ile tespit edilmesi
Table 1. Chi-square test for the relationship between different scabies agents and the sex of the dogs

İLİŞKİSEL TABLO

	Cinsiyet			
Köpek Uyuz Etkenleri	**Erkek**	**Dişi**	**Toplam**	
Sarcoptes scabiei	14	8	22	
Demodex canis	20	14	34	
Otodectes cynotis	7	5	12	
Toplam	41	27	68	**X^2 testi**
				p değeri: 0.927

Uyuz etkenleri ile enfeste köpekler 0-4 ay yavru, 4-12 ay genç ve 12 ay+ erişkin kabul edilerek 3 gruba ayrılmıştır. Köpeklerde farklı uyuz etkenlerinin oluşturduğu enfestasyonların yaşlara göre dağılımı Şekil 5.'de gösterilmiştir. Bu köpeklerin yaşları ve uyuz etkenleri arasındaki ilişki araştırılmış ve istatistiksel açıdan anlamlı bir ilişki olmadığı bulunmuştur (Tablo 2.).



Şekil 5: Farklı uyuz etkenleri ile enfeste köpeklerin yaşlarına göre dağılımı
Figure 5: Distribution of dogs infected with different scabies agents according to age

Tablo 2. Farklı uyuz etkenleri ve köpeklerin yaşları arasındaki ilişkinin ki-kare testi ile tespit edilmesi
Table 2. Chi-square test for the relationship between different mange agents and age of dogs

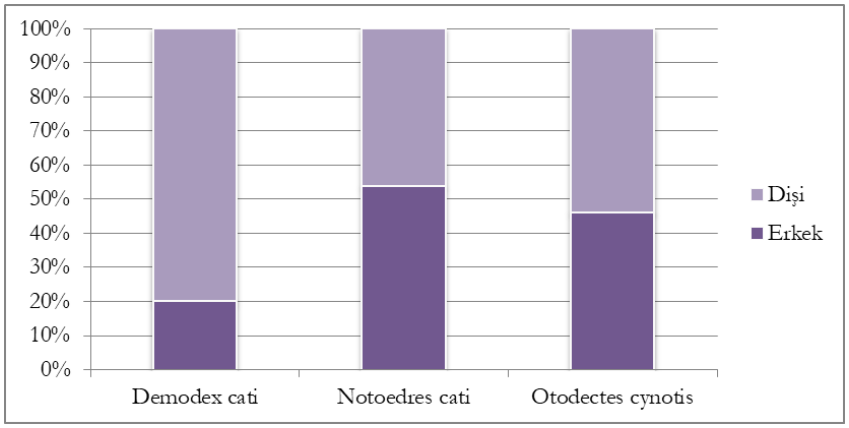
İLİŞKİSEL TABLO

	Yaş Aralığı			
Köpek Uyuz Etkenleri	**1.grup (0-4 ay)**	**2.grup (4-12 ay)**	**3.grup (12 ay +)**	**Toplam**
Sarcoptes scabiei	3	10	9	22
Demodex canis	5	14	15	34
Otodectes cynotis	0	9	3	12
Toplam	8	33	27	68

4 aylık ve 12 aylık yaşta olan hayvanlar 2. gruba dahil edilmiştir.

X^2 testi
p değeri: 0.318

Kliniğe getirilen *Demodex cati* ile enfeste kedilerin %80'inin dişi, %20'sinin erkek, *Notoedres cati* ile enfeste kedilerin %46'sının dişi, %54'ünün erkek, *Otodectes cynostis* ile enfeste kedilerin ise %54'ünün dişi, %46'sının erkek olduğu tespit edilmiştir (Şekil 6.). Yapılan ki-kare testi sonucunda kedilerde uyuz etkenleri ve cinsiyet arasında istatistiksel açıdan anlamlı bir ilişki olmadığı tespit edilmiştir (Tablo 3.).



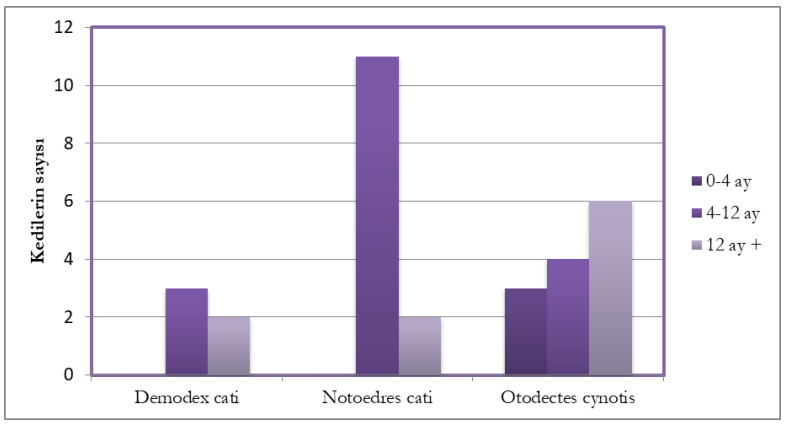
Şekil 6: Farklı uyuz etkenleri ile enfeste erkek ve dişi kedilerin dağılımı
Figure 6: Distribution of male and female cats infected with different scabies agents

Tablo 3. Farklı uyuz etkenleri ve kedilerin cinsiyeti arasındaki ilişkinin ki-kare testi ile tespit edilmesi
Table 3. Chi-square test for the relationship between different scabies causative agents and sex of cats

İLİŞKİSEL TABLO

	Cinsiyet			
Kedi Uyuz Etkenleri	**Erkek**	**Dişi**	**Toplam**	
Demodex cati	1	4	5	
Notoedres cati	7	6	13	
Otodectes cynotis	6	7	13	
Toplam	14	17	31	**X^2 testi**
				p değeri: 0.432

Kliniğe getirilen kediler 0-4 ay yavru, 4-12 ay genç ve 12 ay+ erişkin olarak 3 gruba ayrılmıştır. Kedilerde farklı uyuz etkenlerinin oluşturduğu enfestasyonların yaşlara göre dağılımı Şekil 7.'da gösterilmiştir. Bu kedilerin yaşları ve uyuz etkenleri arasında istatistiksel açıdan anlamlı bir ilişki olmadığı tespit edilmiştir (Tablo 4.).



Şekil 7: Farklı uyuz etkenleri ile enfeste kedilerin yaşlarına göre dağılım grafiği
Figure 7: Distribution graph of cats infected with different mange agents according to age

Tablo 4. Farklı uyuz etkenleri ve köpeklerin yaşları arasındaki ilişkinin ki-kare testi ile tespit edilmesi
Table 4. Chi-square test for the relationship between different mange agents and age of dogs

İLİŞKİSEL TABLO

	Yaş Aralığı				
Kedi uyuz etkenleri	**1.grup (0-4 ay)**	**2. grup (4-12 ay)**	**3. grup (12 ay +)**	**Toplam**	
Demodex cati	0	3	2	5	
Notoedres cati	0	11	2	13	
Otodectes cynotis	3	4	6	13	
Toplam	3	18	10	31	**X^2 testi**
					p değeri: 0.051

4 aylık ve 12 aylık yaşta olan hayvanlar 2. gruba dahil edilmiştir.

Uyuz etkenleri ile enfeste hayvanlarda gözlenen en belirgin davranış kaşınmadır. Kliniğe getirilen uyuz etkenleri ile enfeste hayvanların tamamında kaşınma davranışı olduğu gözlemlenmiştir. Ancak hayvanlarda meydana gelen kaşıntı tek bir hastalığa özgü değildir. Bu nedenle ayırıcı tanı için anamnez, fiziksel muayene ve laboratuvar testleri önemli bir yer tutmaktadır. Hayvanlarda meydana gelen yoğun kaşıntı nedeniyle tüm hayvanlarda huzursuzluk şekillendiği, hayvanların aktivite seviyelerinin düştüğü ve ilgilerinin azaldığı gözlemlenmiştir. Köpeklerde meydana gelen kafa sallama davranışı ve uyuz etkenleri arasında istatistiksel açıdan anlamlı bir ilişki bulunmuştur. Bu duruma, uyuz etkenlerinin boyun, baş ve kulak bölgesinde lokalize olmasının neden olduğu düşünülmektedir. Köpeklerde korkma davranışı ile uyuz etkenleri arasında anlamlı bir ilişki tespit edilirken saldırganlık davranışı ile uyuz etkenleri arasında anlamlı bir ilişki olmadığı tespit edilmiştir. Kedilerde ise tam tersi bir durum söz konusudur. Her iki hayvan türünde de uyuz etkenleri ve iştahsızlık arasında anlamlı bir ilişki olduğu tespit edilirken, hırlama davranışı ile uyuz etkenleri arasında anlamlı bir ilişki olmadığı tespit edilmiştir. Elde edilen bulgular köpeklerde uyuz etkenleri ve kulak-ayak refleksi arasında bir ilişki olduğunu gösterirken kedilerde bu durumun tersi söz konusudur. Elde edilen bu sonuçlar, uyuz hastalığının köpekler ve kediler arasında farklı etkilere sahip olabileceğini göstermektedir (Tablo 5., Tablo 6.)

Tablo 5. Farklı uyuz etkenleri ile enfeste köpeklerde meydana gelen davranış değişiklikleri
Table 5. Behavioral changes in dogs infected with different scabies agents

Davranış değişiklikleri	Sarcoptes scabiei	Demodex canis	Otodectes cynotis	p değeri
Kafa sallama	0/22	0/34	12/12	< .001
Korkma	0/22	1/34	3/12	0.007
Saldırganlık	7/22	12/34	4/12	0.964
İştahsızlık	16/22	7/34	4/12	< .001
Hırlama	4/22	5/34	3/12	0.721
Kulak-ayak refleksi	5/22	0/34	5/12	< .001

Tablo 6. Farklı uyuz etkenleri ile enfeste kedilerde meydana gelen davranış değişiklikleri
Table 6. Behavioral changes in cats infected with different mange agents

Davranış değişiklikleri	Demodex cati	Notoedres cati	Otodectes cynotis	p değeri
Korkma	0/5	0/13	3/13	0.1
Saldırganlık	3/5	10/13	3/13	0.021
İştahsızlık	5/5	11/13	6/13	0.029
Hırlama	3/5	9/13	8/13	0.895
Kulak-ayak refleksi	3/5	7/13	9/13	0.722

TARTIŞMA

Uyuz, kedi ve köpeklerde yaygın görülen paraziter bir deri hastalığıdır. 2000-2011 yılları arasında Arnavutluk (Xhaxhiu ve ark., 2009), Kore (Chee ve ark., 2008), Meksika (Rodriguez-Vivas ve ark., 2003), İran (Mosallanejad ve ark., 2012) dahil olmak üzere dünyanın bir çok bölgesinde uyuz hastalığının insidansı araştırılmıştır. Bu çalışmaların sonucuna göre *Demodex canis* prevalansının %0,2-23; *Sarcoptes scabiei* prevalansının %0,7-35,6 ve *Otodectes cynotis* prevalansının ise %2,8-24,26 olduğu bildirilmiştir (Kaya ve ark., 2018). Türkiyede Van ve yöresinde yapılan bir çalışmada ise köpeklerdeki *Demodex canis* prevalansının %40 olduğu bildirilmiştir (Değer et al. 1994). Uyuz hastalığına sahip hayvanlarda bir takım davranış değişiklikleri meydana gelmektedir. Hayvan sağlığını ve refahını iyileştirmek adına hayvanlarda meydana gelen bu davranış değişikliklerinin altında yatan nedenlerin bilinmesi gerekmektedir. Hasta hayvanların geç fark edilmesi ya da enfestasyonun erken dönemlerinde hastalığın göz ardı edilmesi pet hayvanlarında deri rahatsızlıklarının tedavisini güçleştirmektedir. Bu nedenle, hasta hayvanlarda meydana gelen normal ve anormal davranışların bilinmesi hastalıkların erken safhalarında tespit edilebilmesine ve tedavi edilebilmesine olanak sağlayarak hayvanların sağlığının sürdürülmesi ve refahının iyileştirilmesine katkıda bulunmaktadır. Uyuz etkenlerinin bazıları (*Otodectes cynotis*) hayvanların kulak yoluna yerleşip burada enfeksiyon oluşturmakta ve hayvanlarda duyma kaybına yol açmaktadır. Diğer bazı etkenler (*Sarcoptes scabeii, Demodex* spp.) ise derinin çeşitli katmanlarına yerleşerek burada soyucu-sömürücü etki oluşturmakta ve aynı zamanda sekonder bakteriyel etkenlerin de devreye girmesi ile beraber daha komplike bir tablo oluşturarak hayvanlarda huzursuzluk ve agresyon meydana getirebilmektedir (Yipel, 2015; Aydın, 2017). Enfeste kedilerde ayaklar ile kulağı kaşıma refleksinin yaygın olarak gözlendiği tespit edilmiştir. (Scott ve ark., 2001). Ayrıca uyuz etkenleri ile enfeste hayvanlarda sıklıkla iştahsızlık tablosunun da şekillendiği bildirilmiştir (Behera ve ark., 2011). Yapılan bir çalışmada uyuz enfestasyonları ve cinsiyet dağılımları karşılaştırılmış ve erkeklerin dişilerden %9.4 oranında daha fazla enfeste olduğu ortaya konulmuştur (Chee ve ark., 2008). Bu sonuç her iki cinsiyetin de eşit derecede duyarlı olduğunu öne süren çalışmalar ile çelişmektedir (Nayak ve ark., 1997; Rodriguez-Vivas ve ark., 2003). Benzer bir araştırma Hatay yöresinde bulunan sokak köpeklerindeki uyuz etkenlerinin insidansını tespit etmek amacı ile yapılmıştır. Bu çalışmada da hayvanların yaşı ve cinsiyeti ile uyuz etkenleri arasında istatistiksel açıdan anlamlı bir ilişki olmadığı bildirilmiştir (Kaya ve ark., 2018). Ayrıca Arnavutluk ve İranda yapılan çalışmalarda yaş ve uyuz enfestasyonu arasındaki ilişki araştırılmış ve hayvanların yaşı ve uyuz enfestasyonu arasında bir ilişki olmadığı bildirilmiştir. Ancak Kore'de yapılan diğer bir çalışmada yavru köpeklerdeki prevalansın yetişkinlere göre çok daha yüksek olduğu (%66,7) bildirilmiştir (Chee ve ark., 2008; Xhaxhiu ve ark., 2009; Mosallanejad ve ark., 2012).

SONUÇ

Sonuç olarak köpeklerde bulunan uyuz etkenleri ile kafa sallama, korkma, iştahsızlık, kulak-ayak refleksi arasında anlamlı bir ilişki olduğu, saldırganlık ve hırlama davranışları ile uyuz etkenleri arasında ise anlamlı bir ilişki olmadığı tespit edilmiştir. Kedilerde ise saldırganlık ve iştahsızlık ile uyuz etkenleri arasında bir ilişki tespit edilirken, korkma, hırlama ve kulak-

ayak refleksi arasında ilişki tespit edilmemiştir. Uyuz hastalığının sadece deri belirtileri ile sınırlı olmayıp hayvanlarda davranışsal belirtilere de neden olabilmektedir bu nedenle veteriner hekimlerin, hayvan sahiplerinin ve araştırmacıların hastalığın bu belirtilerini göz ardı etmemeleri gerekmektedir. Hayvanlarda uyuz etkenlerinin meydana getirdiği davranış değişikliklerinin dikkate alınması ile hastalığın erken teşhis edilmesi ve hayvan refahının arttırılması mümkün olabilir.

Etik onay: Bu çalışma "Hayvan Deneyleri Etik Kurullarının Çalışma Usul ve Esasları Hakkında Yönetmelik" 8(k) uyarınca HADYEK'in iznine tabi değildir. Bu makalede sunulan veri, bilgi ve belgeler akademik ve etik kurallar çerçevesinde elde edilmiştir.

Çıkar çatışması: Yazarların rapor edecekleri herhangi bir çıkar çatışması yoktur.

Yazar Katkı Oranları: YP ve EYGA çalışmanın proje fikrine, tasarımına ve yürütülmesine katkıda bulundular. YP, verilerin toplanmasına katkıda bulundu. YP ve EYGA verileri analiz ederek taslağı hazırladı ve metni yazdı. YP ve EYGA metni eleştirel bir gözle incelediler. Tüm yazarlar son halini alan makaleyi okudu ve onayladı.

KAYNAKLAR

Akgöl,J., & Köroğlu, A. Uyuz Hastalığı (Skabiyes) Tedavisi ve Uyuz Hastalığı Tedavisinde Kullanılan Bitkiler (2022). Ankara Eczacılık Fakültesi Dergisi, 46 (2), 600-618.

Arlian, L. G., Runyan, R. A., Achar, S., & Estes, S. A. (1984). Survial and infectivity of sarcoptes scabici var. Canis and var. Journal of the American Academy of Dermatology, 11, 210-5.

Aydın, L. (2017). Akar Enfestasyonları (UYUZ). Veteriner Hekimliğinde Parazit Hastalıkları. İzmir: Türkiye Parazitoloji Derneği.

Basavashree, K., Halmandge, S.C., & Patil, N. A. (2022). Diagnosis and therapeutic management of feline scabies. The Pharma Innovation Journal, 11(10), 1847-1848.

Beerda, B., Schilder, M. B., Van Hooff, J. A., De Vries, H. W., & Mol, J. A. (1998). Behavioural, saliva cortisol and heart rate responses to different types of stimuli in dogs. Applied Animal Behaviour Science, 58(3-4), 365-381.

Behera, S.K., Dimri, U., Singh, S.K., & Mohanta, R.K. (2011). The curative and antioxidative efficiency of ivermectin and ivermectin+ vitamin E-selenium treatment on canine Sarcoptes scabiei infestation. Veterinary Research Communications, 35, 237-244.

Bennett, S.L., Litster, A., Weng, H. Y., Walker, S.L., & Luescher, A.U. (2012). Investigating behavior assessment instruments to predict aggression in dogs. Applied Animal Behaviour Science, 141(3-4), 139-148.

Bhunu, C.P., Mushayabasa, S., & Monero, T. G. (2013). Assessing the impact of vaccination on controlling the spread of human scabies. ISRN Computational Biology, 2013,362973.

Canpolat, İ., Çakır, S., & Aktaş, C. (2018). İstanbul ilindeki Veteriner Kliniklerine Getirilen Kedi ve Köpeklerde Deri Hastalıklarının Görülme Oranlarının Araştırılması, Erciyes Üniversitesi Veteriner Fakültesi Dergisi, 15 (2), 110-116.

Cebecioğlu, V.H., & Aslıhan D.(2012). Deri sağlığı ve tüy kalitesinde beslenmenin önemi. Petinfo, 46:18-25.

Centers for Disease Control and prevention, web cite (2020). https://www.ede.gov/parasites/scabies/prevent.html

Chadwick, A., Mayer, U., Ordeix, L., & Prélaud, P. (2016). Microscopic diagnosis of ectoparasites. https://vetgrad.com/core_modules/entityshow.php?Entity=10MinuteTopUps&ID=119.

Chee, J.H., Kwon, J.K., Cho, H.S., Cho, K.O., Lee, Y.J., Abd El-Aty, A.M., Shin, S.S. (2008). A survey of ectoparasite infestations in stray dogs of Gwang-ju City, Republic of Korea. The Korean Journal of Parasitology, 46(1), 23-27

Curtis, C.F. (2004). Current trends in the treatment of Sarcoptes, Cheyletiella and Otodectes mite infestations in dogs and cats. Veterinary Dermatology, 15 (2), 108–114.

Çakmak A., & Vatansever Z., (1997). Hayvanlarda Uyuz Hastalığı, Artropod Hastalıkları ve Vektörler. Türkiye Parazitoloji Derneği Yayınları, 13, 317- 337.

Deger, S., Taşçı, S., Akgül, Y., Alkan, İ. (1994). Van ve Yöresinde Evcil Hayvanlarda Ektoparaziter Dermatitisler. Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Dergisi, 5(1), 155-161.

Ellis, S. L. (2018). Recognising and assessing feline emotions during the consultation: history, body language and behaviour. Journal of feline medicine and surgery, 20(5), 445-456.

Flint, H. E., Coe, J. B., Pearl, D. L., Serpell, J. A., & Niel, L. (2018). Effect of training for dog fear identification on dog owner ratings of fear in familiar and unfamiliar dogs. Applied Animal Behaviour Science, 208, 66-74.

Ghubash, R. (2006). Parasitic miticidal therapy. Clinical techniques in small animal practice, 21(3), 135-144.

Hayden, K. M. (2016). Sarcoptic mange in dogs: Diagnosis and management. Veterinary Medicine: Research and Reports, 7, 109-115.

Hill, P.B. (2006). Survey of the prevalence, diagnosis and treatment of dermatological conditions in small anımals in general practive. Veterinary Record, 158 (16), 533 -39.

Kandi, V. (2017). Laboratory diagnosis of scabies using a simple saline mount: a clinical microbiologist's report. Cureus, 9(3).

Kaya, Ö.M., Akküçük, Ş., Karagöz, M., Zerek, A., & Yaman, M. (2018). A Survey of Mange-Mite in Stray Dogs from Hatay province. Van Veterinary Journal, 29(2), 67-70.

Malik, R., Stewart, K. M., Sousa, C. A., Krockenberger, M. B., Pope, S., Ihrke, P., ... & Walton, S. (2006). Crusted scabies (sarcoptic mange) in four cats due to Sarcoptes scabiei infestation. Journal of Feline Medicine and Surgery, 8(5), 327-339.

Miller, W. H., Griffin, C. E., & Campbell, K. L. (2012). Muller and Kirk's small animal dermatology. Elsevier Health Sciences.

Mosallanejad, B., Alborzi, A., Katvandi, N. (2012). A Survey on Ectoparasite Infestations in Companion Dogs of Ahvaz District, South-west of Iran. J Arthropod Borne Dis, 6(1), 70-78.

Mueller, R., Bettenay, S. V., & Shipstone, M. (2001). Value of the pinnal- pedal reflex in the diagnosis of canine scabies. Veterinary Record, 148(20), 621-623.

Muir, W. W., & Hubbell, J. A. E. (2009). Handbook of veterinary anesthesia (4th ed.). Mosby Elsevier.

Nayak, D.C., Tripathy, S.B., Dey, P.C., Ray, S.K., Mohanty, D.N., Parida, G.S., ... & Das, M. (1997). Prevalence of canine demodicosis in Orissa (India). Veterinary Parasitology. 73(3-4), 347-352.

Oğuz, K. (2016). Kedi ve Köpeklerin Bakım ve Beslenmesi ile Beslenmeye Bağlı Bazı Problemler ve Davranışlar Arasındaki İlişkiler. Mustafa Kemal Üniversitesi Hatay, Doktora Tezi.

Perego, R., Spada, E., Foppa, C., & Proverbio, D. (2019). Critically appraised topic for the most effective and safe treatment for canine generalised demodicosis. BMC Veterinary Research, 15 (17), 1-7.

Rataj, A.V., Posedi, J., & Bidovec, A. (2004). Ectoparasites: Otodectes cynotis, Felicola subrostratus and Notoedres cati in the ear of cats. Slovenıan Veterınary Research, 41, 89-92.

Rodriguez-Vivas, R.I., Ortega-Pacheco, A., Rosado-Aguilar, J.A., Bolio, G.M. (2003). Factors affecting the prevalence of mange-mite infestations in stray dogs of Yucatan, Mexico. Veterinary Parasitology, 115(1), 61-65

Saygın, B. (2022). Uyuz ile doğal enfeste köpeklerde bazı kimyasal ve esansiyel yağların karşılaştırılması. Bursa Uludağ Üniversitesi Sağlık Bilimleri Enstitüsü, Doktora Tezi.

Scott, D. W., Millier, W.H., & Griffen, C.E. (2001). The prevalence of dermatophytosis in dogs and cats. William, H., Miller, J., Griffin, C.E., Karen, L. Cds. In : Muller and kirk's small animal dermatology. Philadelphia PA : W.B. Saunders company, PP. 432-38.

Sivajothi, S., Sudhakara Reddy, B., Rayulu, V. C., & Sreedevi, C. (2015). Notoedres cati in cats and its management. Journal of Parasitic Diseases, 39, 303-305.

Şimşek, E., Keskin, A., & Dağcıoğlu, B. F. (2019). Sık Rastlanan ve Sık Atlanan Hastalık Uyuz: Olgu Sunumu, Ankara Medical Journal, (1), 205-9

Taylor, M.A., COOP, R. L., & Wall, R.L. (2007). Veterinary Parasitology Fourth Edition: Susssex, UK : Wiley Black Well.

Thomas, C., Coates, S.J., Engelman, D., Chosidow, O., & Chang, A. Y. (2020). Ectoparasites : Scabies. Journal of the American Academy of Dermatology, 82 (3), 533-548.

Xhaxhiu, D., Kusi, I., Rapti, D., Visser, M., Knaus, M., Lindner, T., Rehbein, S. (2009). Ectoparasites of dogs and cats in Albania. Parasitology Research, 105(6), 1577-1587.

Yipel, F.A. (2015). Kedilerde Otodectes Cynotis' in Kulak Problemleri Arasındaki Yeri: Güncel Tanı ve Tedavi Yaklaşımları. Atatürk Üniversitesi Veteriner Bilimleri Dergisi., 10(1).

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RESEARCH ARTICLE

Updated Molecular Characterization of Orf Virus in Türkiye

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ABSTRACT

Orf (ORFV) virus is endemic in sheep and goats in Türkiye and detected with increasing incidence in Spring. Despite this significant prevalence rate of ORFV, a few molecular characterization studies have been conducted in Türkiye and all of them have focused on only B2L gene region so far. The aim of this study was to determine the molecular characterization of different gene regions of ORFV isolated from field studies. In the study, partial genome sequencing of different gene regions (B2L, F1L and VIR) of positive ORFV isolates detected by real-time PCR from seven sheep with suspected Orf in 2018-2020 were performed. Molecular characterizations were determined by bioinformatics studies. After the molecular and in-silico processes, we found remarkable diversification in the evaluation of each gene region. This result showed that different variants are more likely to have circulated in different parts of the Türkiye. When the study is evaluated as a whole, it is thought that the F1L gene region should be considered in addition to the B2L gene region in molecular studies. Molecular data of ORFV should be updated and followed to provide the best efficiency of the prevention and control strategy.

Keywords: Amino acid, molecular, orf virus, phylogeny, Türkiye

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Türkiye'de Orf Virusunun Güncellenmiş Moleküler Karakterizasyonu

ÖZ

Orf virusu Türkiye'de koyun ve keçilerde endemiktir ve ilkbaharda artan oranlarda tespit edilmektedir. ORFV'nun ülkedeki bu önemli prevalans oranına rağmen, Türkiye'de bugüne kadar birkaç moleküler karakterizasyon çalışması yapılmış ve hepsi sadece B2L gen bölgesine odaklanmıştır. Bu çalışmanın amacı saha çalışmalarında izole edilen ORFV'nun farklı gen bölgelerinin moleküler karakterizasyonlarının belirlenmesidir. Çalışmada, 2018-2020 yıllarında Orf şüpheli yedi adet koyundan real-time PCR ile izole edilen pozitif ORFV izolatlarına ait farklı gen bölgelerinin (B2L, F1L ve VIR) kısmi genom dizilemesi yapıldı. Biyoenformatik çalışmalar ile moleküler karakterizasyonları belirlenerek GenBank'ta bulunan izolatlarla ilişkileri değerlendirildi. Moleküler ve in-siliko çalışmalardan sonra, her bir gen bölgesinin değerlendirilmesinde dikkate değer bir çeşitlilik tespit edildi. Bu sonuçlar, farklı varyantların ülkenin farklı bölgelerinde dolaşıma girmiş olma olasılığının yüksek olduğunu göstermiştir. Çalışma bir bütün olarak değerlendirildiğinde, moleküler çalışmalarda B2L gen bölgesinin yanı sıra F1L gen bölgesinin de dikkate alınması gerektiği düşünülmektedir. Hastalıkla ilgili korunma ve kontrol stratejisinin en iyi verimini sağlamak amacıyla ORFV'nun moleküler verileri güncellenmeli ve takip edilmelidir.

Anahtar Sözcükler: Amino asit, filogeni, moleküler, orf virus, Türkiye

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INTRODUCTION

Orf virus (ORFV) causes a significant disease, referred to as contagious ecthyma (CE), in different animal species (sheep, goat, etc.) and human. ORFV belongs to the genus of *Parapoxvirus*, a subfamily of *Chordopoxvirinae,* family of *Poxviridae* (ICTV 2020). ORFV is an epitheliotropic virus, and it causes proliferative lesions on the skin. Lesions are mostly local epitheliotropic; however, it may convert to a life-threating disease in case of complication with a secondary infection or occurrence on mouth, lips, nostrils, and nasal area (Spyrou and Valiakos 2015). Lambs are more sensitive than sheep and, the disease is more severe in the late summer and early fall. Although mortality is low, morbidity is approximately 90% (Fleming and Mercer 2007, Nettleton et al. 1996). The genome of ORFV is linear double-stranded DNA which is 139 kb in length. The genome has high GC content at the rate of nearly 65%; 88 genes are identified for ORFV genome and some of them are the forefront for molecular characterization (Delhon et al. 2004). *B2L* (ORFV011) is the essential gene region for the genetic and immunogenic characterization of ORFV (Abrahão et al. 2009, Hosamani et al. 2006). Similary, F1L (ORFV059) is another immunogenic gene of ORFV (Zhao et al. 2011). Besides these large immunogenic envelope genes, virulence genes were identified which were claimed important for pathogenesis and immune responses such as VIR (ORFV020), VEGF, vIL-10, GIF (Peralta et al. 2018). Considering the molecular characterization studies of orf virus in Türkiye, only the B2L gene region was studied. (Akkutay-Yoldar et al. 2016, Karakas et al. 2013, Şevik 2017 and 2019). Sheep and goat breeding is an important part of animal production in Türkiye. Significant efforts have been spent to improve through projects conducted in recent years. In this context, studies are carried out on many viral diseases including CE. CE is widespread in Türkiye, and especially affects the health of sheep and goat. There hasn't been much notification related to zoonotic cases of orf virus in Türkiye thus far. Different gene regions belonging to ORFV have not yet been characterized in Türkiye (Akkutay-Yoldar et al. 2016, Karakas et al. 2013, Şevik 2017 and 2019). The aim of this study was multiple comparisons using by three gene regions, B2L, F1L, and VIR, to better understand the current genetic circumstances. In this context, we performed phylogenetic analyses and amino acid comparisons for each gene region.

MATERIALS AND METHODS

Samples and preparation

In this study, samples were taken from sheep with suspected CE. Oral swab samples taken from six sheep were added to 2 ml PBS and vortexed, and the supernatant was collected and used for DNA extraction. Tissue samples (liver, spleen, lymph nodes, etc.) taken from a sheep were homogenized by homogenizer. The homogenate was centrifuged at 3500 rpm at +4 °C for 15 minutes, then the supernatant was collected and used for DNA extraction. Information of the samples including location, year, tissue type, isolate name and GenBank acces number are given in Table 1.

Table 1. The information about collected materials and relevant genes for PCR and their accession numbers from GenBank.

Province	Sample type	Date	Isolate name	Region	Accession no
				B2L	MW492036
Denizli	Swap	2018	TR/ORFV/2020/Bor1	F1L	MW492043
				VIR	MW492050
				B2L	MW492037
İzmir	Swap	2019	TR/ORFV/2020/Bor2	F1L	MW492044
				VIR	MW492051
				B2L	MW492038
Manisa	Internal organ	2018	TR/ORFV/2020/Bor3	F1L	MW492045
				VIR	MW492052
				B2L	MW492039
Manisa	Swap	2020	TR/ORFV/2020/Bor4	F1L	MW492046
				VIR	MW492053
				B2L	MW492040
Muğla	Swap	2018	TR/ORFV/2020/Bor5	F1L	MW492047
				VIR	MW492054
				B2L	MW492041
Uşak	Swap	2019	TR/ORFV/2020/Bor6	F1L	MW492048
				VIR	MW492055
				B2L	MW492042
İzmir	Swap	2019	TR/ORFV/2020/Bor7	F1L	MW492049
				VIR	MW492056

DNA Isolation and Real-Time PCR

Viral DNA extraction was performed using the Roche MagNA Pure LC 2.0 Instrument and the MagNA Pure LC Total Nucleic Acid Isolation Kit as recommended by manufacturer's instructions.

Real-time PCR was carried out using primers and probe previously described by Bora et al. (2011). The nucleotide sequence of the primers and probe are given in Table 2. Roche probe master PCR kit and thermal cycler (Roche LightCycler® 480) were used for DNA amplification. PCR was carried out in a 20 µl reaction volume containing 5µl template DNA, 0,75 µl of each primer (10 pmol), 0,5 µl probe (10 pmol), 10 µl LightCycler® 480 Probes Master and 3 µl PCR grade water. The PCR cycling conditions of the Real Time PCR were given in Table 2.

Table 2. Oligonucleotides used in real-time and conventional PCR and conditions in thermal cycler.

Method	Gene	Primers	Cycle	Reference
Real-Time	*Pol*	5 -TACACGGAGTTGGCCGTGATCTTGTA-3	Pre Den: 95 °C/5 min 40 Repeats: (95 °C/10 sec → 64 °C/45 sec → 72°C/1 sec)	Bora *et al.* (2011)
		5 -CGCCAAGTACAAGAAGCTGATGA-3		
		5 HexTGCATCGAGTTGTAGATCTCGCGGT-BHQ-1		
Conventional	B2L	5-ATGTGGCCGTTCTCCTCCATC-3	Pre Den: 95 °C/5 min 35 Repeats: (94 °C/30' → 58 °C/40'→ 72 °C/75')	Yang *et al.* (2014)
		5-TTAATTTATTGGCTTGCAGAACTCC-3		
	F1L	5-ATGGATCCACCCGAAATCACG-3	Pre Den: 95 °C/5 min 35 Repeats: (94 °C/30' → 60 °C/40'→ 72 °C/75')	
		5-TCACACGATGGCCGTGACCA-3		
	VIR	5-ATGGCCTGCGAGTGCGCG-3	Pre Den: 95 °C/5 min 35 Repeats: (94 °C/30' → 55 °C/40'→ 72 °C/60')	
		5-TTAGAAGCTGATGCCGCAG-3		

Sequencing PCR

Conventional PCR were conducted for each gene region (B2L, F1L, and VIR) according to the previous report (Table 2). A commercial kit (Xpert Fast Hotstart Mastermix with Dye) was used for amplification. The reaction mix with a total volume of 25 µl containing of 1 µl Forward and reverese primer (10 mM) 12.5 µl Fast PCR master mix, 5.5 µl water, 5 µl template DNA was prepared for each sample. Amplification conditions were given in Table 2. Amplified products were sent for sequencing in commercial lab (Macrogen, South Korea). Sanger dideoxy sequencing technique was performed and raw sequences belonging to each product were obtained. To correct errors, raw sequence histograms were edited in Tracer implemented in ClustalW method using the MEGAX software (Kumar et al., 2018). We checked the correspondence rates of similar sequences in BLAST, and confirmed.

Phylogenetic Tree and Molecular Characterization

We downloaded reference and genotypical sequences from GenBank. Clustal W algorithm in BioEdit software was used to align sequences. After alignment and trimming, "find best model" was used to determine parameters in MEGA X to be selected for phylogenetic trees. We constructed the phylogenetic trees for each gene region by performing the Maximum-likelihood method with 1000 bootstrap replicates. Kimura-2 parameter was used to calculate of the nucleotide similarities of the obtained sequences.

RESULTS

Positive seven ORFV samples detected by qPCR were also amplified for B2L, F1L, and VIR gene regions. After PCR processes, we viewed relevant amplicons in 1% agarose gel under blue-light transilluminator, which were 1137, 1023, and 552 base pairs for B2L, F1L, and VIR, respectively. These seven samples were uploaded to GenBank and accession numbers were provided for each corresponding sequence (Table 1). Three phylogenetic trees were generated based on three gene regions (Figure 1, 2A, 2B).

In B2L phylogenetic tree, our sequences were clustered in a clade, and they showed monophyletic relatedness to D1701 (HM133903), the reference sequence being the most prominent and essential for phylogenetic evaluation, reported from the USA. Furthermore, all previous ORFV sequences submitted from Türkiye showed diversities between each other and spread out into different clusters. Our sequences also were located far from the previous Turkish sequences in B2L phylogenetic tree (Figure 1).

In F1L phylogenetic tree, the strains that were sequenced for this study fell into three clusters.

TR/ORFV/2020/Bor5 and TR/ORFV/2020/Bor7 were clustered with reference strains belonging to China, which are Assam 2010 and Guizhou (KY412866 and KP057582, respectively). TR/ORFV/2020/Bor1, TR/ORFV/2020/Bor2, TR/ORFV/2020/Bor4, and TR/ORFV/2020/Bor6 were clustered with reference strains belonging to India and China which are referred to as Ludhiana, Jilin, and SY19 (KY412865, FJ808075, and MG712417, respectively). TR/ORFV/2020/Bor3 was located as the furthest strain from others and drew a distinct branch with a reference strain from Canada, named OV/Torino (AY040081) (Figure 2A).

VIR phylogenetic tree showed substantially similar demography with B2L phylogenetic tree. However, TR/ORFV/2020/Bor3, a strain in this study, fell into a distinct cluster located rather than our other strains. TR/ORFV/202/Bor3 was clustered with two reference sequences, which are SBF/Goabal-01 and SBF/Goabal-02 (KU672680 and KU672678, respectively), from a study conducted in Kashmir. TR/ORFV/2020/Bor1, TR/ORFV/2020/Bor2, TR/ORFV/2020/Bor4, TR/ORFV/2020/Bor5, TR/ORFV/2020/Bor6, TR/ORFV/2020/Bor7 were gathered at near of D1701 (HM133903), one of the oldest reference strain from the USA, as polyphyletic taxa as compared to TR/ORFV/2020/Bor3 (Figure 2B).

The identity and similarity matrix based on the nucleotide sequences of B2L showed that seven isolates of this study and previous Turkish sequences available in GenBank were high similarity ranging from 97.6% to 98.4%.

Basis on amino acid alignment, TR strains in this study interestingly corresponded to D1701 and similarity was 97%-99.8% to each three gene regions. The comparison of amino acid composition between reference and our strains revealed D1701 was the most corresponded strain rather than others.

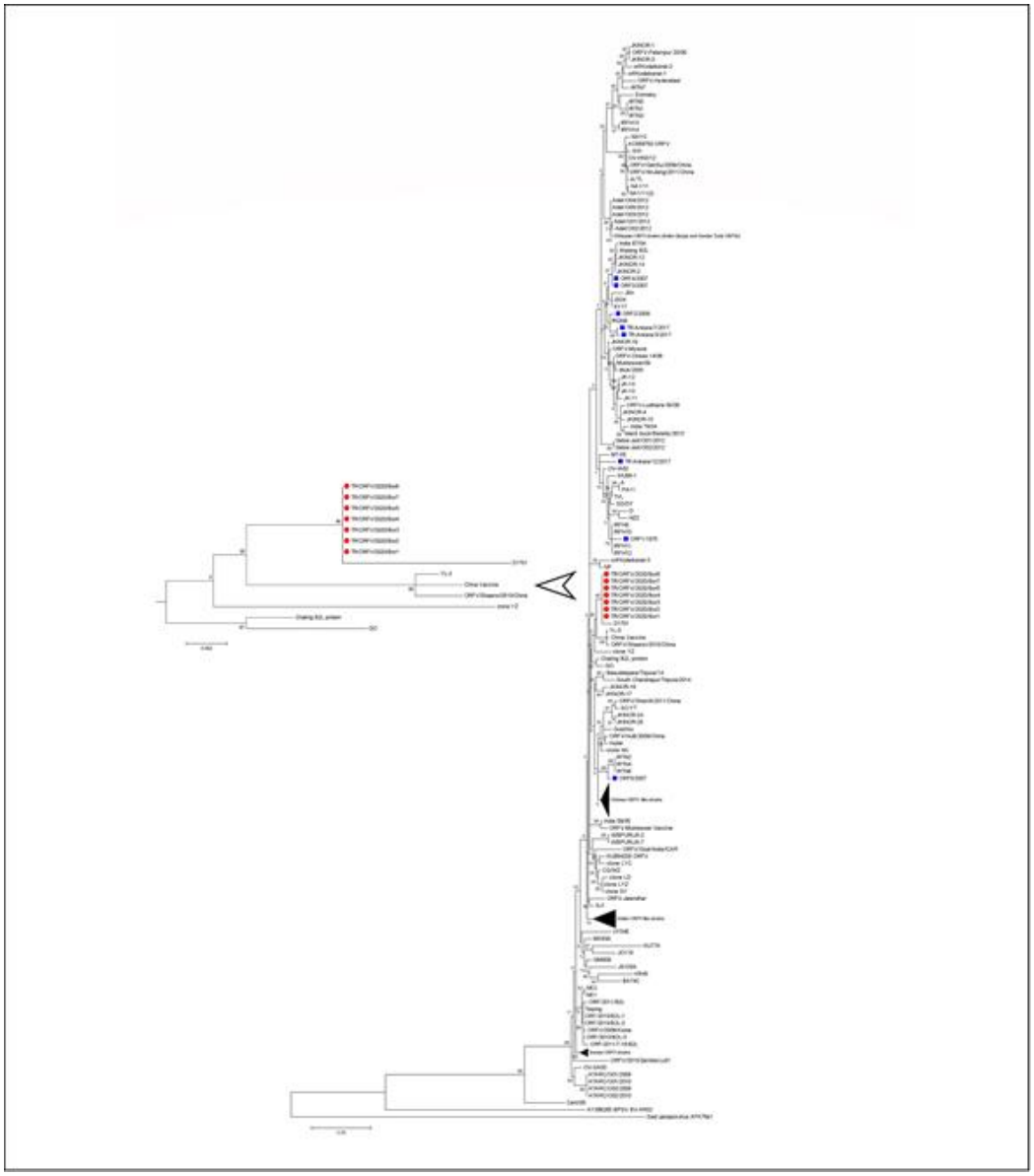

Figure 1: Phylogenetic tree based on the complete B2L gene region of ORFV. Turkish sequences in this study were marked with "●", and previous Turkish sequences belonging complete B2L gene region were marked with "■".

Figure 2: A- Phylogenetic tree based on complete F1L gene region of ORFV. B- Phylogenetic tree based on complete VIR gene region of ORFV. Turkish sequences in this study were marked with "●" in both trees.

DISCUSSION

Türkiye has close to 42 million sheep, making up a significant portion of the country's farm animal population, according to recently updated statistics data. (TUIK, 2020). ORFV is endemic in Türkiye among farm animals and primarily affects sheep and goat flocks (Akkutay-Yoldar et al. 2016, Karakas et al. 2013, Şevik 2017 and 2019). ORFV also has a zoonotic potential, and causes pustular dermatitis in humans mostly appears at the hands (Spyrou and Valiakos 2015). The lambing and shearing season, which lasts from April to June, is the most important risk factor for ORFV infection in both animals and individuals who closely contact with animals, such as farmers or veterinarians. (Spyrou and Valiakos 2015). Although ORFV is endemic in Türkiye, there haven't been enough molecular research to understand virus characterization. These few molecular studies have primarily focused on sequencing the B2L gene region to characterize ORFVs (Akkutay-Yoldar et al. 2016, Karakas et al. 2013, Şevik 2017, Şevik 2019). In this study, we sequenced the F1L and VIR gene sections in addition to the B2L gene region to better understand the molecular dynamics of ORFV in Türkiye. Thus, we presented a preliminary phylogenetic analysis using F1L and VIR (Figure 2) from sheep in Türkiye. Analyzing ORFV phylogenetic trees of this study, it was observed that our sequences were clustered almost similarly according to VIR (Figure 2) and B2L (Figure 1) gene region. However, tree constructed based on the F1L gene showed different demography. TR/ORFV/2020/Bor3 was on a separate branch as in the other trees. But, interestingly TR/ORFV/2020/Bor5 and TR/ORFV/2020/Bor7, located in the same branch, were in different cluster. This demography shows that F1L and B2L are more prominent in terms of molecular diversification and evolutionary assessment. Our findings compatible with previous phylogenetic research from throughout the world suggests that the B2L and F1L gene regions are better suited for phylogenetic analysis (Abdullah et

al. 2015, Peralta et al. 2018, Yang et al. 2015). Obtained data suggested it might be better particularly characterizing B2L and F1L genes in the next molecular investigations of ORFV.

In the assessment of three phylogenetic trees and the composition of amino acids belonging to relevant viruses, a vast majority of TR sequences in this study closely located monophyletic, as a sister group, to ORFV reference strain D1701 (HM133903). Strain D1701 was isolated from a sheep in Germany in 1975. However, the complete genome of D1701 has been available since 2011 in GenBank. Following owing to increasing biotechnological investigations, it has also become the biological platform for vaccines and drugs (McGuire et al. 2012). Cell culture-adapted variants D1701-B and D1701-V have been defined as immunomodulators for stimulation of the immune system, and/or viral vector platforms for delivering different microbial and viral antigens (Rziha et al. 1999 and 2016). TR strains in this study showed high homology to variants D1701-B and D1701-V. Therefore, if TR strains used in this study could be successfully isolated in cell culture, it might provide a novel molecular platform for local research.

Kumar et al. (2014) claimed to lead the host shifting with the existence of serine or glycine on the 249th amino acid of B2L. According to this prediction, if the 249th amino acid is serine, the host is more likely to be sheep, or if the 249th amino acid is glycine (G), the host is most likely to be the goat. As compatible with Kumar et al. (2014), the 249th amino acid of all TR ORFV in this study was serine (S) in this study.

In the Genbank database, sequences of B2L from Türkiye are limited, there are no sequences belonging to F1L and VIR or other gene regions. This fact makes it difficult to evaluate the molecular characterization outputs for Türkiye. A molecular study conducted in goats in the Central region of Türkiye in 2017 compared the amino acids by reference sequences available in GenBank (Şevik 2017). The author mentioned two amino acid substitutions (A134R and V309A) on B2L by comparison of other ORFV TR strains and claimed those substitutions might have occurred because of the host response (Şevik 2017). In another study which was performed in cattle in the same region in 2019, ORFV sequences showed 100% homology to ORFV goat sequences in 2017 (Şevik 2017 and 2019). In this study, we also compared amino acid substitutions on the alignment of all ORFV TR and some reference sequences available in the GenBank. Non-unique amino acid substitutions on several points of B2L already exist. Therefore, mentioned amino acid substitutions could not show what was the effect on virus-host interaction dynamics. It is thought that more detailed studies including larger and different gene regions or complete gene analysis of ORFV strains isolated from various animal species in Türkiye would benefit to a better understanding of the molecular dynamics.

F1L (ORF059), an immunodominant region of ORFV, has been less considered in the evaluation of its molecular dynamics when compared with B2L. We compared motifs of amino acids between reference and our sequences. Highly conserved gene regions of F1L are emphasized in some previous reports, which are KGD (Lys-Gly-Asp) motif, Cx3C motif, D/ExD motif, GAG motif, and KTR motif and completely corresponded to our recent sequences (Scagliarini et al. 2004, Yogisharadhya et al. 2018, Yu et al. 2020). On the contrary to other motifs, the proline-rich region might have affected phylogenetic diversification. Taken together, "PAPA-box" motif between 40th and 45th amino acids and point changes in amino acids might have led to TR/ORFV/2020/Bor5 and TR/ORFV/2020/Bor7 shift to distinct clade near Indian strains (Assam 2010 and Mukteswar Vaccine) (Figure 3). Yu et al. (2020), in the most recent study on amino acid motifs reported, mentioned that F1L is conservative, although the proline-rich region shows heterogeneity. Therefore, they claimed that the molecular characterization of B2L and F1L did not reflect the molecular dynamics of ORFV. However, the phylogenetic tree and amino acids of F1L in this study revealed it is necessary to be considered F1L for evolutionary dynamics.

VIR is one of the virulence genes of ORFV (Delhon et al. 2004, Hautaniemi et al. 2010, Peralta et al. 2018). Two arguments have been asserted on genetic variation of VIR so far. According to one of them, previously reported by Kottaridi et al. (2006), it was claimed that VIR had conservation in amino acid residues, and this did not associate with phylogenetic grouping based on host species, geographical origin, and time of isolation. A second argument by more recently reported by Peralta et al. (2018) mentioned that VIR and vIL10 were virulence factors for ORFV, and these were the most variable regions for all PPV genus viruses, including ORFV. In fact, our results were partially corresponded to these two arguments. TR sequences in this study except TR/ORFV/2020/Bor3 were 100% identical to D1701. TR/ORFV/2020/Bor3 was 98.4% identical to SBF/Goabal-02/Sheep Indian strain in 2016. This situation leads to think a number of variations coming from various geographic regions have already been circulating in the Western part of Türkiye.

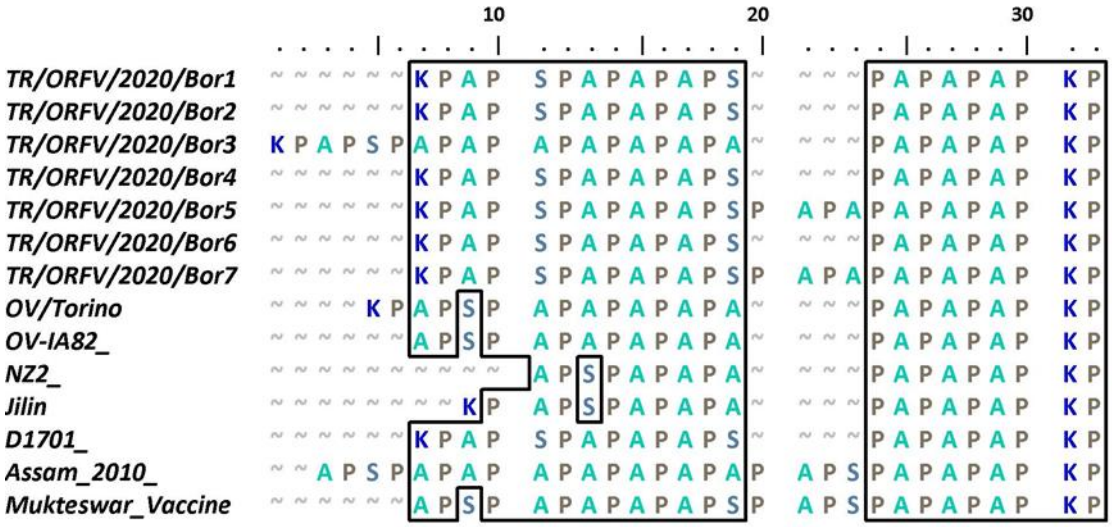

Figure 3: Comparison of residues on the proline-rich region (PAPA-box) of F1L in the alignment of the sequences.

CONCLUSION

In a conclusion, this study is a comprehensive molecular study conducted in three gene regions including B2L, F1L and VIR in recently isolated ORFVs in Türkiye. The study suggests that F1L gene region should be considered in phylodynamic evaluation besides B2L. According to results of the study, molecular knowledge of immunodominant genes might be verified by molecular analysis of virulence genes of ORFVs.

Türkiye is a geographical crossroad for many sectors from a global view. Livestock is highlighted in these sectors and affected from various situation. These clearly cause increasing circulation densities of viruses in the environment. The results exhibiting high homology between TR ORFV strains in this study and both European and Asian, suggested ORFV strains in Türkiye may likely originate from different geographies of the world.

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

Author Contributions Rates: MK:%35, BTK:%35, KM: %10, AAÇ: %10, FA: %10

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REFERENCES

Abdullah, A.A., Ismail, M.F.B., Balakrishnan, K.N., Bala, J.A., Hani, H., Abba, Y., Awang Isa, M.K., Abdullah, F.F.J., Arshad, S.S., Nazariah, Z.A., Abdullah, R., Mustapha, N.M., Mohd-Lila, M.-A. (2015). Isolation and phylogenetic analysis of caprine Orf virus in Malaysia. VirusDisease, 26, 255–259. https://doi.org/10.1007/s13337-015-0278-4

Abrahão, J.S., Campos, R.K., Trindade, G.S., Guedes, M.I.M., Lobato, Z.I.P., Mazur, C., Ferreira, P.C.P., Bonjardim, C.A., Kroon, E.G. (2009). Detection and phylogenetic analysis of Orf virus from sheep in Brazil: a case report. Virology Journal, 6, 47. https://doi.org/10.1186/1743-422X-6-47

Akkutay-Yoldar, Z., Oguzoglu, T.C., Akça, Y. (2016). Diagnosis and phylogenetic analysis of orf virus in Aleppo and Saanen goats from an outbreak in Türkiye. Virologica Sinica, 31, 270–273. http://dx.doi.org/10.1007/s12250-015-3684-2

Bora, D.P., Venkatesan, G., Bhanuprakash, V., Balamurugan, V., Prabhu, M., Sankar, M.S., ve Yogisharadhya, R. (2011). TaqMan real-time PCR assay based on DNA polymerase gene for rapid detection of Orf infection. Journal of Virological Methods, 178 (1-2), 249-252. https://doi.org/10.1016/j.jviromet.2011.09.005

Delhon, G., Tulman, E.R., Afonso, C.L., Lu, Z., de la Concha-Bermejillo, A., Lehmkuhl, H.D., Piccone, M.E., Kutish, G.F., Rock, D.L. (2004). Genomes of the parapoxviruses ORF virus and bovine papular stomatitis virus. Journal of Virology, 78, 168–177. https://doi.org/10.1128/JVI.78.1.168-177.2004

Fleming, S. B., Mercer, A. A. (2007). Genus parapoxvirus in Poxviruses, 127-165. Birkhäuser Basel.

Hautaniemi, M., Ueda, N., Tuimala, J., Mercer, A.A., Lahdenperä, J., McInnes, C.J. (2010). The genome of pseudocowpoxvirus: comparison of a reindeer isolate and a reference strain. Journal of General Virology, 91, 1560–1576. https://doi.org/10.1099/vir.0.018374-0

Hosamani, M., Bhanuprakash, V., Scagliarini, A., Singh, R.K. (2006). Comparative sequence analysis of major envelope protein gene (B2L) of Indian orf viruses isolated from sheep and goats. Veterinary Microbiology, 116, 317–324. https://doi.org/10.1016/j.vetmic.2006.04.028

Karakas, A., Oguzoglu, T.C., Coskun, O., Artuk, C., Mert, G., Gul, H.C., Sener, K., Özkul, A. (2013). First molecular characterization of a Turkish orf virus strain from a human based on a partial B2L sequence. Archives of Virology, 158, 1105–1108. https://doi.org/10.1007/s00705-012-1575-5

Kottaridi, C., Nomikou, K., Teodori, L., Savini, G., Lelli, R., Markoulatos, P., Mangana, O. (2006). Phylogenetic correlation of Greek and Italian orf virus isolates based on VIR gene. Veterinary Microbiology, 116, 310–316. https://doi.org/10.1016/j.vetmic.2006.04.020

Kumar, N., Wadhwa, A., Chaubey, K.K., Singh, S.V., Gupta, S., Sharma, S., Sharma, D.K., Singh, M.K., Mishra, A.K. (2014). Isolation and phylogenetic analysis of an orf virus from sheep in Makhdoom, India. Virus Genes, 48, 312–319. https://doi.org/10.1007/s11262-013-1025-9

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution, 35, 1547-1549. https://doi.org/10.1093/molbev/msy096

McGuire, M.J., Johnston, S.A., Sykes, K.F. (2012). Novel immune-modulator identified by a rapid, functional screen of the parapoxvirus ovis (Orf virus) genome. Proteome Science, 10, 4. https://doi.org/10.1186/1477-5956-10-4

Nettleton, P. F., Brebner, J., Pow, I., Gilray, J. A., Bell, G. D., Reid, H. W. (1996). Tissue culture-propagated orf virus vaccine protects lambs from orf virus challenge. Veterinary record, 138(8), 184-186. https://doi.org/10.1136/vr.138.8.184

Peralta, A., Robles, C.A., Micheluod, J.F., Rossanigo, C.E., Martinez, A., Carosio, A., König, G.A. (2018). Phylogenetic analysis of ORF viruses from five contagious ecthyma outbreaks in Argentinian goats. Frontiers in veterinary science, 5, 134 https://doi.org/10.3389/fvets.2018.00134

ICTV.(2020).https://ictv.global/taxonomy/taxondetails?taxnode_id=202204780. EC 52, Online meeting, October 2020 [Accessed 23.08.2023].

Rziha, H.-J., Henkel, M., Cottone, R., Meyer, M., Dehio, C., Büttner, M. (1999). Parapoxviruses: potential alternative vectors for directing the immune response in permissive and non-permissive hosts. Journal of Biotechnology, 73, 235–242. https://doi.org/10.1016/S0168-1656(99)00141-8

Rziha, H.-J., Rohde, J., Amann, R. (2016). Generation and selection of Orf virus (ORFV) recombinants. Vaccine Technologies for Veterinary Viral Diseases: Methods and Protocols, 1349, 177–200. https://doi.org/10.1007/978-1-4939-3008-1_12

Scagliarini, A., Gallina, L., Dal Pozzo, F., Battilani, M., Ciulli, S., Prosperi, S. (2004). Heparin binding activity of orf virus F1L protein. Virus Research, 105, 107–112. https://doi.org/10.1016/j.virusres.2004.04.018

Şevik, M. (2017). Association of two clusters of Orf virus isolates in outbreaks of infection in goat in the Central Anatolian region of Türkiye. VirusDisease, 28, 345–348. https://doi.org/10.1007/s13337-017-0392-6

Şevik, M. (2019). Orf virus circulation in cattle in Türkiye. Comparative Immunology, Microbiology and Infectious Diseases, 65, 1–6. https://doi.org/10.1016/j.cimid.2019.03.013

Spyrou, V., Valiakos, G. (2015). Orf virus infection in sheep or goats. Veterinary Microbiology, 181, 178–182. https://doi.org/10.1016/j.vetmic.2015.08.010

TÜİK. (2020). https://data.tuik.gov.tr/Bulten/Index?p=Animal-Production-Statistics-June-2020-33874. [Accessed 20.05.2021].

Yang, H., Meng, Q., Qiao, J., Peng, Y., Xie, K., Liu, Y., Zhao, H., Cai, X., Chen, C. (2014). Detection of genetic variations in Orf virus isolates epidemic in Xinjiang China: Detection of genetic variations in Orf virus. Journal of Basic Microbiology, 54, 1273–1278. https://doi.org/10.1002/jobm.201300911

Yogisharadhya, R., Bhanuprakash, V., Kumar, A., Mondal, M., Shivachandra, S.B. (2018). Comparative sequence and structural analysis of Indian orf viruses based on major envelope immuno-dominant protein (F1L), an homologue of pox viral p35/H3 protein. Gene, 663, 72–82. https://doi.org/10.1016/j.gene.2018.04.026

Yu, Y., Duan, X., Liu, Y., Ma, J., Song, B., Lian, Z., Cui, Y. (2020). Laboratory diagnosis of a NZ7-like Orf virus infection and pathogen genetic characterization, particularly in the VEGF gene. Frontiers in Veterinary Science, 7, 538. https://doi.org/10.3389/fvets.2020.00538

Zhao, K., He, W., Gao, W., Lu, H., Han, T., Li, J., Zhang, X., Zhang, B., Wang, G., Su, G., Zhao, Z., Song, D., Gao, F. (2011). Orf virus DNA vaccines expressing ORFV 011 and ORFV 059 chimeric protein enhances immunogenicity. Virology Journal, 8, 562. https://doi.org/10.1186/1743-422X-8-562

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RESEARCH ARTICLE

Investigation of Antioxidant and Histopathological Effects of Aqueous and Ethanol Extracts Obtained from *Sideritis akmanii*

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ABSTRACT

In this study, it was aimed to investigate the antioxidant activities and histopathological effects of the aqueous and ethanolic extracts obtained from the endemic *Sideritis akmanii* plant, which spreads on the Şuhut-Sandıklı road in the Kumalar plateau region of Afyonkarahisar province. The number of 48 Wistar albino male rats were divided into 8 groups; Control Group (Group I), CMC Group (Group II), 0.5% CMC (carboxymethyl cellulose), Aqueous and ethanolic plant extract groups (Group III-IV-V-VI-VII-VIII). Three different doses (1%, 2%, and 4%) extracts from the determined LD50 dose were applied to 6 experimental groups for 30 days. At the end of the experiment, malondialdehyde (MDA), glutathione (GSH) levels, superoxide dismutase (SOD), and catalase (CAT) enzyme activities were measured in healthy rat's blood and tissue (liver, heart, brain, kidney, testis) samples. The whole blood MDA level decreased in all doses of the ethanolic extract group compared to the control group, while the GSH level increased in both extract groups compared to the control group. SOD and CAT enzyme activities were increased in the ethanolic groups compared to the control group, except for erythrocyte and testis tissue ($p<0.05$). On the other hand, no changes were observed in the histopathological examination results. As a result of the study, it was observed that the aqueous and ethanolic extracts of *Sideritis akmanii* had antioxidant properties and the ethanolic extract was more effective on antioxidant enzymes.

Keywords: Antioxidant enzymes, Histopathology, Lipid peroxidation, *Sideritis akmanii* extract (aqueous and ethanolic)

***

Sideritis akmanii'den Elde Edilen Sulu ve Etanol Ekstraktlarının Antioksidan ve Histopatolojik Etkilerinin Araştırılması

ÖZ

Bu çalışmada, Afyonkarahisar ili Kumalar yaylası bölgesinde Şuhut-Sandıklı yolu üzerinde yayılış gösteren endemik *Sideritis akmanii* bitkisinden elde edilen sulu ve etanolik ekstraktların antioksidan aktivitelerinin ve histopatolojik etkilerinin araştırılması amaçlanmıştır. 48 adet Wistar albino erkek rat 8 gruba ayrıldı; Kontrol Grubu (Grup I), CMC Grubu (Grup II), %0,5 CMC (karboksimetil selüloz), Sulu ve etanolik bitki ekstrakt grupları (Grup III-IV-V-VI-VII-VIII). Belirlenen LD50 dozundan uygun oranlarda üç farklı doz (%1, %2 ve %4) ekstraktı 6 deney grubuna 30 gün uygulandı. Deney sonunda sağlıklı ratların kan ve doku (karaciğer, kalp, beyin, böbrek, testis) örneklerinde malondialdehit (MDA), glutatyon (GSH) düzeyleri, süperoksit dismutaz (SOD) ve katalaz (CAT) enzim aktiviteleri ölçüldü.Tam kan MDA düzeyi etanol ekstrakt grubunun tüm dozlarında kontrol grubuna göre azalırken, GSH düzeyi her iki ekstrakt grubunda da kontrol grubuna göre arttı. Etanol gruplarında eritrosit ve testis dokusu dışında SOD ve CAT enzim aktiviteleri kontrol grubuna göre arttı ($p<0.05$). Histopatolojik inceleme sonuçlarında ise herhangi bir değişiklik gözlenmedi. Çalışma sonucunda *Sideritis akmanii*'nin sulu ve etanol ekstraktlarının antioksidan özelliklere sahip olduğu ve etanol ekstraktının antioksidan enzimler üzerinde daha etkili olduğu gözlemlendi.

Anahtar kelimeler: Antioksidan enzimler, Histopatoloji, Lipid peroksidasyonu, *Sideritis akmanii* ekstraktı (sulu ve etanollü)

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INTRODUCTION

The therapeutic properties of plants have been known to humans since ancient times. As in the whole world, medicinally important plants have been used by the Anatolian people for centuries in Turkey as well. Sideritis species growing in the Mediterranean flora, especially in Spain and Turkey, are used as herbal tea due to the unique pleasant smell and aroma of their dried flowers and leaves. Sideritis species are widely used in traditional medicine in Turkey due to their properties such as nervous system stimulant - sedative, gastrointestinal disorders, rheumatism, cough caused by cold, pain reliever, and diuretic (González Burgos et al. 2011). Considering these common features among the public, the effect of extracts obtained from Sideritis species has been a matter of curiosity. In a study, it was revealed that different types of Sideritis exhibit anti-inflammatory, antinociceptive, and antioxidant activities, as well as nervous system stimulant or anti-stress activities (Ozturk et al. 1996, Guvenç et al. 2010). In another study, it was expressed that it has a good inhibition on enzymes that have an important role in Alzheimer's disease (Çarıkçı 2020). It has been determined that the extracts obtained from *S. scardica* have gastroprotective activities as well as anti-inflammatory effects in rats. It has also been demonstrated to have cytotoxic effects on tumor cell lines (Tadić et al. 2012).

There are a few studies on *Sideritis akmanii*, an endemic species belonging to Kumalar Mountain in Afyonkarahisar province. In a chemical content study, it was determined that the species contains essential oils and flavones in its structure, and the basic components are linearol, isolineraol, sideroxol, foliol, isofoliol, sideridiol (Bondi et al. 2000). Thanks to its rich components, it has an antibacterial effect and is effective on some pathogenic bacteria (Temel et al. 2014). In a recent in vitro study, its anticarcinogenic effect was demonstrated (Cigerci et al. 2023). Aksoy et al., in their in vitro study using *Sideritis akmanii* methanol and acetone extract, found that this species has antioxidant properties and can inhibit acetylcholinesterase, α-glucosidase, α-amylase enzymes. In the study, both total phenolic content and DPPH radical scavenging activity of methanol extract were higher in methanol extract compared to acetone extract, and in this context, the importance of solvent was emphasized (Aksoy et al. 2022).

In this study, it was aimed to investigate the antioxidant activity of the aqueous and ethanolic extracts obtained from the plant *Sideritis akmanii*, which is endemic to Afyonkarahisar, in rat blood and tissues, and its histopathological effects on the tissues.

MATERIAL and METHODS

Sideritis akmanii Plant Supply

The plant material of this study, *Sideritis akmanii*, was collected from above-ground parts such as leaves, flowers etc. on 20 July 2016, from the Kumalar plateau region of the Şuhut-Sandıklı highway in Afyonkarahisar. Prof. Dr. Mustafa Kargıoğlu conducted the identification of the plant. The plant sample is stored at the Afyon Kocatepe University herbarium with the registration number AKU8314. The aerial parts of *Sideritis akmanii* were dried in the shade and at room temperature and mechanically cut into small pieces. Dried plant samples were pulverized in a blender device.

Preparation of *Sideritis akmanii* Aqueous and Ethanolic Extracts

The aerial parts of *Sideritis akmanii* were ground into powder, and 50 g was taken and added to a 2-liter volumetric flask. A cooling system was set up by adding twenty times the amount of ethanol (1000 ml) of the sample for ethanolic extract and distilled water (1000 ml) twenty times the sample for water extract on the volumetric flask. In this way, it was kept in a

container filled with water at 50 °C for 24 hours. The filtered extracts were removed separately with ethanol and water at 50 °C in a rotary device (Şerbetçi 2007). Afterward, 4.9 g of greenish-black, moist, sticky ethanolic extract and 1.5 g of milky-brown, moist, sticky water extract were obtained. The obtained extracts were dissolved in 0.5% CMC (Carboxymethyl Cellulose) / distilled water solution and used for the experiment.

Determination of Lethal Dose 50 (LD50)

The lethal dose 50 (LD50) was determined with these two extracts. For this, the OECD (2001) (Organization for Economic Co-operation and Development) method was used. According to this method, 10 male Wistar albino rats weighing 250-350 g were divided into two groups.

To the rats in the 1st Group, in order of 175 mg/kg, 550 mg/kg, 1000 mg/kg, 2000 mg/kg, and 5000 mg/kg ethanolic extract, (n: 5)

Similarly, 175 mg/kg, 550 mg/kg, 1000 mg/kg, 2000 mg/kg, and 5000 mg/kg aqueous extract was given to the rats in the Group 2, (n: 5)

It was administered as a single dose via gastric gavage on the same day and time. After the application, the general clinical conditions and behaviors of the rats were followed for 48 hours. In the evaluation, If no animals died at the doses administered to the rats, the LD50 dose was interpreted as greater than the highest dose given to the rats (OECD, 2001). As a result of the application of ethanolic and water extracts prepared from the aerial parts of the *Sideritis akmanii* plant to the rats, the lethal dose was found to be 50 (LD50) > 5000 mg/kg. The amounts of 1% (50 mg/kg), 2% (100 mg/kg), and 4% (200 mg/kg) of the OD50 dose determined from the aqueous and ethanolic extracts were determined and administered to the rats (Çelik and Küçükkurt 2016).

Experimental Protocol

For this study, study approval was obtained from Afyon Kocatepe University Experimental Animals Ethics Committee with reference number 79-18. Wistar albino male rat weighing 200-300 g was used as animal material. Rats in their cages in the experimental animals unit were kept at a temperature of 24±1 °C, a 12 hours light/dark, and a regularly ventilated environment. In feeding the rats, standard rat food and drinking water were given fresh every day.

Rats were divided into 8 groups with 6 in each group. The Control Group (Group I) was fed only with standard rat chow. CMC Group (Group II) was fed with standard rat chow and 0.5% CMC was given by gastric gavage. In addition to the standard rat feed, the other six groups were given plant extracts (1%, 2%, and 4%) by preparing the plant extracts in accordance with the LD50 dose. The groups given the aqueous extract were divided into S1, S2, and S3 (Groups III, IV, V), and the groups given the ethanolic extract were divided into E1, E2, and E3 (Groups VI, VII, VIII). These determined doses were given to the rats by gastric gavage for 30 days. At the end of 30 days, 24 hours after the last application, blood and tissue (liver, heart, kidney, brain, testis) samples were taken from animals under xylazine and ketamine anesthesia.

Preparation of Whole Blood, Erythrocyte Lysate, and Tissue Homogenates

Blood samples taken into heparinized tubes were divided into two parts. Some of the blood was used as whole blood for the measurement of MDA and GSH, and some of it was prepared for the measurement of SOD and CAT activities. For this, erythrocyte and plasma were separated by centrifugation at 3500 g for 15 minutes at 4 C within 30 minutes. The precipitated erythrocytes were washed three times with isotonic saline and the fluffy layer was removed. Then, isotonic saline and erythrocytes were added in the same volume and stored at -20 C (Winterbourn et al. 1975).

After the animals were sacrificed, liver, kidney, heart, brain, and testis tissues were removed and thoroughly washed with cold 0.9% NaCl. Tissues were homogenized 1:40 w/v in 0.1 M phosphate buffer, pH 7.4, containing 1 mM EDTA. After centrifugation at 18000xg for 15 min at 4 C obtained supernatants were stored at -20 C until analysis (Kucukkurt et al. 2008).

Biochemical Analyses

Whole blood MDA levels, which are an important marker for lipid peroxidation, were determined by Draper and Hadley (Draper and Hadley, 1990), and tissue homogenates by Ohkawa et al. (1979) were determined by the method. MDA in the catabolite of lipid peroxide reacts with thiobarbituric acid (TBA) to produce a pink compound with maximum absorption at 532 nm. GSH level was measured in blood and tissue according to Beutler et al. (1993). The amount of GSH is determined by reading the optical density of this yellow compound at 412 nm in the spectrophotometer. Antioxidant enzyme activity of SOD in erythrocyte lysate and tissue homogenate Sun et al. (1988) was measured according to the method. CAT activity was determined by Luck (1955)'s method in erythrocyte lysate and by Aebi's (1974) method in tissue homogenate. CAT activity was measured by the rate of reduction in H2O2 at 240 nm for 45 seconds at 25°C. Protein content in tissue Lowry et al. (1951) and the amount of hemoglobin were measured by the Drabkin method (Drabkin and Austin 1935). All spectrophotometric measurements were performed using the Shimadzu 1601 UV-VIS spectrophotometer (Tokyo, Japan).

Preparation of Tissues for Histopathological Analysis

Histopathological examination was performed in order to see the possible damage of the extracts on healthy tissues due to oxidative stress. At the end of the study, the liver, kidney, heart, brain, and testis tissues of the sacrificed rats were placed in 10% formalin solution for histopathological analysis and fixed in 10% formalin solution for 48 hours. Tissues were dehydrated by passing through graded alcohol (70% to 100%). After cleaning the tissues in xylene, they were embedded in paraffin, cut into 5-6 μm sections, and stained with hematoxylin-eosin (H&E). As a result, each section was examined under a light microscope (Microscopic Digital Picture Analysis System with Olympus BX51 and DP20 attached, Tokyo, Japan).

Statistical Analysis

The results obtained from the research, one-way ANOVA test were applied in the SPSS 20.0 statistical package program. Duncan's test was applied to the statistically different results, and the data were expressed as "mean ± standard deviation". $P < 0.05$ was accepted for statistical significance.

RESULTS

Effect on Lipid Peroxidation and Reduced Glutathione

MDA level is widely used as a marker of free radical-mediated LPO. It was determined that the extracts obtained from *Sideritis akmanii* did not cause a statistical change ($p>0.05$) in the MDA levels in both aqueous and ethanolic groups in other tissues, except blood, compared to the control group (Table 1). Blood MDA was significantly decreased in the ethanolic extract groups compared to the control and CMC groups ($p < 0.005$), the decrease in the aqueous extract groups was not statistically significant. GSH is a non-enzymatic antioxidant substance in detoxification and reduces the toxic effect of xenobiotic metabolites. The GSH levels obtained in the study are given in Table 2. It is seen that the extracts obtained from *Sideritis akmanii* have a positive effect on GSH, but this is not statistically significant in other tissues except blood

(p>0.05). Blood GSH levels increased significantly in all extract groups (p < 0.001).

Effect on Antioxidant Enzymes

The antioxidant enzymes SOD and CAT activities were determined in the erythrocyte, liver, kidney, heart, brain, and testis tissues of the rats as shown in Tables 3 and 4. When the CMC group was compared with the control group, there was no difference in both SOD and CAT enzyme activities. A statistically significant increase was observed in SOD enzyme activity when compared to all ethanolic groups of liver, heart, and brain tissues and the E1 group of kidney tissue, control, and other groups. When the S1 and S2 groups of brain tissue were compared to the control group, an increase was observed, while there was no difference in the aqueous extracts of other tissues compared to the control group. An increase in CAT enzyme activity was observed in the whole ethanolic extract group of kidney tissue compared to the control group. The increase in E2 groups in liver tissue and E2 and E3 groups in heart and brain tissues is significant. A significant increase was observed in kidney tissue S1 and S2 groups. No statistically significant difference was observed in all groups of erythrocyte and testis tissue in both analyzes.

Histopathological Changes

Histopathological examination was performed for the following changes in each organ;

- In the brain, neuron degeneration, edema and myelin degeneration,
- In the liver, sinusoidal dilatation and hyperemia, Kupffer cell activation, degenerative changes in hepatocytes,
- Degenerative changes in the kidney, tubules, and changes in Bowman's capsule in the glomerulus,
- In the heart muscle, hyaline degeneration and Zenker's necrosis.
- Decreased spermatozoid density in the testicular tubulous seminiferous contortus (TSC) lumen vacuolization areas in the TSC lumen.

The histopathological changes in the organs of the animals in the experimental groups were examined in detail and are shown in Figure 1. No pathological changes were observed in the histopathological analysis of all groups.

Table 1. Effect of *Sideritis akmanii* aqueous and ethanolic extracts on whole blood and tissue MDA levels in rats

MDA	Blood (nmol/mL)	Liver (nmol/g tissue)	Kidney (nmol/g tissue)	Heart (nmol/g tissue)	Brain (nmol/g tissue)	Testis (nmol/g tissue)
Control	7.07±1.10^{a}	2.98±0.86	2.17±0.21	2.29±0.33	2.29±0.20	2.76±0.86
CMC	7.05±0.86^{a}	3.14±0.18	2.88±1.77	2.58±0.63	2.19±0.25	3.27±0.96
S1	6.06±0.76^{ab}	3.41±0.64	2.78±0.81	2.93±0.91	2.14±0.21	2.70±0.57
S2	6.27±0.96^{ab}	2.73±0.51	2.16±0.23	2.20±0.17	2.21±0.32	2.75±1.01
S3	6.12±0.38^{ab}	2.37±0.27	2.63±0.59	3.19±0.99	2.63±0.29	2.09±0.14
E1	5.49±0.54^{b}	3.33±1.03	3.95±1.02	3.83±1.14	2.90±0.63	3.36±0.87
E2	5.63±0.93^{b}	3.61±1.01	3.19±1.12	3.47±2.17	2.45±0.55	2.78±0.77
E3	5.37±0.90^{b}	2.87±0.35	2.58±1.07	3.26±0.14	2.52±0.71	2.71±0.61
p	0.004	0.073	0.063	0.061	0.064	0.198

Mean ± standard deviation; n=6

a,b Values with different letters in the same column are statistically significant

Table 2. Effect of *Sideritis akmanii* aqueous and ethanolic extracts on whole blood and tissue GSH levels in rats

GSH	Blood (nmol/mL)	Liver (nmol/g tissue)	Kidney (nmol/g tissue)	Heart (nmol/g tissue)	Brain (nmol/g tissue)	Testis (nmol/g tissue)
Control	13.83±1.16^{b}	7.57±0.46	8.45±0.68	7.41±0.31	8.11±0.42	8.60±0.58
CMC	14.53±1.11^{b}	8.02±0.55	7.70±0.39	7.37±0.56	8.23±0.90	8.71±1.10
S1	18.43±1.36^{a}	8.04±0.23	8.53±0.38	8.23±0.53	8.63±1.20	8.13±0.66
S2	19.73±3.90^{a}	8.02±1.39	9.01±0.63	8.53±0.87	8.35±0.34	9.23±0.63
S3	20.23±3.22^{a}	8.93±1.39	8.35±0.91	9.04±0.58	8.29±1.21	9.64±0.92
E1	21.00±2.70^{a}	7.88±1.77	8.16±1.44	8.80±1.09	8.95±1.35	8.18±3.06
E2	20.83±2.82^{a}	9.40±2.40	8.44±1.16	8.81±1.81	8.66±1.73	7.41±0.87
E3	21.36±3.61^{a}	9.03±1.46	8.75±1.39	9.43±2.71	8.78±1.01	7.98±1.83
p	0.000	0.227	0.456	0.093	0.885	0.207

Mean ± standard deviation; n=6

a,b Values with different letters in the same column are statistically significant

Table 3. Effect of aqueous and ethanolic extracts of *Sideritis akmanii* on erythrocyte and tissue SOD levels in rats

SOD	Erythrocyte U/mgHb	Liver U/µg protein	Kidney U/µg protein	Heart U/µg protein	Brain U/µg protein	Testis U/µg protein
Control	8.7±1.87	2.90±0.33^{d}	4.15±0.29^{bc}	4.46±0.97^{d}	7.21±1.19^{d}	6.40±1.22^{ab}
CMC	6.82±2.9	3.56±0.27^{d}	3.45±0.53^{c}	5.87±1.43^{cd}	8.53±1.78^{cd}	7.03±2.43^{ab}
S1	6.52±4.93	4.24±0.95^{cd}	3.71±3.70^{bc}	5.88±1.82^{cd}	10.10±2.52^{bc}	5.71±1.04^{b}
S2	6.92±1.98	5.43±3.03^{bcd}	5.01±3.34^{abc}	5.98±2.08^{cd}	10.58±1.25^{abc}	6.09±1.63^{a}
S3	6.61±1.48	5.33±2.48^{bcd}	3.65±0.73^{bc}	6.66±1.76^{bcd}	7.08±1.85^{d}	5.24±0.52^{b}
E1	9.46±5.05	7.65±3.21^{ab}	7.74±1.34^{a}	7.98±1.57^{abc}	10.86±1.68^{abc}	8.21±1.69^{a}
E2	9.02±3.61	6.52±1.96^{abc}	6.34±1.41^{abc}	8.77±3.14^{ab}	12.87±2.76^{a}	8.31±2.26^{a}
E3	9.38±1.26	8.92±2.48^{a}	6.47±3.36^{ab}	10.02±2.86^{a}	11.91±2.77^{ab}	8.18±2.39^{a}
p	0.148	0.000	0.011	0.001	0.000	0.009

Mean ± standard deviation; n=6

a,b,c,d, Values with different letters in the same column are statistically significant ($p<0.05$)

Table 4. Effect of aqueous and ethanolic extracts of *Sideritis akmanii* on erythrocyte and tissue CAT levels in rats

CAT	Erythrocyte U/mgHb	Liver U/µg protein	Kidney U/µg protein	Heart U/µg protein	Brain U/µg protein	Testis U/µg protein
Control	53.05±16.01	3.91±0.57^{bc}	2.39±1.05^{c}	3.09±0.73^{c}	5.52±0.89^{c}	4.64±0.82^{abc}
CMC	41.49±15.39	3.85±0.84^{c}	1.96±0.43^{c}	3.75±0.74^{c}	5.63±0.34^{c}	4.52±2.01^{abc}
S1	52.83±24.02	2.83±0.55^{b}	3.77±0.49^{b}	4.15±0.57^{bc}	6.82±1.01^{bc}	4.27±0.56^{bc}
S2	31.09±24.09	3.76±0.46^{c}	3.92±0.81^{b}	5.86±1.87^{abc}	6.67±1.15^{bc}	5.04±0.55^{ab}
S3	32.45±13.81	2.98±0.31^{b}	2.49±0.61^{c}	3.40±0.73^{c}	6.79±1.09^{bc}	3.96±0.29^{c}
E1	54.77±25.87	3.96±0.68^{bc}	5.28±1.01^{a}	5.22±1.39^{abc}	7.78±1.79^{abc}	4.86±1.11^{ab}
E2	43.02±30.22	5.91±0.36^{a}	4.36±1.06^{ab}	7.23±3.39^{a}	8.79±1.81^{ab}	5.67±1.27^{ab}
E3	54.51±25.25	4.80±1.41^{b}	5.17±1.28^{a}	6.06±1.67^{ab}	9.67±3.94^{a}	6.06±1.66^{a}
p	0.120	0.000	0.000	0.001	0.000	0.009

Mean ± standard deviation; n=6

a,b,c,d, Values with different letters in the same column are statistically significant

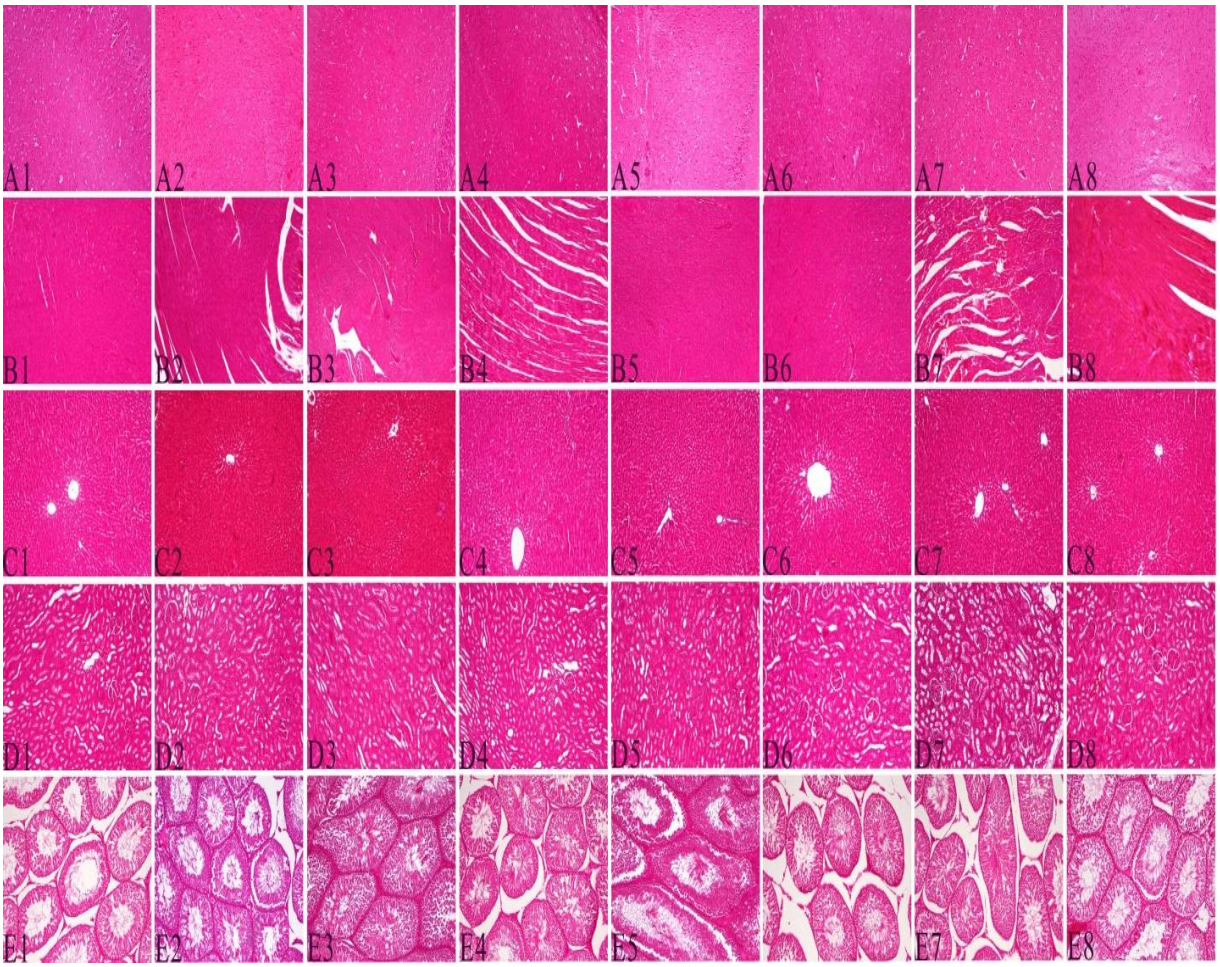

Figure 1. Effect of aqueous and ethanolic extracts from *Sideritis akmanii* on the brain (A), heart (B), liver (C), kidney (D), and testis (E) tissues of male rats. All figures are painted with H&E. 20x and 100 µm were used as the original magnification. (1) Control, (2) CMC, (3) Aqueous 1%, (4) Aqueous 2%, (5) Aqueous 4%, (6) Ethanol 1%, (7) Ethanol 2%, (8) Ethanol 4%.

DISCUSSION

Antioxidants protect the organism from harmful effects by inhibiting the formation of reactive oxygen metabolites. Cells maintain their vital functions against oxidative damage with the help of the antioxidant system (Dündar and Aslan 2000). Many chemicals found in plants, especially phenolic acids and flavonoids, contribute to this system by showing strong antioxidant activity. Phenolic substances, flavonoids, and glycosides in the chemical structure of Sideritis species have an antioxidant effect (González Burgos et al. 2011). Biological systems remove free radicals by means of enzymatic antioxidants such as CAT, and SOD (Şerbetçi 2007) and non-enzymatic antioxidants such as GSH. The increase in free oxygen radicals causes tissue damage resulting in the formation of MDA, a product of lipid peroxidation (Özden et al. 2004).

Many in vitro studies have been carried out to measure the antioxidant activity of Sideritis species. Sagdic et al. (2008) determined in their study that methanolic extracts obtained from *S. ozturkii* and *S. caesarea* have antioxidant effects. Zengin et al. (2014) reported that methanol and water extracts obtained from *Sideritis galatica* had higher phenolic content and antioxidant capacity. In a study, ethanolic and aqueous extracts of 5 different Sideritis species were compared in terms of phenolic acid and flavonoid components. As a result of this comparison, it was seen that ethanol showed higher performance in extracting phenolics (Ozkan 2009). In general, it is seen that the antioxidant effects of Sideritis species have been investigated in vitro. Few in vivo studies show the effect of its

components and antioxidant properties on living organisms. In an in vivo study, the researchers administered an infusion of the above-ground parts of *Sideritis caesarea* in rats in which a model of oxidative stress was established. As a result of the study, it was stated that the MDA level, which increased due to oxidative damage in the tissues, decreased significantly with the infusion, and the GSH level increased significantly. It has been emphasized that Sideritis caesarea provides protection against chemical-induced oxidative damage (Celik and Kaya 2011). In another in vivo study, they reported that herbal tea obtained from *S. clandestina* increased antioxidant capacity in mice, especially in brain tissue (Linardaki et al. 2008). Contrary to these studies, there was no statistically significant difference in MDA level in tissue homogenates, but a statistical decrease was observed in whole blood MDA level only in the ethanolic extract group. This situation between the ethanolic extract and the aqueous extract suggests that different solvents may have different effects. In this study, GSH level in whole blood increased in both water extract and ethanol extract compared to the control group, but no difference was observed in tissue homogenates. It is seen that two different extracts are effective on the GSH level.

Free radicals that are constantly produced in the cell are destroyed by the antioxidant defense system. Antioxidant defense systems produced during normal metabolism can also be taken into the body through food. Antioxidant defense systems have complex enzymatic and non-enzymatic systems. The first line of defense is in the class of SOD and CAT enzymatic antioxidant defense systems that suppress the formation of free radicals (Lobo et al. 2010, Surai et al. 2019). The superoxide dismutase enzyme removes the superoxide radical, but as a result, hydrogen peroxide, another highly toxic substance, is formed. One of the enzymes effective for the breakdown (detoxification) of hydrogen peroxide is CAT (Day 2009).

The effects of Sideritis species on some enzymes have recently attracted attention and various studies have been presented. In an in vivo study, the effect of aqueous and ethanolic extract obtained from the *Sideritis akmanii* plant on biochemical parameters in rats was investigated, and it was observed that ethanolic extract caused a decrease in liver enzymes. (Coğuplugil 2019). Similarly, it was stated that antioxidant enzyme activities such as SOD and CAT of Sideritis caesarea infusion increased significantly in the brain, liver, and kidneys of rats against the harmful effects of TCA, but decreased significantly in the group given Sideritis (Celik and Kaya 2011).

In another in vitro study, the total phenolic content, DPPH radical scavenging effect, total antioxidant capacities, and bio element levels were investigated by using different solvent extracts of the Sideritis akmanii plant. Researchers have emphasized that the amount of phenolic substance in the methanolic extract is higher than that of the acetone extract and that *S. akmanii* contains elements that participate in the antioxidant enzyme (SOD and CAT) structure (Güzey 2017). In this study, it was observed that the enzyme levels were affected by the ethanolic extract causing an increase in both SOD and CAT enzyme activities.

When the literature is examined, there are limited studies on the histopathological effects of Sideritis species. In a study investigating acute and repeated dose oral toxicity in mice, *Sideritis scardica* ethanolic extract reported that all animals survived and no pathological abnormalities were observed. (Feistel et al. 2018). Similarly, no pathological changes were observed in the tissues examined histopathologically in this study.

CONCLUSION

In conclusion despite the large number of studies examining the antioxidant properties of Sideritis species, especially Sideritis akmanii, in vitro, it is noteworthy that the number of in vivo studies is insufficient. Although the effect of the extracts on the MDA level in the tissue homogenate was not observe it caused a decrease in the ethanolic extract in whole blood. While no effect was observed in tissue homogenates in GSH level, both extracts increased in whole blood compared to the control group. The effect of sideritis species on enzymes has been demonstrated by some studies, albeit in limited numbers. In this study, a significant increase in antioxidant enzymes, especially ethanolic extract, is striking. In this study, the effect of Sideritis akmanii aqueous and ethanolic extracts on healthy tissues was investigated, and further studies are needed to observe the effect in case of oxidative stress.

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Ethical Statement: The experimental protocols were approved by the Animal Care and Use Committee of Afyon Kocatepe University and were by the National Institutes of Health Guide for the Care and Use of Laboratory Animals (AKUHADYEK-79-18).

Conflict of Interest: There is no real or potential conflict of interest to declare.

REFERENCES

Aebi H. Catalase in vitro, in: U. Bergmeyer (Ed.), Methods of enzymatic analysis. Academic Press, New York and London. 1974; pp. 673-677. https://doi.org/10.1016/B978-0-12-091302-2.50032-3

Aksoy L, Güzey I, Düz M. Essential Oil Content, Antioxidative Characteristics and Enzyme Inhibitory Activity of Sideritis akmanii Aytaç, Ekici & Dönmez. Turkish Journal of Pharmaceutical Sciences 2022; 19(1): 76. https://doi.org/10.4274/tjps.galenos.2021.86422

Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. Journal of Laboratoty and Clinical Medicine 1993; 61: 882-888.

Bondì ML, Bruno M, Piozzi F, Baser KHC, Simmonds, MS. Diversity and antifeedant activity of diterpenes from Turkish species of Sideritis. Biochemical systematics and ecology 2000; 28(4): 299-303.

Celik I, Kaya MS. The antioxidant role of Sideritis caesarea infusion against TCA toxicity in rats. British journal of nutrition 2011; 105(5) 663-668. https://doi.org/10.1017/S0007114510004265

Cigerci IH, Turhan H, Hazman O, Isitez N. The effects of Sideritis akmanii on endoplasmic reticulum stress, inflammation, and DNA damage in experimentally ER-stress-induced MCF-7 cancer cells. International Journal of Plant Based Pharmaceuticals 2023; 3(1): 47-53. https://doi.org/10.29228/ijpbp.15

Coğuplugil A. The investigation of effects of extracts obtain from *Sideritis akmanii* on lipid profile and some biochemical parameters in rats. MSc, Afyon Kocatepe University Health Science Institute, Afyon, 2019.

Çarıkçı S. Antioxidant and Anticholinesterase Properties of Sideritis perfoliata subsp. athoa (Papan. &Kokkini) Baden and Sideritis trojana Bornm. Teas from Mount Ida-Turkey and Their Phenolic Characterization by LC-MS/MS. Journal of the Turkish Chemical Society Section A: Chemistry, 2020; 7(2): 617-634. https://doi.org/10.18596/jotcsa.718274

Çelik Y, Küçükkurt İ. Investigation of the Antioxidant Effects of Extract Obtained from Thermopsis turcica Plant in Rats. Kocatepe Veterinary Journal 2016; 9(4): 259-2650. https://doi.org/10.5578/kvj.29164

Day BJ. Catalase and glutathione peroxidase mimics. Biochemical pharmacology 2009; 77(3): 285-296. https://doi.org/10.1016/j.bcp.2008.09.029

Draper HH, Hadley M. Malondialdehyde determination as index of lipid Peroxidation. In Methods in enzymology 1990; 186: 421-431. https://doi.org/10.1016/0076-6879(90)86135-I

Drabkin DL, Austin JH. Spectrophotometric Studies. II. Preparations From Washed Blood Cells; Nitric Oxide Hemoglobin and Sulfhemoglobin Journal of Biological Chemistry 1935; 112: 51–65. https://doi.org/10.1016/S0021-9258(18)74965-X

Dündar Y, Aslan R. Hekimlikte Oksidatif Stres ve Antioksidanlar. Afyon, Turkey: Afyon Kocatepe Üniversitesi Yayınları, 2000.

Feistel B, Wegener T, Rzymski P, Pischel I. Assessment of the Acute and Subchronic Toxicity and Mutagenicity of Sideritis scardica Griseb. Extracts. Toxins 2018; 10(7): 258. https://doi.org/10.3390/toxins10070258

González-Burgos E, Carretero ME, Gómez-Serranillos MP. Sideritis spp.: Uses, chemical composition and pharmacological activities—A review. Journal of Ethnopharmacology 2011; 217-219. https://doi.org/10.1016/j.jep.2011.03.014

Güvenç A, Okada Y, Akkol EK, Duman H, Okuyama T, Çalış İ. Investigations of anti-inflammatory, antinociceptive, antioxidant and aldose reductase inhibitory activities of phenolic compounds from Sideritis brevibracteata. Food Chemistry 2010; 118(3): 686-692. https://doi.org/10.1016/j.foodchem.2009.05.034

Güzey İ. Determınatıon of Afyon endemıc plant *Sideritıs akmanii* free radical scavening,the total phenolic substances, total antıoxidant and an oxidant status and mineral matter content. MSc, Afyon Kocatepe University Science Institute, Afyon, Turkey, 2017.

Kucukkurt I, Ince S, Fidan AF, Ozdemir A. The effects of dietary supplementation of different amount of Yucca schidigera powder (Sarsaponin 30®) on blood and tissue antioxidant defense systems and lipid peroxidation in rats. Journal of Animal and Veterinary Advances 2008; 7: 1413-1417.

Linardaki Z, Papandreou MA, Iatrou G, Lamari FN, Margarity M. Ethnobotanical notes about some uses of medicinal plants in Alto Tirreno Cosentino area (Calabria, Southern Italy). Journal of Ethnobiology and Ethnomedicine 2008; 3: 1-6. https://doi.org/10.1186/1746-4269-3-34

Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 2010; 4(8): 118–126. https://doi.org/10.4103/0973-7847.70902

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. The Journal of Biological Chemistry 1951; 193: 265-75. https://doi.org/10.1016/S0021-9258(19)52451-6

Luck H. Catalase. (Bergmeyer, H.U. (ed). Methods in analysis London: Academy Press 1955.

OECD – (Organisation for Economic Co-operation and Development) Guidelines for the Testing of Chemicals, Oecd 423. Acute Oral ToxicityAcute Toxic Class Method. Paris: Organisation for Economic Cooperation and Development, 2001.

Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry 1979; 95: 351-358. https://doi.org/10.1016/0003-2697(79)90738-3Get rights and content

Ozturk Y, Aydın S, Ozturk N, Başer KHC. "Effects of Extracts fromCertain Sideritis species on Swimming Performance in Mice". Phytother. Res, 1996; 10: 70-73. https://doi.org/10.1002/(SICI)1099-1573(199602)10:1<70::AID-PTR785>3.0.CO;2-%23

Ozkan G. Comparison of antioxidant phenolics of ethanolic extracts and aqueous infusions from Sideritis species. Asian Journal of Chemistry 2009; *21*(2): 1024-1028.

Özden TA, Ömer B, Saner G, Saner H, Müslümanoğlu M, Tuzlalı S. MDA, ZN and GSH Levels in Breast Cancer. Türk Klinik Biyokimya Dergisi 2004; 2(2): 59-64.

Sagdic O, Aksoy A, Ozkan G, Ekici L, Albayrak S. Biological activities of the extracts of two endemic Sideritis species in Turkey. Innovative Food Science and Emerging Technologies 2008; 9: 80–84. https://doi.org/10.1016/j.ifset.2007.06.001

Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clinical Chemistry 1988; 34: 497-500. ISSN: 0009-9147

Surai PF, Kochish II, Fisinin VI, Kidd MT. Antioxidant defence systems and oxidative stress in poultry biology: An update. Antioxidants 2019; 8(7): 235. https://doi.org/10.3390/antiox8070235

Şerbetçi H. Determınation of antioxidant capacity of licorice (Glycyrrhiza glabra L.). MSc, . Atatürk University, Erzurum, Turkey, 2007.

Tadić VM, Jeremic I, Dobric S, Isakovic A, Markovic I, et al. Anti-inflammatory, gastroprotective, and cytotoxic effects of *Sideritis scardica* extracts. Planta medica 2012; *78*(05): 415-427. https://doi.org/10.1055/s-0031-1298172

Temel M, Kara R, Müdüroğlu R, Akkaya L. Research Paper Healthcare Antibacterial Activity of Turkish Endemic Sideritis Akmanii (Lamiaceae). Healthcare 2014; 3: 4.

Winterbourn CC, Hawkins RE, Brian M, Carrell RW. The estimation of red cell superoxide dismutase activity. The Journal of laboratory and clinical medicine 1975; 85(2): 337-341. https://doi.org/10.5555/uri:pii:0022214375904394

Zengin G, Sarikurkcu C, Aktumsek A, Ceylan R. Sideritis galatica Bornm.: a source of multifunctional agents for the management of oxidative damage, Alzheimer's's and diabetes mellitus. journal of functional foods, 2014; 11: 538-547. http://dx.doi.org/10.1016/j.jff.2014.08.011).

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RESEARCH ARTICLE

Prevalence of Diroflariasis, Lyme, Anaplasmosis, and Ehrlichiosis in Dogs in Afyonkarahisar Province

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ABSTRACT

This study aimed to determine the prevalence of dirofilariasis, Lyme, anaplasmosis, and ehrlichiosis in sheltered dogs in Afyonkarahisar. The data were obtained and evaluated from 400 dogs (female, n = 246; male, n = 154) of different breeds and at different ages that were referred to Afyon Kocatepe University Animal Hospital.. Accordingly, ovariohysterectomy (n = 196), castration (n = 113), skin disease (n = 113), diarrhea, vomiting, fatigue and anorexia (n = 37), lameness (n = 13), transmissible venereal tumor (n = 9), ascites (n = 2), pregnancy diagnosis (n = 2), mammary tumor (n = 1) and conjunctivitis (n = 1) were determined in dogs. Blood samples of dogs were taken to hematology and biochemical analyses after the first clinical examination of dogs. SNAP 4Dx (Idexx) Quick test kit was used to diagnose dirofilariasis, Lyme, anaplasmosis, and ehrlichiosis. The prevalence of anaplasmosis, ehrlichiosis, dirofilariasis, and Lyme was 1.25%, 0.75%, 0.75%, and 0%, respectively. Although the prevalence of anaplasmosis, ehrlichiosis, and dirofilariasis is low, the results of the study suggested that these zoonotic diseases should still be evaluated as a risk factor for both humans and animals which live in this region as the disease spreads through vectors.

Keywords: Afyon region, Borrelliosis, heartworm, seroprevalence, zoonoses

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Afyonkarahisar Yöresi Köpeklerinde Diroflariozis, Lyme, Anaplazmozis ve Ehrlichiozisin Prevalansı

ÖZ

Bu çalışmada Afyonkarahisar'da yaşayan köpeklerde dirofilariazis, Lyme, anaplasmozis ve ehrlichiozis prevalansının belirlenmesi amaçlanmıştır. Veriler, Afyon Kocatepe Üniversitesi Hayvan Hastanesi 'ne sevk edilen farklı ırk ve yaştaki 400 köpekten (dişi, n=246; erkek, n=154) elde edildi ve değerlendirildi. Buna göre ovariohisterektomi (n=196), kastrasyon (n=113), deri hastalığı (n=113), ishal, kusma, yorgunluk ve anoreksi (n=37), topallık (n=13), bulaşıcı zührevi tümör (n= 9), asites (n=2), gebelik tanısı (n=2), meme tümörü (n=1) ve konjonktivitli (n=1) köpeklerde saptanmıştır. Köpeklerin ilk klinik muayenesinin ardından hematolojik ve biyokimyasal analizler için kan örnekleri alındı. SNAP 4Dx (Idexx) Hızlı test kiti, dirofilariasis, Lyme, anaplasmosis ve ehrlichiosis'i teşhis etmek için kullanıldı. Anaplazmoz, ehrlichiosis, dirofilariasis ve Lyme prevalansı sırasıyla %1.25, %0.75, %0.75 ve %0 olarak tespit edildi. Anaplazmoz, ehrlichiosis ve dirofilariazis prevalansı düşük olmasına rağmen, çalışmanın sonuçları, bu zoonotik hastalıkların, hastalık vektörler yoluyla yayıldığı için hem insanlar hem de bu bölgede yaşayan hayvanlar için bir risk faktörü olarak değerlendirilmesi gerektiğini göstermiştir.

Anahtar Kelimeler: Afyon yöresi, Borreliosis, kalp kurdu, seroprevalans, zoonoz

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INTRODUCTION

Vector-borne pathogens (VBPs) are globally distributed and rapidly spreading, which are transmitted by arthropods, including ticks, fleas, mosquitoes, and phlebotomine sandflies (Otranto et al., 2009). Bacteria such as *Anaplasma platys*, *Anaplasma phagocytophilum*, *Ehrlichia canis*, and *Borrelia burgdorferi* complex; protozoans such as *Babesia canis*, *Hepatozoon canis*, and *Leishmania infantum;* and the nematode *Dirofilaria immitis* are among the major VBPs in dogs (Day, 2011). Different serological methods, including immunofluorescent antibody test (IFAT), enzyme-linked immunosorbent assay, and SNAP4Dx, were used to assess the distribution of VBPs among European countries. Recent studies show that the prevalence of VBPs may vary depending on the method (Maggi et al., 2014; Sainz et al., 2015).

Scientists and public health officials have reported an alarming increase in infectious diseases (Woolhouse & Gowtage, 2005). The possible occurrence of zoonotic pathogens is approximately 1.9 times higher than the non-zoonotic pathogens among the infectious pathogens. In comparison, the possible occurrence of VBPs is approximately 2.3 times higher than non-VBPs (Taylor et al., 2001). Several factors can contribute to the increase in the incidence of diseases caused by VBPs. These factors include changing climate leading to warmer winters (Eisen et al., 2015), increasing the ways people come in contact with nature, suburbanization bringing pets and pathogens together, migratory birds carrying ticks or pathogens to new areas, reducing insecticide use (Loh et al., 2015), increasing the import of different breed dogs from foreign countries and the travel of dogs from Europe or other continents to the tropics along with their owners has increased the prevalence of diseases (Özata, 2012; Unvar et al., 2006). Human-canine bonding that provides several benefits to people has led to some problems. Among these, some risks, such as the transmission of zoonotic diseases seen in dogs to humans, can be included (Doğanay & Şahal, 1987).

The expansion of international tourism and increase in travel activities may have increased tick-borne infections in dogs, which is known as 'emerging infectious disease' (Düzlü et al., 2014). Pets are known as reservoirs in the transmission of some VBPs, even when they do not show any clinical signs (Skotarczak, 2018).

A total of 107 zoonotic infections (37 bacteria, 13 fungi, 29 viral, and 28 parasites) have been reported in Turkey. Of these infections, 19 are transmitted by arthropod vectors. Therefore, 21 of these zoonotic infections are considered a high-priority disease in Europe (Düzlü et al., 2020). Afyonkarahisar is located in the migration routes of migratory birds passing through Turkey from different areas and is a junction point between big cities and holiday destinations in Turkey. The millions of visitor birds use shelters or nests in Turkey during their annual migration, and this leads to a constant threat of spreading new infectious agents. Despite the high endemicity of tick-borne pathogens and the availability of suitable tick habitats in Turkey, no in-depth epidemiological studies and research are available on tick-borne diseases (Inci et al., 2016).

The study aimed to reveal the relationship between the prevalence of zoonosis and common vector-borne diseases in Afyonkarahisar, as well as the predisposing reasons that may cause the presence of these diseases in Afyonkarahisar.

MATERIAL AND METHODS

Animals

The data were evaluated in 400 dogs (female, n = 246; male, n = 154) of different breeds and at different ages that were taken to the xx University Animal Hospital. The age of dogs was as follows: 281 dogs were 1–3 years old, 93 were 4–6 years old and 26 were older than 7 years old. Accordingly, ovariohysterectomy in dogs (n = 196); castration (n = 113); skin problem (n = 113); diarrhea, vomiting, weakness and anorexia (n = 37); lameness (n = 13); transmissible venereal tumor (n = 9); ascites (n = 2); pregnancy examination (n = 2); breast tumor (n = 1); and conjunctivitis (n = 1) complaints were determined.

Rapid Test Kit

We used rapid test kits (IDEXX Snap 4Dx, Westbrook, Maine 04092 United State). The kit functions based on the principle of antigen testing for *D. immitis* and antibody testing for other factors. Three drops of sample and four drops of conjugate were dropped separately into the Eppendorf tubes, and the tube was mixed gently 3–5 times. The final mixture was dropped into the sample compartment, and when the mixture reached the activation section of the kit, the activator part was pressed and waited for 8–10 min to read the result. Lastly, the results were read and recorded (Figure 1).

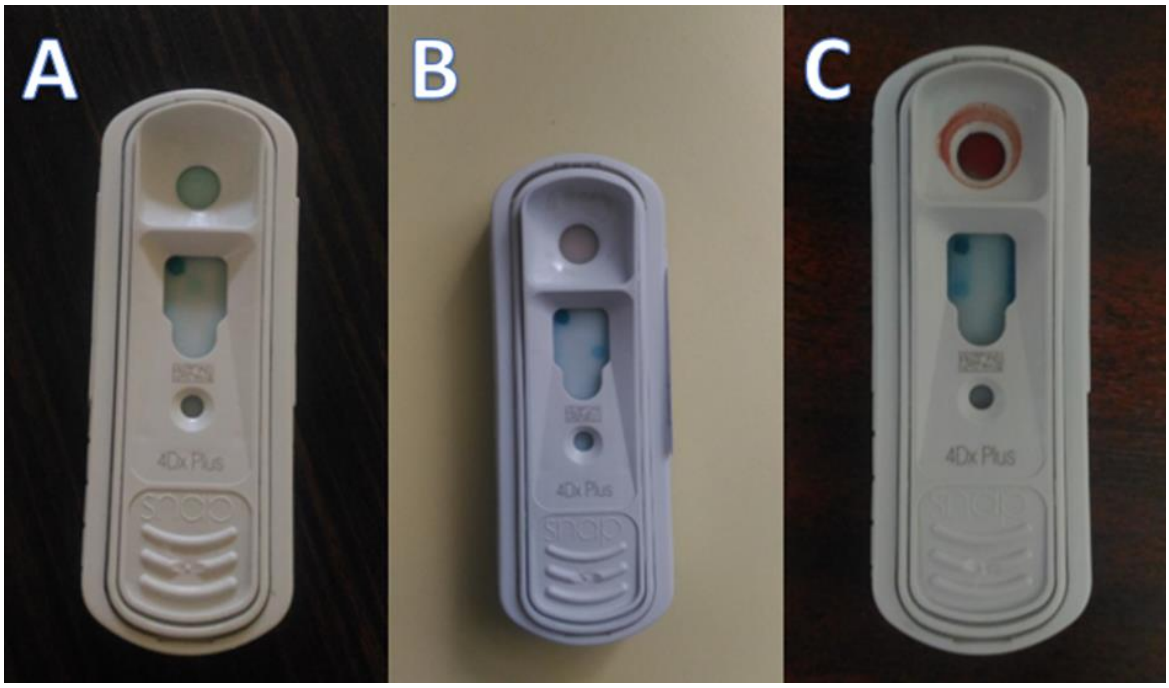

Figure 1: Examples of positive kits (A: A. Phagocytophilum, B: D. immitis, C: E. canis)

Hematological Examination

Blood samples were taken into tubes with K3EDTA, and simultaneously measured using the full automatic hematology device (Mindray BC-2800 Vet, Mindray, Shenzhen, China) in the internal medicine laboratory of our hospital for hematological examinations.

Statistical Analysis

Dogs are classified according to different ages, sexes, and housing patterns. The data obtained from the dogs included in the study were evaluated by the percentage analysis method. Descriptive evaluation of hematological findings in dogs were analyzed with the Windows SPSS 16.0 Package Program (SPSS Inc., Chicago, IL, USA).

RESULTS

The ages and diagnoses of the dogs are presented in Table 1. A total of 400 dogs were included in the study; 33 were indoor fed, and 367 were outdoor fed. Three dogs diagnosed with *D. immitis* infection and five dogs diagnosed with *A. phagocytophilum* infection were all females. In the case of *E. canis* infection, two dogs were female, and one was male. No dog was diagnosed with *B. burgorferi* infection in our study. The prevalences of *D. immitis* was 0.75% (indoor 0%, outdoor 0.82%), *E. canis* was 0.75% (indoor 9.1%, outdoor 0%), *A. phagocytophilum* was 1.25% (indoor 0%, outdoor 1.36%) and *B. burgorferi* was 0% (Table 1 and 2). Two dogs diagnosed with *Ehrlichia* were of Rottweiller (indoor), and one was of Setter (outdoor) breeds. One of the dogs had fainting problems, the other had epistaxis and respiratory problems, and the third one was brought to the obstetrics clinic for pregnancy examination. Leukocytosis and anemia were detected in two of the three dogs. No hematologically abnormal result was seen in the third dog.

Five dogs included in our study were diagnosed with *Anaplasma phagocytophilum*. All five dogs were fed outdoors. One of the dogs was of Terrier breed, one of them was of Kangal breed, and the other three were cross breeds. Four out of the five dogs were brought to our hospital for ovariohysterectomy, and a positive diagnosis was made, although no clinical findings were detected. One dog was brought to our hospital with complaints of loss of appetite and chronic weight loss.

D. immitis was diagnosed in three dogs brought to our hospital for ovariohysterectomy. No abnormal findings were found in the clinical examination of the dogs. The hematological examination results revealed anemia in one dog and leukocytosis in the other dog.

As a result of the statistical evaluation of the blood samples taken from the dogs included in the study, there was no statistically significant difference between *D. immitis, Anaplasma phagocytophilum*, *E. canis* and dogs without disease (Table 3).

Table 1. Prevalence and seroprevalences using the 4DX SNAP test (IDEXX Laboratories, Inc., Westbrook, Maine, USA) for 400 dogs analyzed for *Dirofilaria immitis* antigens and *Ehrlichia* spp., *Anaplasma* spp., and *Borrelia burgdorferi* antibodies in the Afyonkarahisar region of Turkey, according to age and sex. (%) percentage of dogs; (+) positive dogs; (N) number of dogs sampled.

	D. immitis %(+/N)	**E. canis %(+/N)**	**A. phagocytophilum %(+/N)**	**B. burgorferi %(+/N)**	**Total %(+/N)**
Age					
1–3	0,71% (2/281)	0% (0/281)	0,71% (2/281)	0% (0/281)	1,42% (4/281)
4–6	1,08% (1/93)	3,23% (3/93)	3,23% (3/93)	0% (0/93)	7,5% (7/93)
> 7	0% (0/26)	0% (0/26)	0% (0/26)	0% (0/26)	0% (0/26)
Sex					
Male	0% (0/154)	0,65% (1/154)	0% (0/154)	0% (0/154)	0,65% (1/154)
Female	1,22% (3/246)	0,81% (2/246)	2,03% (5/246)	0% (0/246)	4,07% (10/246)
Total	0,75% (3/400)	0,75% (3/400)	1,25% (5/400)	0% (0/400)	2,75% (11/400)

Table 2. Prevalence and seroprevalences using the 4DX SNAP test (IDEXX Laboratories, Inc., Westbrook, Maine, USA) for 400 dogs analyzed for *Dirofilaria immitis* antigens and *Ehrlichia* spp., *Anaplasma* spp., and *Borrelia burgdorferi* antibodies in the Afyonkarahisar region of Turkey, according to habitus. (%) percentage of dogs; (+) positive dogs; (N) number of dogs sampled.

	D. immitis %(+/N)	**E. canis %(+/N)**	**A. phagocytophilum %(+/N)**	**B. burgorferi %(+/N)**	**Total %(+/N)**
Habitus					
İndoor	0% (0/33)	9,1% (3/33)	0% (0/33)	0% (0/33)	9,1% (3/33)
Outdoor	0,82% (3/367)	0% (0/367)	1,36% (5/367)	0% (0/367)	2,18% (8/367)
Total	0,75% (3/400)	0,75% (3/400)	1,25% (5/400)	0% (0/400)	2,75% (11/400)

Table 3. Descriptive analysis of the hematological findings of the dogs included in the study (Mean ± SD)

	D. immitis	A. phagocytophilum	E. canis	Negative
WBC (10^9/l)	16,95±8,13	16,98±6,32	20,57±7,45	19,88±8,45
Lym (10^9/l)	4,70±5,23	1,78±0,95	2,57±0,49	3,16±2,86
Mon (10^9/l)	0,55±0,35	0,50±0,16	0,37±0,32	0,55±0,28
Gran (10^9/l)	11,70±2,55	14,70±5,47	17,43±7,05	16,18±6,75
Lymp %	22,75±20,01	10,28±2,12	12,93±2,59	15,41±8,73
Mon %	3,15±0,49	3,43±2,07	3,13±1,11	2,99±1,15
Gran %	74,10±20,51	86,30±3,33	83,93±3,44	81,84±8,42
EOS %	8,40±10,32	3,10±1,00	1,90±0,53	7,56±10,79
RBC 10^{12}/l	5,46±2,91	5,73±0,72	4,48±2,13	6,34±1,20
Hb g/dl	12,70±7,21	13,15±2,46	10,07±6,13	14,37±2,79
HCT (%)	39,70±22,20	38,53±6,00	30,37±18,05	43,61±8,52
MCV (fl)	72,20±2,26	67,22±4,39	65,20±9,97	68,93±4,54
MCH (pg)	22,95±0,92	22,85±3,04	21,40±3,54	22,65±1,81
MCHC (g/dl)	31,85±0,35	34,00±2,50	32,90±0,52	32,93±1,83
RDW (fl)	10,90±0,28	14,95±0,89	14,83±3,15	14,27±4,21
PLT (10^9/l)	313,00±45,25	404,00±159,61	199,33±114,61	379,14±220,06
MPV (fl)	9,60±0,28	8,15±0,79	8,60±1,10	8,58±1,16
PDW (fl)	16,00±0,00	15,80±0,24	16,30±0,30	15,98±0,39
PCT (10^9/l)	0,30±0,03	0,33±0,13	0,17±0,09	0,31±0,12

WBC: White Blood Cell, Lym: Lymphocyte, MID: Monocyte, Gra: Granulocyte, Hb: Hemoglobin, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, RBC: Red Blood Cell, RDW: Red Cell Distribution Width, HCT: Hematocrit, PLT: Platelet, PCT: Procalcitonin, PDW: Platelet Distribution Width, MPV: Mean Platelet Volume

DISCUSSION

Although Afyonkarahisar is located in the Aegean region, the annual average temperature values are much lower than in the coastal Aegean cities. During the meteorological data examination, the temperature decreases to −27 °C in the winter months, with an annual average of 11.3 °C. Similarly, yearly precipitation is at the lowest level with 443.6 mm compared to other Aegean cities. Vectors are adversely affected by low-temperature values and dryness (Aydın et al., 2021).

There is increasing evidence that changes in land use affect the microclimate conditions and the habitat suitability for both ticks and their hosts (Asghar et al., 2016; Jones et al., 2011; Estrada- Peña, 2001). For example, the abandonment of farmland and regrowth of native vegetation may provide a suitable environment for ticks (Hrkľová et al., 2008; Medlock et al., 2013); however, intensive agricultural activities, loss of native vegetation, and reduced rodent host diversity combined with lack of leaf litter may lead to unsuitable conditions for tick development (Michel et al., 2006; Perez et al., 2016). Sheltering of the majority of stray animals in the settlements, the regular spraying of the landscape areas of the settlements by the boroughs to combat ticks and mosquitoes, and the regular removal of leaf litter where ticks can live may reduce vector-borne diseases.

Researchers in different countries and Turkey have used different test kits and methods to determine the prevalence of vector-mediated zoonotic diseases such as *D. immitis, B. burgdorferi, E. canis, and A. phogocytophilum* (Bell & Patel, 2005; Küçüker, 2016; Gokmen, 2019). In a study in the Aegean region, 307 dogs were screened with Snap 4Dx. The prevalences of the diseases were as follows: *E. canis,* 24.42%; *E. canis* + *A. phagocytophilum* co-infection, 10–42; *A. Phagocytophilum,* 7.49%; and *D. immitis,* 2.28% (Ural K et al., 2014). In a study on 100 dogs in Igdır province, *D. immitis* (40%) and *E. canis* (1%) were reported (Sarı et al., 2013). *D. immitis* (2.4%) and *E. canis* (4.8%) were found in a study with 82 dogs in Diyarbakır (İçen et al., 2011). In a study conducted on 100 dogs

in the Osmaniye region, it was found that E.canis / E. ewingii was 3%, D.immitis was 1%, *A.platys* / *A.phagocytophilum* and Lyme were 0% (26). In this study, the prevalence of *D. immitis* was 0.75%, *E. canis* was 0.75%, *A. phagocytophilum* was 1.25%, and *B. burgforferi* was 0%. Although Afyonkarahisar province is located in the Aegean region, we found that the prevalences are significantly lower than the studies performed in the other areas of the Aegean region, which may be because Afyonkarahisar is in the Central Anatolia continental climate region.

The relationship between vector-borne diseases and gender is still being investigated. Although no effect of gender on *D. immitis* infection has been stated in dogs, some researchers have reported that this parasite is more common in male dogs than females (Theis et al., 2001; Fan et al., 2001; Aranda et al., 1998; Montoya et al., 1998 ; Martin & Collins, 1985; Selby et al., 1980; Graham, 1974). This may be because females are more stable and less active than males. In contrast to other studies, we determined that the number of female animals was higher in the groups with positivity. We believe that gender has no role in predisposition to vector-borne diseases.

Dogs infected by *A. phagocytophilum* typically present with sluggishness, fever, and loss of appetite. Furthermore, lameness and reluctance to move because of neutrophilic polyarthritis may be observed. In addition to these, vomiting, diarrhea, and bleeding disorders such as epistaxis have been reported. However, the majority of dogs appear healthy. In the endemic regions of the disease, 60% of the dogs were seropositive, and no clinical findings were found (Özcel, 2013). Similar to other studies, cachexia and stagnation were observed in only one of the five reported dogs with anaplasmosis in this study. In contrast, no clinical abnormality was found in the other four dogs.

Coagulation disorders may occur in the chronic form of ehrlichiosis because of thrombocytopenia and thrombocytopathy. As a result of the coagulation disorders, epistaxis, melena, petechiae, hematuria, intraocular, brain, and lung hemorrhages can be observed (Aytuğ, 2012). Similarly, in this study, one of the dogs diagnosed with *Ehrlichia* was fainting, the other had epistaxis, and the third one had no clinical pathology. The disease spreads widely in Turkey, and the prevalence of the disease in different regions has been reported to be between 0.06% and 20% (Coşkun et al., 1992; Zeybek, 1989; Alkan & Sarınç, 1986). The prevalence of the disease is higher in village dogs than in both sheltered and pet dogs. This may be because of the presence of reservoir dogs in villages and anti-parasitic pesticides in shelters (Voyvoda et al, 2004). Similarly, in this study, all positive dogs were fed outdoors. The high prevalence of these dogs can be explained by the fact that they may be more in contact with the vector.

Although Lyme disease in dogs is zoonotic, very few studies have been found on the presence and prevalence of the disease in Turkey. In Turkey, Gulanber et al. (Gulanber et al., 2007) reported the first clinical case in dogs which was a St. Bernard breed in Istanbul. Esendal et al. (Esendal et al., 1996) found that the seroprevalence of Lyme disease was 78.4% in 74 dogs using the IFAT method in Ankara. Satir (Satir, 2006) performed polymerase chain reaction in 96 dogs in Istanbul, and no positivity was detected in any dogs. Bhide et al. (Bhide et al., 2008) determined that the disease was seropositive in 93 dogs (23.2%) using the enzyme-linked protein A/G assay test in 400 dogs that were taken to Bursa Uludag University Veterinary Faculty Internal Medicine Clinic. No ticks of the genus *Ixodes*, which is the vector of *B. burgdorferi*, were found in 10,303 ticks collected from the Sivas region (Gunes et. al., 2005). A study conducted in the Aydın region reported that the seroprevalence of the disease was 40.8% (Uslu, 2008). *B. burgdorferi* factor was not found in the study performed on 100 dogs in Osmaniye region (Gokmen, 2019). *B. burgdorferi* was not detected in 400 dogs examined in this study.

CONCLUSIONS

As a result, the prevalence of vector-borne diseases in the Afyonkarahisar region is similar to areas with similar climatic conditions. Even if the prevalence is low, the disease poses a risk to both dogs and humans. It needs attention by veterinarians and researchers not only for regions with tropical climates but also for other regions.

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Authors Contribution Rate: The authors declare that they have contributed equally to the article.

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REFERENCES

Aranda, C., Panyella, O., Eritja, R., Castella, J. (1998). Canine filariasis importance and transmission in the Baix Llobregat area, Barcelona (Spain). Vet Parasitol, 77, 267-275. doi:10.1016/s0304-4017(98)00109-5

Asghar, N., Petersson, M., Johansson, M., & Dinnetz, P. (2016). Local landscape effects on population dynamics of Ixodes ricinus. Geospatial health, 11(3). doi:10.4081/gh.2016.487

Aydın, L., Çetin, H., Çırak, V.Y., Girişgin, A.O., & Özüiçli, M. (2021). Artropodoloji. 1. Baskı. Bursa: Dora Yayıncılık.

Aytuğ N. (2012). Köpek ve Kedilerin İç Hastalıkları. 2. Baskı. Malatya: Medipres Yayınevi.

Bell, C. A., & Patel, R. (2005). A real-time combined polymerase chain reaction assay for the rapid detection and differentiation of Anaplasma phagocytophilum, Ehrlichia chaffeensis, and Ehrlichia ewingii. Diagnostic microbiology and infectious disease, 53(4), 301-306. doi: 10.1016/j.diagmicrobio.2005.06.019

Bhide, M., Yilmaz, Z., Golcu, E., Torun, S., & Mikula, I. (2008). Seroprevalence of anti-Borrelia burgdorferi antibodies in dogs and horses in Turkey. Ann Agric Environ Med, 15(1), 85-90.

Coşkun, S.Z., Tınar, R., Akyol, Ç.V., Aydın, L., Demir, C. (1992). Doğal enfekte köpeklerde Dirofilaria immitis mikrofilerlerine ivermektinin etkisi. Uludag Universitesi Veteriner Fakültesi Dergisi, 11(2), 121-128.

Day, M. J. (2011). One health: the importance of companion animal vector-borne diseases. Parasites & vectors, 4, 1-6. doi:10.1186/1756-3305-4-49

Doğanay, A. (1987). Türkiye'de Köpeklerdeki Dirofilariasis Sorunu ve İnsan Sağlığı Açısından Önemi. Ankara Üniversitesi Veteriner Fakültesi Dergisi, 34(02), 277-287. doi:10.1501/Vetfak_0000001091

Düzlü, Ö., Inci, A., Yıldırım, A., Doğanay, M., Özbel, Y., & Aksoy, S. (2020). Vector-borne zoonotic diseases in Turkey: rising threats on public health. Türkiye Parazitolojii Dergisi, 44(3), 168. doi:10.4274/tpd.galenos.2020.6985

Düzlü, Ö., İnci, A., Yıldırım, A., Önder, Z., Çiloğlu, A. (2014). Köpeklerde kene kaynaklı bazı protozoon ve rickettsial enfeksiyonların Real Time PCR ile araştırılması ve saptanan izolatların moleküler karekterizasyonları. Ankara Universitesi Veteriner Fakültesi Dergisi, 61, 275-282. doi:10.1501/Vetfak_0000002642

Eisen, R.J., Eisen, L., Ogden, N.H., Beard, C.B. (2015). Linkages of weather and climate with Ixodes scapularis and Ixodes pacificus (Acari: Ixodidae), enzootic transmission of Borrelia burgdorferi, and Lyme disease in North America. The Journal of Medical Entomology, 53: 250–261. doi:10.1093/jme/tjv199

Esendal, Ö.M., Özgür, M., Arda, M., Akay, Ö., Keskin, O. (1996). Köpeklerde Borrelia burgdorferi antikorlarının floresan antikor tekniği ile saptanması. In: I. Uluslararası Veteriner Mikrobiyoloji Kongresi, İstanbul, Türkiye. 1996. p. 128–129.

Estrada-Peña, A. (2001). Distribution, abundance and habitiat preferences of Ixodes ricinus (Acari: ixodiae) in Northern Spain. The Journal of Medical Entomology, 38, 361–370. doi:10.1603/0022-2585-38.3.361

Fan, C. K., Su, K. E., Lin, Y. H., Liao, C. W., Du, W. Y., & Chiou, H. Y. (2001). Seroepidemiologic survey of Dirofilaria immitis infection among domestic dogs in Taipei City and mountain aboriginal districts in Taiwan (1998–1999). Veterinary Parasitology, 102(1-2), 113-120. doi:10.1016/S0304-4017(01)00511-8

Gokmen, T.G., Günaydin, E., Turut, N., Akın, B., Koç, Ö., Ütük, A.E. (2019). A serosurvey on some canine vector-borne zoonoses (Anaplasma spp., Ehrlichia spp., Borrelia burgdorferi, Dirofilaria immitis and Leishmania spp.) in Osmaniye. Ataturk Universitesi Veteriner Bilim Dergisi, 14(2), 151-158.

Graham, J. M. (1974). Canine filariasis in northeastern Kansas. The Journal of Parasitology, 322-326.. doi: 10.2307/3278475

Gülanber, E. G., Gülanber, A., Albayrak, R., Gülanber, N. G., & Polat, E. (2007). Lyme disease (Borreliosis) in a Saint Bernard dog: First clinical case in Turkey. Turkish Journal of Veterinary & Animal Sciences, 31(5), 367-369. doi: 10.3906/vet-0504-19

Güneş, T., Poyraz, Ö., Kaya, S., Gençer, L., & Alim, A. (2005). Sivas yöresinde Borrelia burgdorferi vektörlerinin ve lyme seropozitifliğinin araştırılması. Mikrobiyoloji Bülteni, 39(4), 503-508.

Hrkľová, G., Nováková, M., Chytra, M., Kostova, C., & Petko, B. (2008). Monitoring the distribution and abundance of Ixodes ricinus ticks in relevance of climate change and prevalence of Borrelia burgdorferi sensu lato in Northern Slovakia (Liptovska valley). Folia Veterinaria, 52(2), 62-63.

Inci, A., Yildirim, A., Duzlu, O., Doganay, M., & Aksoy, S. (2016). Tick-borne diseases in Turkey: A review based on one health perspective. PLoS neglected tropical diseases, 10(12), e0005021. doi:10.1371/journal.pntd.0005021

İçen, H., Sekin, S., Simsek, A., Kochan, A., Celik, O. Y., & Altas, M. G. (2011). Prevalence of Dirofilaria immitis, Ehrlichia canis, Borrelia burgdorferi infection in dogs from Diyarbakir in Turkey. Asian Journal of Animal and Veterinary Advances, 6(4), 371-378. doi:10.3923/ajava.2011.371.378

Jones, E. O., Webb, S. D., Ruiz-Fons, F. J., Albon, S., & Gilbert, L. (2011). The effect of landscape heterogeneity and host movement on a tick-borne pathogen. Theoretical Ecology, 4, 435-448.. doi: 10.1007/s12080-010-0087-8

Küçüker, S., & Şahinduran, Ş. (2018). Antalya İlinde bulunan köpeklerde Dirofilariazis, Borrelioziz, Ehrlichiazis ve Anaplazmozis' in hızlı test kitleri ile teşhisi ve insidansı üzerine araştırmalar. Atatürk Üniversitesi Veteriner Bilimleri Dergisi, 13(2), 191-200.

Loh, E. H., Zambrana-Torrelio, C., Olival, K. J., Bogich, T. L., Johnson, C. K., Mazet, J. A., ... & Daszak, P. (2015). Targeting transmission pathways for emerging zoonotic disease surveillance and control. Vector-Borne and Zoonotic Diseases, 15(7), 432-437. doi:10.1089/vbz.2013.1563

Maggi, R. G., Birkenheuer, A. J., Hegarty, B. C., Bradley, J. M., Levy, M. G., & Breitschwerdt, E. B. (2014). Comparison of serological and molecular panels for diagnosis of vector-borne diseases in dogs. Parasites & vectors, 7, 1-9.. doi:10.1186/1756-3305-7-127.

Martin, T. E., & Collins, G. H. (1985). Prevalence of Dirofilaria immitis and Dipetalonema reconditum in greyhounds. Australian veterinary journal, 62(5), 159-163. doi:10.1111/j.1751-0813.1985.tb07278.x

Medlock, J. M., Hansford, K. M., Bormane, A., Derdakova, M., Estrada-Peña, A., George, J. C., ... & Van Bortel, W. (2013). Driving forces for changes in geographical distribution of Ixodes ricinus ticks in Europe. Parasites & vectors, 6, 1-11. doi:10.1186/1756-3305-6-1

Michel, N., Burel, F., & Butet, A. (2006). How does landscape use influence small mammal diversity, abundance and biomass in hedgerow networks of farming landscapes?. Acta Oecologica, 30(1), 11-20. doi:10.1016/j.actao.2005.12.006

Montoya, J. A., Morales, M., Ferrer, O., Molina, J. M., & Corbera, J. A. (1998). The prevalence of Dirofilaria immitis in gran canaria, Canary Islands, Spain (1994–1996). Veterinary parasitology, 75(2-3), 221-226. doi:10.1016/s0304-4017(97)00175-1

Otranto, D., Dantas-Torres, F., & Breitschwerdt, E. B. (2009). Managing canine vector-borne diseases of zoonotic concern: part one. Trends in parasitology, 25(4), 157-163. doi:10.1016/j.pt.2009.01.003

Özata, F. (2012). Ehrlichia canis ve anaplasma phagocytophilum ile infekte köpeklerde trombosit indeksleri; plateletkrit, ortalama trombosit hacmi ve trombosit dağılım genişliği (Master's thesis, Adnan Menderes Üniversitesi, Sağlık Bilimleri Enstitüsü).

Özcel, M.A. (2013). Veteriner Hekimliğinde Parazit Hastalıkları. 1.Baskı. İzmir: Meta Basım Matbaacılık.

Perez, G., Bastian, S., Agoulon, A., Bouju, A., Durand, A., Faille, F., ... & Butet, A. (2016). Effect of landscape features on the relationship between Ixodes ricinus ticks and their small mammal hosts. Parasites & vectors, 9(1), 1-18. doi:10.1186/s13071-016-**1296-9**

Sainz, Á., Roura, X., Miró, G., Estrada-Peña, A., Kohn, B., Harrus, S., & Solano-Gallego, L. (2015). Guideline for veterinary practitioners on canine ehrlichiosis and anaplasmosis in Europe. Parasites & vectors, 8, 1-20. doi:10.1186/s13071-015-0649-0

Sarı, B., Taşçı, G.T., Kılıç, Y. (2013). Seroprevalence of Dirofilaria immitis, Ehrlichia canis and Borrelia burgdorferi in Dogs in Iğdır Province, Turkey. Kafkas Universitesi Veteriner Fakültesi Dergisi, 19, 735-739. doi:10.9775/kvfd.2012.8466

Sarnıç, H., & Alkan, M. (1986). Köpeklerde Dirofilaria immitis olguları ve insan sağlığı yönünden önemi. Türkiye Parazitoloji Dergisi, 11, 169-174.

Satir, E. (2006). Köpeklerde Borrelia burgdorferi infeksiyonunun PCR ile araştırılması.

Selby, L. A., Corwin, R. M., & Hayes Jr, H. M. (1980). Risk factors associated with canine heartworm infection. Journal of the American Veterinary Medical Association, 176(1), 33-35.

Skotarczak, B. (2018). The role of companion animals in the environmental circulation of tick-borne bacterial pathogens. Annals of Agricultural and Environmental Medicine, 25(3), 473-480. doi:10.26444/aaem/93381

Taylor, L. H., Latham, S. M., & Woolhouse, M. E. (2001). Risk factors for human disease emergence. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 356(1411), 983-989.doi:10.1098/rstb.2001.0888

Theis, J. H., Stevens, F., & Law, M. (2001). Distribution, prevalence, and relative risk of filariasis in dogs from the state of Washington (1997-1999). Journal of the American Animal Hospital Association, 37(4), 339-347. doi:10.5326/15473317-37-4-339

Unver, A., Huang, H., & Rikihisa, Y. (2006). Cytokine gene expression by peripheral blood leukocytes in dogs experimentally infected with a new virulent strain of Ehrlichia canis. Annals of the New York Academy of Sciences, 1078(1), 482-486.doi:10.1196/annals.1374.090

Ural, K., Gultekin, M., Atasoy, A., & Ulutas, B. (2014). Spatial distribution of vector borne disease agents in dogs in Aegean region, Turkey. Revista MVZ Córdoba, 19(2), 4086-4098. doi: 10.21897/rmvz.102

Uslu, O. (2008). Köpeklerde Lyme hastalığının araştırılması (Master's thesis, Adnan Menderes Üniversitesi, Sağlık Bilimleri Enstitüsü).

Voyvoda, H., & Serdar, P. A. Ş. A. (2004). Aydın'ın bazı ilçe ve köyleri ile İzmir'in Selçuk İlçesindeki köpeklerde Leishmaniosis ve Dirofilariasis' in prevalansı. Turkish Journal of Veterinary and Animal Sciences, 28(6), 1105-1111.

Woolhouse, M. E., & Gowtage-Sequeria, S. (2005). Host range and emerging and reemerging pathogens. Emerging infectious diseases, 11(12), 1842. doi:10.3201/eid1112.050997

Zeybek, H. (1989). Ankara yöresi köpeklerinde Dirofilaria immitis olguları. Etlik Veteriner Mikrobiyoloji Dergisi, 6(5), 1-9.

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RESEARCH ARTICLE

Determining The Optimum Voluntary Waiting Period and Synchronization Protocol in Simmental Cows

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ABSTRACT

The objective of the present study was to compare four different synchronization protocols at the end of various voluntary waiting period (VWP) and evaluate the effect of parity and milk yield on pregnancy rates with synchronization in Simmental cows. All animals were randomly allocated into four synchronization groups and one control group. Parity, milk yield (≤23 kg/d; >23 kg/d), and days in milk (DIM; 45-60, 61-90, >90 days) were compared according to synchronization protocols. Ovsynch (Group I; n=81), Ovsynch+Progesterone (Group II; n=30), G6G (Group III; n=66), 2xG6G (Group IV; n=45), and a control group (n=35) were designed for the study. The parity significantly affected the pregnancy rates in the primiparous cows, especially in Group II ($P<0.05$). The pregnancy rates between synchronization and control groups did not differ significantly ($P>0.05$). There was no significant difference between milk yield and pregnancy rates ($P>0.05$). In conclusion, the synchronization of Simmental cows with Ovsynch, Ovsynch+Progesterone, G6G, or 2xG6G did not affect pregnancy rates across various DIM. These data suggest that the suitable VWP could be planned according to the farm's economic and local market targets, with veterinarians able to choose a protocol based on practicality, effectiveness and cost, as well as the specific requirements of the herd.

Keywords: Simmental cow, Synchronization, Voluntary waiting period

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Simmental Irkı İneklerde Optimum Gönüllü Bekleme Süresi ve Senkronizasyon Programının Belirlenmesi

ÖZ

Bu çalışmanın amacı, Simental ırkı ineklerde farklı gönüllü bekleme sürelerinin (GBS) sonunda dört farklı senkronizasyon programının etkinliğinin karşılaştırılması, doğum sayısı ve süt veriminin bu senkronizasyon protokolleri ile gebelik oranlarına etkilerinin değerlendirilmesidir. Hayvanlar rastlantısal olarak dört farklı senkronizasyon ve bir kontrol grubuna ayrıldı. Doğum sayısı, süt verimi (≤23 kg/gün; >23 kg/gün) ve sağılan gün sayısının (SGS; 45-60, 61-90, >90 günler) uygulanan senkronizasyon protokollerine etkileri karşılaştırıldı. Ovsynch (Grup I; n=81), Ovsynch+Progesteron (Grup II; n=30), G6G (Grup III; n=66), 2xG6G (Grup IV; n=45) ve kontrol (n=35) grupları oluşturuldu. Doğum sayısının gebelik oranlarına etkisi karşılaştırıldığında, ilk laktasyondaki primipar ineklerde multipar ineklere göre özellikle Grup II'de istatistiksel önemin ($P<0,05$) yüksek olduğu tespit edildi. Senkronizasyon ve kontrol grupları arasında gebelik oranları bakımından fark gözlenmezken ($P>0,05$), süt veriminin de gebelik oranı üzerinde istatistiksel öneme yol açmadığı ($P>0,05$) belirlendi. Sonuç olarak, Simental ırkı ineklerin Ovsynch, Ovsynch+Progesteron, G6G ve 2xG6G protokolleri ile senkronizasyonunun farklı sağılan gün sayısı (SGS)'na göre gebelik oranlarını etkilemediği belirlendi. Sunulan çalışmadan elde edilen veriler veteriner hekimlerin pratik, etkili, uygun maliyetli ve çiftliğin özel ihtiyaçlarını karşılayabilecek yukarıda belirtilen herhangi bir senkronizasyon protokolünü seçebileceğini, en uygun gönüllü bekleme süresinin ise çiftliğin ekonomik ve bölgesel piyasa hedeflerine göre planlanmasının daha etkili ve verimli olacağını ortaya koymaktadır.

Anahtar Kelimeler: Gönüllü bekleme süresi, Senkronizasyon, Simental inek

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INTRODUCTION

Reproductive efficiency is the main objective in cattle breeding. However, genetic selections for milk production and pressure to increase milk yield have adversely affected reproductive performance in dairy cattle over recent decades (Thatcher et al. 2006, Shahzad et al. 2019). One of the most crucial challenges in reproductive efficiency is managing the VWP and high pregnancy rates in the first service. Fertility parameters such as calving interval, number of days open, average service per conception, and heat detection are in excess in high milk yielding dairy cows in the postpartum period. In dairy herds, insufficient heat detection is the principal management problem affecting reproductive profitability because of poor visual detection of estrous signs, insemination at the wrong time, lack of reproductive records, reproductive health problems, nutrition deficiencies, and housing factors (Ben Salem et al. 2006, Stangaferro et al. 2018, Lindley et al. 2021). Nowadays, different estrus and ovulation synchronization protocols are using to improve pregnancy rates without any estrous detection in dairy cows. Ovulation synchronization protocols allow the insemination of cows in a fixed time, called Fixed Time Artificial Insemination (FTAI); it also helps veterinarians schedule a predefined breeding period and calving time in a suitable environment and season for calves (Pursley et al. 1995, Bo et al. 2016).

Pregnancy rates have decreased from around 65% to 35% in the past 40 years (Fleming et al. 2018). The Ovsynch program was developed to solve this problem and synchronize ovulation time, FTAI and increase AI success but it could not enhance the pregnancy rate per AI (Pursley et al. 1997, Nak et al. 2011, Yılmaz et al. 2011, Fricke et al. 2014). New strategies related to gonadotropin-releasing hormone (GnRH) and prostaglandin F2α (PGF2α) treatments, frequently Ovsynch based developed for ovulation control and increasing pregnancy/AI success.

protocols such as G6G, Double Ovsynch (DO), Presynch/Ovsynch, and Progesterone+Ovsynch aim to improve reproductive management success and pregnancy rates at the end of the VWP. However, each protocol has its advantages and disadvantages: herd size, labor, parity, milk yield, breed of animals, drug cost, etc. (Astiz and Fargas 2013, Wiltbank et al. 2015, Heidari et al. 2017, Shahzad et al. 2019). One of these presynchronization protocols is G6G, which involves PGF2α and GnRH two days apart, and then six days later, following the standard Ovsynch protocol. The results showed that the ovulation rates and circulatory progesterone levels were increased with higher synchronization rates following G6G synchronization (Ribeiro et al. 2012). Double Ovsynch is a more extended protocol than Ovsynch and G6G (28 days vs. 10 and 18 days), and uses two GnRH and PGF2α administrations before Ovsynch to achieve pregnancy rates. Studies suggest that the DO protocol gives better results in primiparous cows (Astiz and Fargas 2013). Progesterone integration to the classic Ovsynch protocol hypothesis to improve synchronization prevents premature estrus and ovulation before PGF2α injection (Larson et al. 2006).

Many studies using the modified Ovsynch protocol give different synchronization and pregnancy rates, and it is still unclear which environmental and/or endocrine factors affect the synchronization success. The objective of this study was to compare four different synchronization protocols at the end of various VWPs days and evaluate the effect of parity, milk yield, and the number of services per conception on pregnancy rates with synchronization in Simmental cows.

MATERIAL and METHODS

All procedures performed with cows were approved by the Experimental Animal Ethics Committee of Afyon Kocatepe University (approval number: 167-20).

Animals

This experiment was performed on a commercial farm with 257 healthy, lactating Simmental cows (aged between two and nine years) housed in free-stall barns. Cows were milked twice daily, and TMR was presented twice daily with ad libitum access to water (NRC 2001). All cows were evaluated to body condition score (BCS) on the day of enrollment (one=emaciated, five=obese).

Experimental Design

All animals were randomly allocated into four synchronization groups and one control group. Parity, milk yield (≤23 kg/d; >23 kg/d), and DIM (45-60; 61-90; >90 days) were compared according to synchronization protocols.

Group I (Ovsynch; n=81): Cows in Group I received GnRH analogue (2.5 ml, I.M; 0.004 mg/ml Buserelin acetate, Receptal, Intervet International GmbH, Germany) on day 0; 500 μg PGF2α (2 ml, I.M.; Cloprostenol, Dalmazin, FATRO, Italy) was injected to all cows on day 7, and second GnRH treatment was administered with the same dose on day 9. Cows were FTAI 16 hours after the second GnRH administration (Figure 1a).

Group II (Ovsynch+Progesterone; n=30): Cows in this group were treated the same as with the Ovsynch protocol, but CIDR (1.38 g progesterone, Easy Bred, Zoetis, Turkey) was inserted on day 0 and removed on day 7 (Figure 1b).

Group III (G6G; n=66): **Animals in this group received** 500 μg PGF2α (2 ml, IM.; Cloprostenol, Dalmazin, FATRO, Italy) on day 0, two days later GnRH (2.5 ml, I.M; 0.004 mg/ml Buserelin acetate, Receptal, Intervet International GmbH, Germany) was injected, 6 days later classic Ovsynch protocol was applied (Figure 1c).

Group IV (2xG6G; n=45): Cows in Group IV received G6G protocol with a modification by two times more GnRH doses (5 ml, I.M; 0.004 mg/ml Buserelin acetate, Receptal, Intervet International GmbH, Germany) (Figure 1d). The effect of GnRH dose on pregnancy rates was studied in this group.

Control Group (n=35): The cows in this group were observed for estrus detection without any hormone administration previously and inseminated.

The animals in the study groups were inseminated at a fixed time, and the animals in the control group were inseminated once at the specified time after the estrus detection.

Pregnancy Diagnosis

Pregnancy status was determined by ultrasonography (HT838, Hasvet, Turkey) on day 45 after FTAI. The presence of amniotic vesicle, positive hearth beat, and intraluminal uterine fluid was used as a determinant of pregnancy.

Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software, Version 20.0. The findings are presented as means and percentages, and mean values are expressed with their standard error and standard deviation. The differences in age, milk production, DIM, and parity between groups were determined using ANOVA. The Kruskall-Wallis test was used to determine the impact of synchronization protocols on primiparous and multiparous cows, VWP groups, and milk production

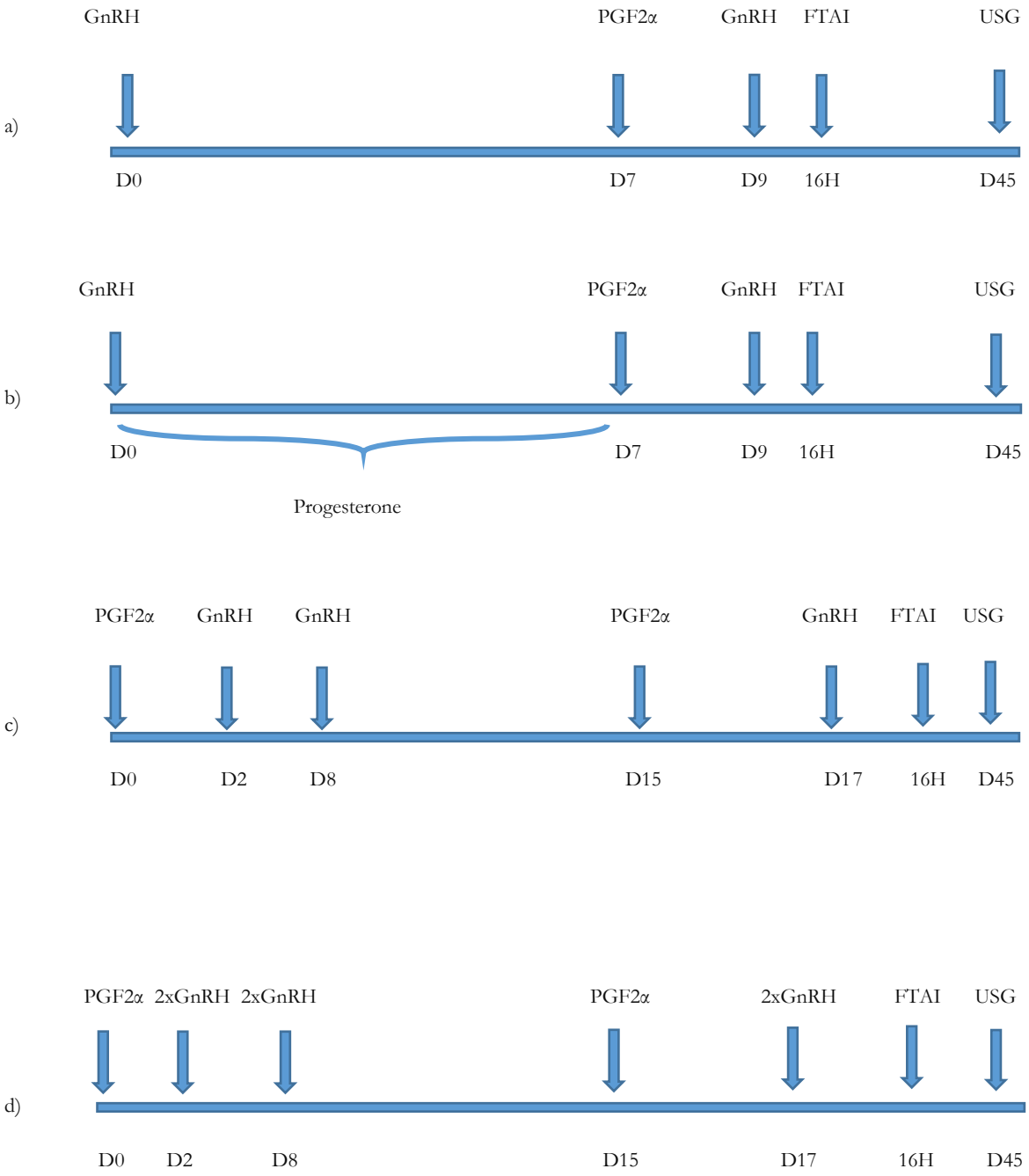

Figure 1. Schematic representation of synchronization protocols during the study in lactating Simmental cows. a) Ovsynch, b) Ovsynch+Progesterone, c) G6G, d) 2xG6G protocols.

RESULTS

The average age of the cows was 4.2±2.0 (2 to 9 years old) in the study. The mean milk production (kg/d), DIM (days), and parity were 23.6±4.1, 106.9±68.0, and 2.6±1.8, respectively (Table 1).

Number and proportions of animals to be first inseminated postpartum in groups; Group I (45/81, 55.6%), Group II (19/30, 63.3%), Group III (37/66, 50.1%), Group IV (22/45, 48.9%) and Control (20/35, 57.1%) respectively. The pregnancy rates on day 45

after FTAI in Group I, Group II, Group III, Group IV, and Control were 55.6%, 53.3%, 54.5%, 51.1%, and 57.1%, respectively. Pregnancy rates following the first AI and two or more AI did not differ significantly between groups (P>0.05). One pregnancy loss was observed in Group I in the fourth month, three aborts in Group III between the second and fifth months, and one abort in Group IV in the fifth month.

Parity significantly affected the pregnancy rates in the primiparous cows, especially in Group II (P<0.05) (Table 2). The pregnancy rates in the other study groups were also higher than in the control group. However, the synchronization protocols did not increase the pregnancy rates compared with the control group in the multiparous cows; the control group rates were significantly higher than those in the study groups (P<0.05).

Pregnancy rates at the end of the VWP of 45-60 DIM were 61.5% in Group I, 60% in Group II, 42.8% in Group III, 50.0% in Group IV, and 53.8% in Control group (Table 3). At the 61-90 days the pregnancy rates were 48.3%, 33.3%, 52.0%, 40.0%, and 33.3%, respectively (Table 3); the rates were 58.9%, 55.5%, 58.8%, 60.9%, and 68.8%, respectively at >91 days at the end of VWP (Table 3). The pregnancy rates between synchronization and control groups did not differ significantly (P>0.05). The relationship between milk production (≤23 kg/d and >23 kg/d) and pregnancy rates is shown in Table 4 and Table 5, and there was no significant difference between milk yield and pregnancy (P>0.05).

Table 1. The descriptive statistics of age, milk production, DIM, and parity of Simmental cows in the study.

Variables	Mean	Standart Deviation
Age	4.2	2.0
Milk production (kg/d)	23.6	4.1
DIM (days)	106.9	68.0
Parity (number)	2.6	1.8

Table 2. The effect of parity on pregnancy numbers according to synchronization protocols

Parity Groups	Synchronization Groups	N	P value
Primiparous	Group I[ab]	39	
	Group II[b]	14	
	Group III[ab]	25	
	Group IV[ab]	13	0.05
	Control[a]	11	
Multiparous	Group I[b]	42	
	Group II[b]	16	
	Group III[b]	41	0.03
	Group IV[b]	32	
	Control[a]	24	

Table 3. The pregnancy rates between synchronization and control groups.

	Postpartum 45-60 days				Postpartum 61-90 days				Postpartum >91 days				P Value
Groups	**n**	**Pregnant**	**Non-pregnant**	**Pregnancy rate (%)**	**n**	**Pregnant**	**Non-pregnant**	**Pregnancy rate (%)**	**n**	**Pregnant**	**Non-pregnant**	**Pregnancy rate (%)**	
Group I	13	8	5	61.5	29	14	15	48.3	39	23	16	58.9	0.612
Group II	15	9	6	60.0	6	2	4	33.3	9	5	4	55.5	0.547
Group III	7	3	4	42.8	25	13	12	52.0	34	20	14	58.8	0.708
Group IV	12	6	6	50.0	10	4	6	40.0	23	13	10	56.5	0.686
Control	13	7	6	53.8	6	2	4	33.3	16	11	5	68.8	0.323

Table 4. The relationship between milk production (≤23 kg/d) and pregnancy rates.

Groups	**n**	**Pregnancy Number**	**Non-pregnancy Number**	**Pregnancy Rate (%)**	**P Value**
Group I	45	24	21	53.3	
Group II	11	5	6	45.5	
Group III	39	23	16	59.0	0.885
Group IV	19	12	7	63.2	
Control	21	12	9	57.1	

Table 5. The relationship between milk production (>23 kg/d) and pregnancy rates.

Groups	**n**	**Pregnancy Number**	**Non-pregnancy Number**	**Pregnancy Rate (%)**	**P Value**
Group I	36	21	15	58.3	
Group II	19	11	8	57.9	
Group III	27	13	14	48.2	0.840
Group IV	26	11	15	42.3	
Control	14	8	6	57.1	

DISCUSSION

Determining the duration of the VWP is still unclear in dairy cows. Various studies suggest the optimal VWP is between 42 to 150 days for primiparous and multiparous dairy cows (Arbel et al. 2001, Inchaisri et al. 2011, Toledo-Alvarado et al. 2017, Stangaferro etal. 2018). In most dairy farms, reducing the VWP is the

primary purpose of having a calving interval of 12 to 13 months. However, in the early days at the end of

VWP might need more services per conception due to anovulatory cows before 60 days postpartum, and the other disadvantage of early VWP is drying off of cows too early that have still relatively high milk yield. On the other hand, the simulation models and field data showed that every delayed week increases economic losses in dairy farming (Inchaisri et al. 2011). In the present study, we evaluated the effect of different VWP of 45-60, 61-90, and >90 days on pregnancy rates using different synchronization protocols in Simmental cows. No differences between groups were observed in terms of pregnancy rates. Our results showed that the early or late services did not give significant differences in pregnancy rates compared with the control group, and these data suggest the VWP could be planned according to the farm's economic and local market targets.

Ovulation synchronization and FTAI programs can be used for any kind of herd system (pasture/confinement based; seasonal/year-round herds) to eliminate the oestrus detection requirement or to treat reproductive diseases such as anovulatory anoestrus (Lindley et al. 2021a). Ovsynch protocol is one of the earliest ovulation synchronization programs used in cows (Pursley et al. 1997). However, this protocol does not ensure satisfactory pregnancy rates in anoestrous cows with low conception rates and high embryo mortality or heifers due to frequent follicular waves and dampens LH response to GnRH because of circulating progesterone levels (Kaçar et al. 2008, Herlihy et al. 2013, Lindley et al. 2021a). All presynchronization protocols aim to control the estrous cycle stage to improve synchronization success rates when the ovulation synchronization protocol begins (Butler et al. 2019). The present study compared three different presynchronization protocols and classic Ovsynch with the non-synchronized group in a confinement based year-round herd. Pregnancy rates were similar in all groups following the first and second FTAI, and the presynchronization did not affect synchronization success. The results of this study confirm that the selection of ideal protocol should be planned according to individual herd nuances, as stated by Lindley et al. (2021b). The synchronization protocols have their advantages and disadvantages, and the veterinarian should determine the ideal protocol as the most practical, most successful, least cost path, and herd's specific requirement.

Exogenous GnRH is one of the primary hormones used for ovulation synchronization, and it induces LH surge and affects ovarian follicular development during the program (Sartori et al. 2001, Butler et al. 2019). An optimal standard dose of GnRH is 100 μg for the synchronization of cows (Pursley et al. 1997, Souza et al. 2009). However, it was detected that the progesterone environment at GnRH treatment affects GnRH-induced LH secretion, and a 200 μg dose of GnRH increases LH secretion (Giordano et al. 2012). Giordano et al. (2013) studied the higher dose of GnRH (100 μg vs. 200 μg) with the Double Ovsynch protocol to increase ovulatory response in cows. They reported that the higher GnRH dose increased ovulation rates only in cows with elevated progesterone, but no effect of higher dose of GnRH on fertility was detected. In this study, two different GnRH doses (100 μg-Group III vs. 200 μg-Group IV) were compared with the G6G synchronization protocol in Simmental cows. Pregnancy rates did not differ significantly, either with WVPs or parity in either GnRH doses. Studies have reported that G6G protocol increases ovulation rates, corpus luteum formation in anovulator cows, and higher progesterone levels (Ribeiro et al. 2011, Kohsari and Benjamin 2022). Nevertheless, two different G6G protocols in the present study did not increase fertility compared with Ovsynch and Ovsynch+Progesterone protocols. The circulatory progesterone levels or ovulation rates were not evaluated in this study. However, the reports cited above showed that the synchronization protocols could increase ovulation rates and circulatory progesterone, but pregnancy rates were not shown increase satisfactorily. Hence, future researches could determine the factors affecting final pregnancy rates.

Several factors influence fertility and the success of synchronization and presynchronization protocols. The conditions such as breed, nutrition, parity, postpartum diseases, and milk yield may affect the pregnancy rates following synchronization. In primiparous cows, ovarian cyclicity could be delayed, and there could be longer intervals from parturition to the first service and conception; in addition, multiparous cows might tend to have lower luteolysis rates (Astiz and Fargas 2013, Meikle et al. 2004, Toledo-Alvarado et al. 2017, Lindley et al. 2021a). Some studies stated that the Double Ovsynch program gives higher pregnancy results in primiparous cows than in multiparous cows (Herlihy et al. 2012, Astiz and Fargas 2013). Our research showed that parity affects the pregnancy rates, and the Ovsynch+Progesterone protocol was given a statistically higher pregnancy rate in primiparous cows.

It is known that there is a strong interaction between milk yield and fertility, higher milk yielding breeds such as Holstein and Brown Swiss have worsened fertility records than the dual-purpose Simmental and Alpine Grey breeds. Also, milk yield may vary across the individual cows within the same breeds (Toledo-Alvarado et al. 2017, Martinez-Castillero et al. 2020). Toledo-Alvarado et al. (2017) reported that reproductive efficiency and fertility rates increase at least up to a milk yield about 25 kg/d. The present study was conducted on Simmental cows with mean milk production of about 23.6 kg/d (10 to 35 kg/d). In the case of milk yield of ≤23 kg/d and >23 kg/d, there was no significant difference between synchronization and control groups in pregnancy rates in our study.

CONCLUSION

In conclusion, the synchronization of Simmental cows either with Ovsynch, Ovsynch+Progesterone, G6G, or 2xG6G did not affect pregnancy rates across various DIM. These data suggest that the suitable VWP could be planned according to the farm's economic and local market targets, with veterinarians able to choose a protocol based on practicality, effectiveness and cost, as well as the specific requirements of the herd.

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

Authors Contribution Rate: UK, MD, and DBA planned the study, designed and performed the experiments, and helped with manuscript writing; HH analyzed the statistics data. All authors read and approved the final manuscript.

Ethical Approval: This study has received permission with Experimental Animal Ethics Committee of Afyon Kocatepe University (Decision number: 167-20).

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REFERENCES

Arbel, R., Bigun, Y., Ezra, E., Sturman, H., Hojman, D. (2001). The effect of extended calving intervals in high lactating cows on milk production and profitability. Journal of Dairy Science, 84, 600–608.

Astiz, S., Fargas, O. (2013). Pregnancy per AI differences between primiparous and multiparous high-yield dairy cows after using Double Ovsynch or G6G. Theriogenology, 79, 1065-1070.

Ben Salem, M., Djemali, M., Kayouli, C., Majdoup. (2006). A review of environmental and management factors affecting the reproductive performance of Holstein-Friesian dairy herds in Tunisia. Livestock Research for Rural Development, 18, 53.

Bo, G.A., de la Mata, J.J., Baruselli, P.S., Menchaca, A. (2016). Alternative programs for synchronizing and resynchronizing ovulation in beef cattle. Theriogenology, 86, 388-396.

Butler, S.T., Cummins, S.B., Herlihy, M.M., Hutchinson, I.A., Moore, S.G. (2019). Optimizing productive and reproductive performance in the grazing cow. Bioscientifica Proceedings, 8, 179-191.

Fleming, A., Ebdalla, E.A., Maltecca, C., Baes, C.F. (2018). Invited review: Reproductive and genomic technologies to optimize breeding strategies for genetic progress in dairy cattle. Archives Animal Breeding, 61, 43–57.

Fricke, P.M., Giordano, J.O., Valenza, A., Amundson, V.C., Carvalho, P.D. (2014). Reproductive performance of lactating dairy cows managed for first service using timed artificial insemination with or without detection of estrus using an activity-monitoring system. Journal of Dairy Science, 97, 2771-2781.

Giordano, J.O., Fricke, P.M., Guenther, J.N., Lopes Jr, G., Herlihy, M.M., Nascimento, A.B., Wiltbank, MC. (2012). Effect of progesterone on magnitude of the luteinizing hormone surge induced by two different doses of GnRH in lactating dairy cows. Journal of Dairy Science, 95, 3781–3793.

Giordano, J.O., Wiltbank, M.C., Fricke, P.M., Bas, S., Pawlisch, R., Guenther, J.N., Nascimento, A.B. (2013). Effect of increasing GnRH and PGF2α dose during Double-Ovsynch on ovulatory response, luteal regression, and fertility of lactating dairy cows. Theriogenology, 80, 773-783.

Heidari, F., Dirandeh, E., Ansari Pirsaraei, Z., Colazo, M.G. (2017). Modifications of the G6G timed-AI protocol improved pregnancy per AI and reduced pregnancy loss in lactating dairy cows. Animal, 11, 2002-2009.

Herlihy, M.M., Giordano, J.O., Souza, A.H., Ayres, H., Ferreira, R.M., Keskin, A., Nascimento, A.B., Guenther, J.N., Gaska, J.M., Kacuba, S.J., Crowe, M.A., Butler, S.T., Wiltbank, M.C. (2012). Presynchronization with Double-Ovsynch improves fertility at first postpartum artificial insemination in lactating dairy cows. Journal of Dairy Science, 95, 7003-7014.

Herlihy, M.M., Crowe, M.A., Berry, D.P., Diskin, M.G., Butler, S.T. (2013). Factors associated with fertility outcomes in cows treated with protocols to synchronize estrus and ovulation in seasonal-calving, pasture-based dairy production systems. Journal of Dairy Science, 96, 1485-1498.

Inchaisri, C., Jorritsma, R., Vos, P., van der Weijden, G.C., Hogeveen, G. (2011). Analysis of the economically optimal voluntary waiting period for first insemination. Journal of Dairy Science, 94, 3811–3823.

Kaçar, C., Kamiloglu, N.N., Ucar, O., Arı, U.C. (2008). İneklerde β-karoten+ E vitamini uygulamasıyla kombine edilen Ovsynch ve Cosynch senkronizasyon programlarının gebelik oranı üzerine etkisi. Kafkas Universitesi Veteriner Fakültesi Dergisi, 14(1), 45-50.

Kohsari, H., Berenjian, K. (2022). Comparison of the effects of Presynch-Ovsynch, Presynch-Ovsynch+ CIDR, and G6G protocols on the reproductive performance of lactating Holstein cows in the postpartum period. Veterinarska Stanica, 53, 419-430.

Larson, J.A., Lamb, G.C., Stevenson, J.S., Johnson, S.K., Day, M.L., Geary, T.W., Kesler, D.J., DeJarnette, J.M., Schrick, F.N., DiCostanzo, A., Arseneau, J.D. (2006). Synchronization of estrus in suckled beef cows for detected estrus and artificial insemination using gonadotropin-releasing hormone, prostaglandin F, and progesterone. Journal of Animal Science, 84, 332-342.

Lindley, G., Willshire, J., Martin, M. (2021a). Controlled breeding in dairy cows. Part 1: bovine oestrus cycle and synchronization. In Practice, 43, 445-456.

Lindley, G., Willshire, J., Martin, M. (2021b). Controlled breeding in dairy cows. Part 2: selection and ethical implications. In Practice, 43, 511-518.

Martinez-Castillero, M., Toledo-Alvarado, H., Pegolo, S., Vazquez, A.I., de los Campos, G., Varona, L., Finocchiaro, R., Bittante, G., Cecchinato, A. (2020). Genetic parameters for fertility traits assessed in herds divergent in milk energy output in Holstein-Friesian, Brown Swiss, and Simmental cattle. Journal of Dairy Science, 103, 11545-11558.

Meikle, A., Kulcsar, M., Chilliard, Y., Febel, H., Delavaud, C., Cavestany, D., Chilibroste, P. (2004). Effects of parity and body condition at parturition on endocrine and reproductive parameters of the cow. Reproduction, 127, 727-737.

Nak, Y., Tuna, B., Nak, D., Karakas, E., Simsek, G. (2011). The effects of Ovsynch, Ovsynch with progestin and progestin plus double TAI on pregnancy rates in unobserved oestrus dairy cows and heifers. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 17(6), 917-922.

NRC. (2001). Nutrient Requirements of Dairy Cattle. 7th Rev Ed Natl Acad Sci, Washington, DC.

Pursley, J.R., Mee, M.O., Wiltbank, M.C. (1995). Synchronization of ovulation in dairy cows using PGF2α and GnRH. Theriogenology, 44, 915–923.

Pursley, J.R., Wiltbank, M.C., Stevenson, J.S., Ottobre, J.S., Garverick, H.A., Anderson, L.L. (1997). Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation or synchronized estrus. Journal of Dairy Science, 80, 295-300.

Ribeiro, E.S., Bisinotto, R.S., Favoreto, M.G., Martins, L.T., Cerri, L.R.A., Silvestre, F.T., Greco, L.F., Thatcher, W.W., Santos, J.E.P. (2012). Fertility in dairy cows following presynchronization and administering twice the luteolytic dose of prostaglandin F2a as one or two injections in the 5-day timed artificial insemination protocol. Theriogenology, 78, 273–284.

Ribeiro, E.S., Cerri, R.L.A., Bisinotto, R.S., Lima, F.S., Silvestre, F.T., Greco, L.F., Thatcher, W.W., Santos, J.E.P. (2011). Reproductive performance of grazing dairy cows following presynchronization and resynchronization protocols. Journal of Dairy Science, 94, 4984-4996.

Sartori, R., Fricke, P.M., Ferreira, J.C., Ginther, O.J., Wiltbank, M.C. (2001). Follicular deviation and acquisition of ovulatory capacity in bovine follicles. Biology of Reproduction, 65, 1403-1409.

Shahzad, A.H., Sattar, A., Ahmad, N., Ahmad, I., Nak, D., Nak, Y. (2019). Evaluation of Ovsynch and CIDR+Ovsynch protocols to improve reproductive efficiency in lactating dairy cows. Pakistan Journal of Zoology, 51, 1607-1614.

Souza, A.H., Cunha, A.P., Silva, E.P., Gümen, A., Ayres, H., Guenther, J.N., Wiltbank, M.C. (2009). Comparison of gonadorelin products in lactating dairy cows: efficacy based on induction of ovulation of an accessory follicle and circulating luteinizing hormone profiles. Theriogenology, 72, 271–279.

Stangaferro, M.L., Wijma, R., Masello, M., Thomas, M., Giordano, J.O. (2018). Economic performance of lactating dairy cows submitted for first service timed artificial insemination after a voluntary waiting period of 60 or 88 days. Journal of Dairy Science, 101, 7500-7516.

Thatcher, W.W., Bilby, T.R., Bartolome, J.A., Silvestre, F., Staples, C.R., Santos, J.E.P. (2006). Strategies for improving fertility in the modern dairy cow. Theriogenology, 65, 30–44.

Toledo-Alvarado, H., Cecchinato, A., Bittante, G. (2017). Fertility traits of Holstein, Brown Swiss, Simmental, and Alpine Grey cows are differently affected by herd productivity and milk yield of individual cows. Journal of Dairy Science, 100, 1-12.

Wiltbank, M.C., Baez, G.M., Cochrane, F., Barletta, R.V., Trayford, C.R., Joseph, R.T. (2015). Effect of a second treatment with prostaglandin F2α during the Ovsynch protocol on luteolysis and pregnancy in dairy cows. Journal of Dairy Science, 98, 8644–8654.

Yılmaz, C., Yılmaz, O., Ucar, M. (2011). Effect of PGF2α and GnRH injections applied before Ovsynch on pregnancy rates in cows and heifers. Kafkas Universitesi Veteriner Fakültesi Dergisi, 17(4), 641-644.

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RESEARCH ARTICLE

Prevalence of Enteropathogens in Neonatal Calves with Acute Diarrhea in Aydın Province

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ABSTRACT

Neonatal calf diarrhea is an important cause of morbidity and mortality that is frequently reported worldwide and can cause serious direct and indirect economic losses. In this study, it was aimed to determine the prevalence of *E. coli*, *Cryptosporidium* spp., *Rotavirus*, *Coronavirus* and *Giardia* spp. which play a role in the etiology of diarrhea in neonatal calves in Aydın province and to evaluate the severity of clinical findings caused by etiological agents. The study included 167 calves with diarrhea, 86 males and 81 females, aged 1-28 days in Aydın province. Calf health scores (CHS) were determined on the basis of clinical examination results. Detection of *E.coli* K99, *Cryptosporidium* spp., *Rotavirus*, *Coronavirus* and *Giardia* spp. in fecal samples was performed using a rapid diagnostic test kit. The prevalence of the causative agents of diarrhea were *Cryptosporidium* spp. (55.09%), *Rotavirus* (37.13%), *Coronavirus* (19.76%), *Giardia* spp. (7.19%) and *E. coli* K99 (5.39%) regardless of whether they were mono or co-infection. Among the co-infections, *Cryptosporidium* spp. + *Rotavirus* (16.77%) was the most common combination. According to CHS, the highest score (6.5) was found in *Cryptosporidium* spp. + *Rotavirus* + *Coronavirus* combination and the lowest score (4) was found in *Cryptosporidium* + *Giardia* spp. combination. The results revealed that *Cryptosporidium* spp. and Rotavirus were the most common diarrhea agents in neonatal calves in Aydın province and that there may be different variables affecting CHS. These results are significant for developing effective control strategies for the leading etiological causes of diarrhea in calves.

Keywords: Calf, Clinic Score, Diarrhea, Etiology, Prevalance

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Aydın İlinde Akut İshalli Neonatal Buzağılarda Enteropatojenlerin Prevalansının Araştırılması

ÖZ

Neonatal buzağı ishalleri, dünya çapında sık bildirilen, doğrudan ve dolaylı ciddi ekonomik kayıplara neden olabilen önemli morbidite ve mortalite nedenidir. Bu çalışmada, Aydın ilindeki neonatal buzağılarda ishalin etiyolojisinde rol oynayan *E. coli*, *Cryptosporidium* spp., *Rotavirus*, *Coronavirus* ve *Giardia* spp. etkenlerinin prevalansının belirlenmesi ve etiyolojik etkenlerin oluşturduğu klinik bulguların şiddetinin değerlendirilmesi amaçlandı. Araştırmaya, Aydın ilinde bulunan 1-28 günlük yaşta, 86 erkek ve 81 dişi olmak üzere 167 ishalli buzağı dahil edildi. Klinik muayene sonuçları temelinde buzağı sağlık skorları (BSS) belirlendi. Dışkı örneklerinde *E.coli* K99, *Cryptosporidium* spp., *Rotavirus*, *Coronavirus* ve *Giardia* spp. etkenlerinin belirlenmesi hızlı tanı test kiti kullanılarak gerçekleştirildi. İshale neden olan etkenlerin mono ya da koenfekte olmalarına bakılmaksızın yaygınlıkları sırasıyla *Cryptosporidium* spp. (%55,09), *Rotavirus* (%37,13), *Coronavirus* (%19,76), *Giardia* spp. (%7,19) ve *E. coli* K99 (%5,39) olarak tespit edildi. Koenfeksiyonlar arasında *Cryptosporidium* spp. + *Rotavirus* (%16,77) ile en yaygın kombinasyon olarak belirlendi. BSS'ye göre en yüksek skorun (6,5), *Cryptosporidium* spp. + *Rotavirus* + *Coronavirus* kombinasyonunda, en düşük skorun (4) ise *Cryptosporidium* spp. + *Giardia* spp. kombinasyonunda olduğu saptandı. Sonuçlar, Aydın ilinde neonatal buzağılarda en yaygın ishal etkenlerinin *Cryptosporidium* spp. ve *Rotavirus* olduğunu ve BSS'yi etkileyen farklı değişkenlerin olabileceğini ortaya koymaktadır. Bu sonuçlar, buzağılarda ishalin öne çıkan etiyolojik nedenlerine yönelik etkin kontrol stratejileri geliştirmek açısından önemlidir.

Anahtar Kelimeler: Buzağı, Etiyoloji, İshal, Klinik skor, Prevalans

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GİRİŞ

Buzağılarda neonatal dönem, fizyolojik fonksiyonların oluşup geliştiği, doğumdan sonraki hayata uyum sağladığı oldukça kritik bir yaşam dönemi olarak tanımlanmaktadır (Izzo ve ark. 2015; Constable ve ark. 2017). Bu dönemde görülen ishaller buzağıların vücut sıvılarının yetişkinlere oranla yüksek olmasına rağmen, kompenzasyon ve regülasyon yeteneğinin sınırlılığı ve agamaglobulinemik doğmaları sebebiyle sıvı-elektrolit kayıplarının hızlı şekillenmesi sonucu önemli sağlık problemlerine neden olabilmektedir (Aydoğdu ve Güzelbekteş 2018).

Neonatal buzağı ishali, enfeksiyöz ve nonenfeksiyöz (yönetimsel, konakçı faktörleri, beslenme ve çevresel faktörler vb.) nedenlere bağlı ortaya çıkabilmektedir. Çoğunlukla polifaktöriyel bu sorunda önemli enfeksiyöz nedenler arasında bakteriyel (*Cl. perfringens*, *E. coli*), viral (*Coronavirus, Rotavirus*) ve paraziter (*Cryptosporidium parvum*, *Eimeria* sp., *Giardia* sp.) etkenler yer almaktadır (Bendali ve ark. 1999; Kaske ve Kunz 2003; Güzelbekteş ve ark. 2007; Blanchard 2012; Aydoğdu ve ark. 2018a).

İshal, neonatal buzağılarda en yüksek mortalite ve morbiditeye neden olan sağlık sorunları arasında yer alır (Aygün ve Yıldız 2018; Yıldız ve ark. 2018). Ölümlerle doğrudan, buzağıların büyüme ve verimleri üzerinde olumsuz etkileri ve sağaltım giderleri ile de dolaylı önemli ekonomik kayıplar oluşmaktadır (Aydoğdu ve ark. 2018a, 2018b). Bu kapsamda, buzağılarda ishale yol açan nedenlerin hızlı bir şekilde tespit edilmesi ve uygun tedavilerin uygulanması, neonatal dönemde ortaya çıkan kayıpların azaltılmasına katkı sağlayacağı belirtilmektedir (Kalınbacak 2003).

Bu araştırmada, neonatal buzağılarda ishal etiyolojisinde rol oynayan etkenlerin BSS üzerinde farklı değişimlere neden olacağı ve bu değişimlerin de hem tanısal hem de sağaltım stratejileri gelişimine katkı sağlayacağı hipotezi ile yola çıkılarak, Aydın ilindeki neonatal buzağılarda ishalin etiyolojisinde rol oynayan *E.coli, Cryptosporidium* spp., *Rotavirus, Coronavirus* ve *Giardia* spp. etkenlerinin prevalansının belirlenmesi ve etiyolojik etkenlerin oluşturduğu klinik bulguların şiddetinin değerlendirilmesi amaçlandı.

MATERYAL ve METOD

Hayvan Materyalli

Çalışmanın hayvan materyalini, 2016-2017 yıllarında Aydın Adnan Menderes Üniversitesi Veteriner Fakültesi Hayvan Hastanesi İç Hastalıkları Anabilim Dalı Kliniğine akut ishal şikâyeti ile getirilen 1-28 günlük yaşta Holstein ırkı 167 buzağı oluşturdu. Akut ishal, klinik muayenede dışkı kıvamı, içeriği ve rengi, defekasyon sıklığı (günde 4 defadan fazla), yoğunluğu (bol sulu, mukuslu, kanlı) ve ishalin süresi değerlendirilerek ortaya konuldu (Izzo ve ark. 2015).

Araştırmaya Dahil Etme/Çıkarma Kriterleri

Etiyolojik tespitlerin yapılmasında hatalara yol açabilecek, ishal kapsamında önceden bir tedavi girişiminde bulunulmuş buzağılar çalışmaya dahil edilmedi.

Klinik ve Laboratuvar Muayeneler

Çalışma kriterlerine uygun olup, çalışmaya dahil edilmesine karar verilen her bir buzağının palpe edilebilir lenf yumrularının değerlendirilmesi, kalp ve akciğer seslerinin dinlenmesi, nabız ve solunum sayılarının ölçülmesi ile Buzağı Sağlık Skoru (BSS) kapsamında bulunan parametrelerin (McGuirk 2013) (Şekil 1) değerlendirilmesini içeren klinik muayeneleri yapıldı. Klinik muayeneler sonucu her bir buzağının BSS hesaplanıp kaydedildi.

İshalli neonatal buzağılarda klinik muayeneler ve BSS hesaplamasından sonra etiyolojiyi belirleme olarak toplanan dışkı örnekleri, immunokromatografik temelli hızlı tanı test kiti (BOVID-5 Ag Test Kit, Anigen, Bionote) kullanılarak üretici firmanın önerileri doğrultusunda beş etiyolojik etkene (*Cryptosporidium* spp.*, Rotavirus, Coronavirus, E. coli K 99* ve *Giardia* spp.) yönelik incelendi.

BULGULAR

Çalışmada değerlendirilen toplam 167 neonatal ishalli buzağıda etiyolojik prevalans, BSS, yaş ve cinsiyet verileri Tablo 1'de özetlendi. Çalışma kapsamında değerlendirilen 167 neonatal ishalli buzağının %80,84'ü en az bir etken yönünden pozitif belirlendi. İki ve üç etken yönüyle pozitiflik veren olgu yüzdeleri sırasıyla %30,54 ve %5,99 olarak saptandı. Araştırma kapsamında değerlendirilen neonatal ishalli buzağılarda dört ve üzeri etkene rastlanılmadı. Pozitif olguların ortalama BSS skoru 5,32 ve yaş ortalamaları 11,71 gün amacıyla rektumdan masaj yolu ile ve/veya spontan olarak gerçekleşen dışkılama sırasında tekniğine uygun olarak kaydedildi. Toplam 167 buzağının 86'sı erkek, 81'i ise dişi olduğu not edildi.

Bu çalışmada, Aydın ilinde, neonatal buzağılarda ishale neden olan enfeksiyöz etkenlerden *Cryptosporidium* spp. (%23,35), *Rotavirus* (% 9,58), *E.coli K99* (% 5,39), *Coronavirus* (% 4,19) ve *Giardia* spp. (% 1,80) monoenfeksiyonları tespit edildi. Koenfeksiyonlardan ise en yüksek *Cryptosporidium* spp. + *Rotavirus*'u (%16,77) takiben *Cryptosporidium* spp. + *Coronavirus* (%4,79), *Rotavirus* + *Coronavirus* (%4,79), *Cryptosporidium* spp. + *Giardia* spp. (%4,19), *Cryptosporidium* spp. + *Rotavirus* + *Coronavirus* (%4,79) ve *Cryptosporidium* spp. + *Rotavirus* + *Giardia* spp. (%1,20) belirlendi (Tablo 1, Şekil 2).

Çalışmada değerlendirilen neonatal buzağılarda ishale neden olan enfeksiyöz etkenlerin mono ya da koenfekte olmalarına bakılmaksızın yaygınlıkları sırasıyla *Cryptosporidium* spp. (%55,09), *Rotavirus* (%37,13), *Coronavirus* (%19,76), *Giardia* spp. (%7,19) ve *E. coli* K99 (%5,39) olarak tespit edildi.

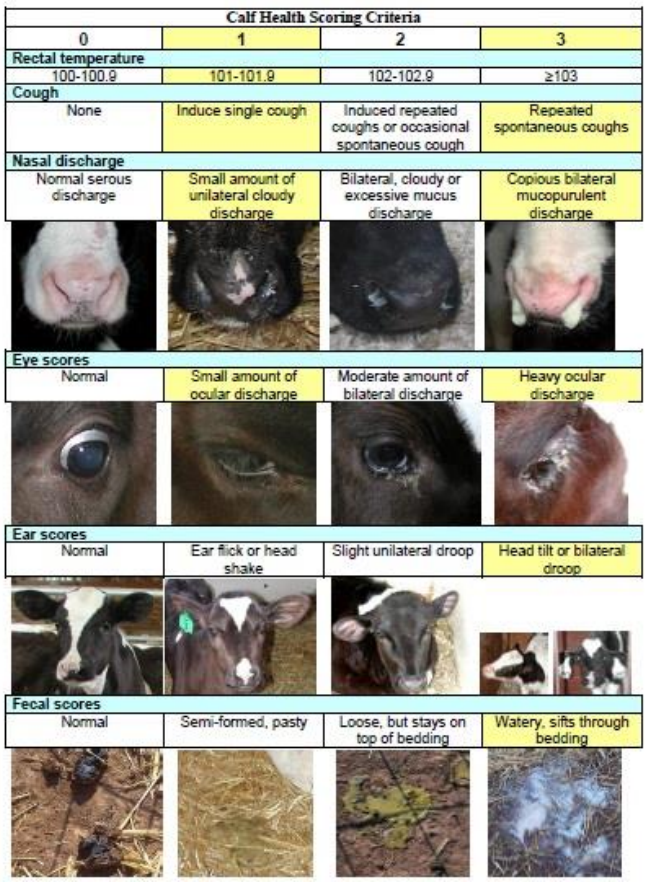

Figure 1. Calf Health Scoring (McGuirk 2013)

Şekil 1. Buzağı Sağlık Skorlaması (McGuirk 2013)

Table 1. Etiological prevalence, BSS, age and gender data of neonatal calves with acute diarrhea

Tablo 1. Akut ishalli neonatal buzağılarda etiyolojik prevalans, BSS, yaş ve cinsiyet verileri

Etken	**Olgu sayısı (n=167)**	**Prevalans (%)**	**BSS Ortalama (Min-Max)**	**Yaş (Gün) Ortalama (Min-Max)**	**Cinsiyet (Erkek/Dişi)**
Negatif	32	19,16	4,72 (2-7)	9,5 (2-20)	18/14
Cryptosporidium spp.	39	23,35	5,19 (3-9)	12,42 (4-28)	17/22
Rotavirus	16	9,58	4,23 (3-6)	9,77 (2-21)	9/7
E. Coli K99	9	5,39	6,29 (5-8)	2,7 (1-5)	3/6
Coronavirus	7	4,19	5,6 (3-8)	17,4 (3-26)	3/4
Giardia spp.	3	1,80	5 (5-5)	26 (24-28)	1/2
Cryptosporidium spp. + *Rotavirus*	28	16,77	6,28 (2-9)	12,48 (5-30)	17/11
Cryptosporidium spp. + *Coronavirus*	8	4,79	5 (4-6)	8,67 (7-15)	5/3
Rotavirus + *Coronavirus*	8	4,79	5,67 (4-8)	12,3 (6-24)	5/3
Cryptosporidium + *Giardia* spp.	7	4,19	4 (3-5)	19,6 (11-27)	4/3
Cryptosporidium spp. + *Rotavirus* + *Coronavirus*	8	4,79	6,5 (4-9)	14,8 (7-21)	3/5
Cryptosporidium spp. + *Rotavirus* + *Giardia* spp.	2	1,20	5 5-5	10 (8-12)	1/1

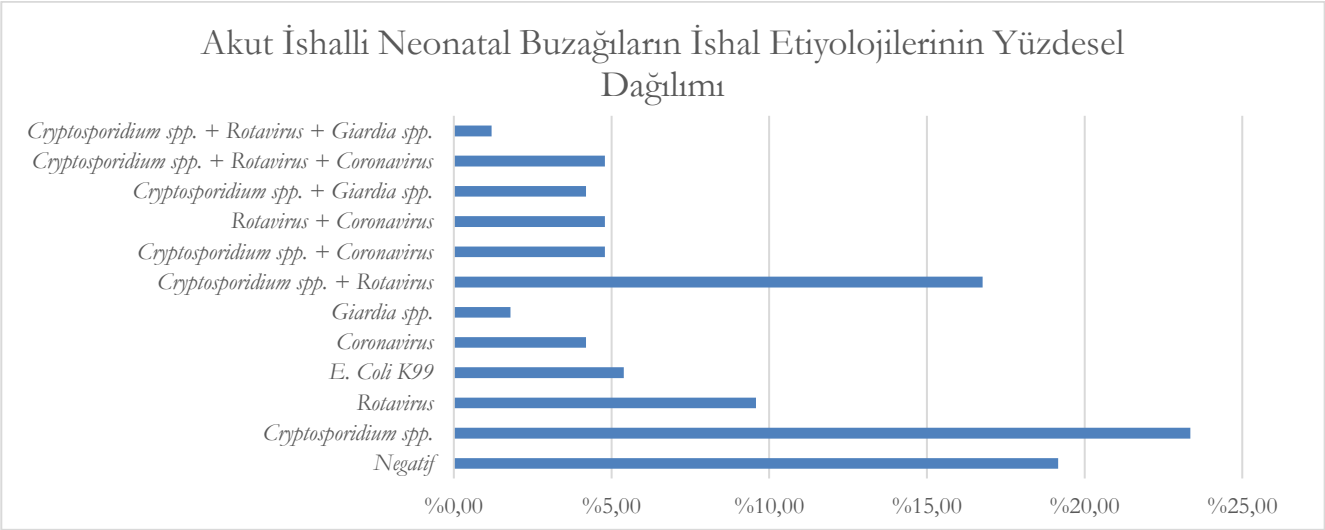

Figure 2. Percentage distribution of diarrhea etiologies of neonatal calves with acute diarrhea

Şekil 2. Akut ishalli neonatal buzağıların ishal etiyolojilerinin yüzdesel dağılımı

TARTIŞMA

İshal, neonatal buzağıların en önemli sağlık problemlerinden birisi olarak görülmektedir (Uhde ve ark. 2008). Bu dönemdeki buzağılarda gelişen ishalde etiyolojinin hızlı bir şekilde belirlenip etkin sağaltımlar uygulamalarıyla doğrudan ve dolaylı ekonomik kayıpların azaltılmasındaki önemine vurgu yapılmaktadır (Kalınbacak 2003). Bu çalışmanın sonuçları, Aydın ilinde neonatal buzağılarda ishale neden olan etkenlerin mono ya da koenfekte olmalarına bakılmaksızın yaygınlıkları sırasıyla *Cryptosporidium* spp. (%55,09), *Rotavirus* (%37,13), *Coronavirus* (%19,76), *Giardia* spp. (%7,19) ve *E. coli* K99 (%5,39) olarak tespit edildi. Koenfeksiyonlar arasında ise *Cryptosporidium* spp. + *Rotavirus* (%16,77) ile en yaygın kombinasyon olarak belirlendi. Değerlendirilen 167 buzağının 32'sinde (%19,16) ise araştırılan beş enfeksiyöz etken dışında diğer enfeksiyöz etkenler ve/veya nonenfeksiyöz faktörler ishale neden olmuştur. Bu sonuçlar, Aydın ilindeki buzağılarda ishalin etiyolojik nedenlerini anlamak ve etkin kontrol stratejileri geliştirmek açısından önemlidir.

Cryptosporidium spp., neonatal dönemdeki buzağılarda sık görülen ishal etkenlerinden biridir ve bu konuda kapsamlı çalışmalar yapılmıştır (Chen ve ark. 2023; Gao ve ark. 2023; Kuraa ve Malek 2023). Dünya genelinde farklı coğrafyalarda yapılan araştırmalara göre *Cryptosporidium* spp. ishal vakalarında %2,8 ile %65,1 arasında bir yaygınlık bildirilmiştir (Chen ve ark. 2023). Bu çalışmada, neonatal ishalli buzağılarda, mono ve koenfeksiyon durumlarına bakılmaksızın *Cryptosporidium* spp. yaygınlığı %55,09 olarak belirlenmiştir. Bu sonuç, diğer dünya genelindeki çalışmalar göz önüne alındığında yüksek kabul edilebilir. Ülkemiz genelinde *Cryptosporidium* spp. yaygınlığını araştıran bir çalışmada, 333 buzağıda %27,4 oranında bildirilmiştir (Kabir ve ark. 2020). İl bazında yapılan değerlendirmelere göre ise; Kars ilinde %4 (Öztürkler, 2023), Sivas ilinde %7 (Kuliğ ve Coşkun 2019), Tokat ilinde %11 (Coşkun ve Kaya 2018), Uşak ilinde %18 (Sezer ve Akgül 2022), Siirt ilinde %22 (Kozat ve Tuncay 2018), Burdur ilinde %38,6 (Atcalı ve Yıldız 2020) ve Güneydoğu bölgesinde %47,7 (İçen ve ark. 2013) olarak tespit edilmiştir. Elazığ ilinde yapılan bir araştırmada (Al ve Balıkçı 2012), *Cryptosporidium* spp.'ye rastlanılamadığı belirtilirken, diğer bir çalışmada ise enfeksiyonun varlığı %7,2 olarak bildirilmektedir (Özer ve ark. 1990). Bu çalışmada, ülkemizin farklı bölgelerinde ve illerinde yapılan diğer çalışmalara kıyasla daha yüksek bir yaygınlık belirlenmesi dikkat çekicidir ve Aydın ilinde buzağılarda ishal önlemlerinin alınmasında mutlaka göz önünde bulundurulmalıdır.

Rotavirus, dünya çapında en yaygın hastalık etkenlerinden biridir ve kapsamlı bir şekilde incelenmiştir (Pesavento ve ark. 2003; Sezer ve Akgül 2022; Ateş ve Yeşilbağ 2023; Değirmençay ve ark. 2023). Farklı çalışmalarda ishalli buzağılarda Bovine *Rotavirus* yaygınlığı %20-60 oranında bildirilmiştir (Björkman ve ark. 2003; Al ve Balıkçı 2012; İçen ve ark. 2013; Coşkun ve Kaya 2018; Kozat ve Tuncay 2018; Kuliğ ve Coşkun 2019; Atcalı ve Yıldız 2020; Sezer ve Akgül 2022). Bu çalışmada neonatal ishalli buzağılarda mono ve koenfeksiyon durumuna bakılmaksızın *Rotavirus* yaygınlığı %37,13 olarak belirlendi. Bu oran ülkemizin farklı bölgelerinde aynı amaca yönelik çalışmaların bulgularıyla büyük ölçüde uyumludur. Bu kapsamda ishalli buzağılarda *Rotavirus*'un yaygınlığı Kars ilinde %14 (Öztürkler 2023), Burdur ilinde %22,7 (Atcalı ve Yıldız 2020), Uşak ilinde %31 (Sezer ve Akgül 2022), Sivas ilinde %22 (Kuliğ ve Coşkun 2019), Siirt ilinde %25 (Kozat ve Tuncay 2018), Elazığ ilinde

%30 (Al ve Balıkçı 2012), Tokat ilinde %44,86 (Coşkun ve Kaya 2018) ve Güneydoğu bölgesinde %55,2 (İçen ve ark. 2013) tespit edilmiştir. İçen ve ark. (2013) tarafından gerçekleştirilen araştırmada saptanan *Rotavirus* prevalansı, diğer illere göre en fazla görülen il olduğu görülmektedir. Bu çalışmada ise Aydın ili *Rotavirus* yönünden iller arasında en yüksek prevalansa sahip üçüncü il olarak tespit edilmiştir. *Rotavirus*, Aydın ilinde ise ikinci yüksek prevalansa sahip (*Cryptorporidium* spp.'den sonra) etken olarak tespit edilmiştir. Bu nedenle, Aydın ilinde tanı ve sağaltım stratejilerinde ihmal edilmemesi gereken bir etken olduğu düşünülmektedir.

E. coli, özellikle neonatal dönemin başlangıcında karşılaşılan ishalde en yaygın hastalık etkenlerinden biridir (Brunauer ve ark. 2021; Aydın ve ark. 2022; Higgs ve ark. 2023). Dünya genelinde farklı coğrafyalarda yapılan çalışmalarda *E. coli* ishalli buzağılarda %0-23,56 oranında bildirilmiştir (Brunauer ve ark. 2021). Bu çalışmada neonatal ishalli buzağılarda mono ve koenfeksiyon durumuna bakılmaksızın *E. coli* K99 yaygınlığı %5,39 olarak belirlendi. Dünya genelinde bildirilen yaygınlıklara göre alt sıralarda yer aldığını görülmektedir. Ülkemizin farklı bölge ve illerinde yapılan diğer çalışmalara göre de daha az yaygınlıkta olduğu görülmektedir. İshalli buzağılarda *E. coli* yaygınlığı Kars ilinde %15 (Öztürkler 2023) Burdur ilinde %9,1 (Atcalı ve Yıldız 2020), Uşak ilinde %15 (Sezer ve Akgül 2022), Sivas ilinde %26 (Kuliğ ve Coşkun 2019), Siirt ilinde %18 (Kozat ve Tuncay 2018), Elazığ ilinde %17 (Al ve Balıkçı 2012), Tokat ilinde %7,48 (Coşkun ve Kaya 2018) ve Güneydoğu bölgesinde %26 (İçen ve ark. 2013) belirlenmiştir. Bu çalışmada hem diğer illere göre hem de araştırmada değerlendirilen diğer etkenlere göre en düşük prevalansa sahip olarak *E. coli* monoenfeksiyonu belirlenmiştir. Diğer illere ve diğer etiyolojik etkenlere göre en az görülen etken olarak yorumlanması doğru olacaktır. Ancak, en az görülmesine rağmen bu çalışma kapsamında en şiddetli klinik bulgulara neden olduğu BSS'de (6,29) görülmektedir. Az görülmesine rağmen şiddetli semptomlara neden olabileceğinden dolayı tanı ve sağaltım stratejilerinde göz ardı edilmemesi gereken bir etken olarak yorumlanabilmektedir.

Coronavirus, neonatal buzağı ishal patojenleri arasında önemli etkenlerden birisidir (Abbasov ve Zeynalova 2023; Du ve ark. 2023; Geng ve ark. 2023;). Dünya genelinde ishalli buzağılardaki yaygınlığı %0-47,48 arasında bildirilmiştir (Brunauer ve ark. 2021). Bu çalışmada neonatal ishalli buzağılarda mono ve koenfeksiyon durumuna bakılmaksızın *Coronavirus* yaygınlığı %19,76 olarak belirlendi. Bu oran ülkemizin farklı bölgelerinde aynı amaca yönelik çalışmaların bulgularına göre yüksektir. İshalli buzağılarda *Coronavirus*'un yaygınlığı Kars ilinde %29 (Öztürkler 2023), Burdur ilinde %11,4 (Atcalı ve Yıldız 2020), Uşak ilinde %10 (Sezer ve Akgül 2022), Sivas ilinde %9 (Kuliğ ve Coşkun 2019), Siirt ilinde %7 (Kozat ve Tuncay 2018), Elazığ ilinde %13 (Al ve Balıkçı 2012), Tokat ilinde %9,35 (Coşkun ve Kaya 2018), Şanlıurfa ilinde %5,32 (Abikoğlu ve Özgünlük 2022) ve Güneydoğu bölgesinde %5,1 (İçen ve ark. 2013) bildirilmiştir. Bu bildirimlere ve araştırmamıza göre Aydın ilinin *Coronavirus* prevalansı iller arası en yüksek ikinci sırada yer almaktadır. Aydın ilinde tespit edilen etiyolojik etkenler arasında ise *Cryptosporidium* spp. ve *Rotavirus*'dan sonra gelerek, üçüncü en çok görülen etken olarak tespit edilmiştir. Özellikle diğer illere göre, Aydın ilinde tanı ve tedavi stratejilerinde göz önünde bulundurulması gereken etkenler arasında görülmektedir.

Giardia spp., dünya çapında yaygın ve zoonotik önemiyle öne çıkan hastalık etkenlerinden biridir (Taghipour ve ark. 2022; Öztürkler 2023; Park ve ark. 2023). Dünyada farklı coğrafyalarda yapılan çeşitli çalışmalarda *Giardia* spp. ishalli buzağılarda %0-76,5 oranında bildirilmiştir (Taghipour ve ark. 2022). Bu çalışmada ise neonatal ishalli buzağılarda mono ve koenfeksiyon durumuna bakılmaksızın *Giardia* spp. yaygınlığı %7,19 olarak belirlendi. Bu oran dünya

geneline göre alt sıralarda yer alırken, ülkemizin farklı bölgelerinde aynı amaca yönelik çalışmaların bulgularıyla büyük ölçüde uyumludur. Bu kapsamda ishalli buzağılarda Giardiasisin yaygınlığı Kars ilinde %12 (Öztürkler 2023), Burdur ilinde %22,7 (Atcalı ve Yıldız 2020), Siirt ilinde %4 (Kozat ve Tuncay 2018), Sivas ilinde %4,13 (Değerli ve ark. 2005), Tokat ilinde %16,82 (Coşkun ve Kaya 2018), Van ilinde %48,7 (Ayan ve ark. 2019) ve Aydın ilinde %17,67 (Gültekin ve ark. 2017) tespit edilmiştir. Aydın ve Van illerinde gerçekleştirilen çalışmalarda bu çalışmaya göre belirgin yüksek oran belirlenmiştir. Fakat anılan çalışmalarda 3 aylık yaşa kadar olan ishalli buzağılar değerlendirilirken, bu çalışmaya ise 28 gün yaşa kadar olan buzağılar dahil edilmiştir. Gültekin ve ark. (2017), *Giardia* spp. enfeksiyonunun neonatal dönem sonrasında daha yaygın görülebileceğini belirtmektedir.

Bu çalışmaya dahil edilen akut ishalli neonatal buzağıların %19,16'sı değerlendirilen beş yaygın enteropatojen yönünden negatif olarak belirlenmiştir. Neonatal dönemdeki buzağılarda ishale neden olan etiyolojilerin arasında non-enfeksiyöz nedenlerin (yönetimsel, konakçı faktörü, beslenme ve çevresel faktörler vb.) oldukça önemli olduğu bildirilmektedir (Bendali ve ark. 1999; Blanchard 2012). Enfeksiyöz nedenlerden öne çıkanlar ise bakteriyel (*Cl. perfringens, E. coli*), viral (*Coronavirus*, *Rotavirus*) ve paraziter (*Cryptosporidium parvum*, *Eimeria* spp., *Giardia* spp.) olarak bildirilmektedir (Kaske ve Kunz 2003; Güzelbekteş ve ark. 2007; Aydoğdu ve ark. 2018b). Sık rastlanılan enfeksiyöz nedenlerin dışında *Salmonella* spp.*, Adenovirus, Bovin Viral Diyare Virus, Torovirus, Calicivirus, Nebovirus, Norovirus* ve *Candida* spp. etkenlerinin de neonatal buzağı ishallerine neden olduğu bildirilmektedir (Cho ve Yoon 2014). Bu çalışmadaki negatif olguların hem non-enfeksiyöz, hem de çalışmada değerlendirilmeyen diğer etiyolojik etkenler tarafından oluşabilmesi muhtemeldir.

Özetle, ülkemizde ishalli buzağılarda etiyolojik değerlendirmeye yönelik çalışmalar incelendiğinde, etkenlerin yaygınlıklarının farklılık ya da benzerlik gösterebildiği görülmektedir. Bu sonuçlar birçok faktörden etkilenebilmektedir. Özellikle çalışmalara katılan buzağıların yaş grupları ve etiyolojik etkenlerin farklı bölgelerdeki yaygınlığı, değerlendirmelerdeki farklılıklarda önemli bir rol oynayabilir (Blanchard 2012, Izzo ve ark. 2015).

Neonatal dönemde ishal görülen buzağılarda, etiyolojiye göre klinik bulgu şiddetlerinde gelişebilecek değişim ile ilişkili kısıtlı sayıda veri bulunmaktadır. Siirt ilinde gerçekleştirilen ishalli buzağılarda etiyolojik prevalans araştırması kapsamında olgular dehidrasyon şiddetine göre hafif, orta ve şiddetli olarak 3 sınıfa ayrılmış ve etiyolojik etkenler, bu dağılıma göre incelenmiştir. İnceleme sonucunda, hafif dehidrasyonu bulunan olguların %28,6'sı monoenfeksiyon, %71,4'ü negatif (diğer nedenler) olduğu bildirilmektedir. Orta dereceli dehidrasyonlu olguların %60'ı koenfeksiyon, %40'ında ise negatif (diğer nedenler) olduğu bildirilmektedir. Şiddetli dehidrasyonlu olguların ise %64,3'ü koenfeksiyon, %35,7'sinin negatif (diğer nedenler) olduğu bildirilmektedir (Kozat ve Tuncay 2018). Tarafımızca yapılan araştırmada ise, dehidrasyon derecesini de kapsayan ve güncel çalışmalarda kullanılan buzağı sağlık skorlaması (McGuirk 2013) kullanılarak, etiyolojik etkenlerin oluşturduğu klinik şiddeti ortaya konulmaya çalışılmıştır (Tablo 1). Çalışma kapsamındaki monoenfeksiyonlu olgularda en şiddetli bulguların (yüksek skor) *E. coli* monoenfeksiyonunda (6,29), en hafif bulguların (düşük skor) *Rotavirus* monoenfeksiyonunda (4,23) görüldüğü saptanmıştır. Koenfeksiyonlu buzağılarda ise en şiddetli bulguların (yüksek skor) *Cryptosporidium* spp. + *Rotavirus* + *Coronavirus* (6,5) olduğu, en hafif bulguların (düşük skor) ise *Cryptosporidium* spp. + *Giardia* spp. (4) olduğu saptanmıştır. Bu sonuçlara göre, koenfeksiyonların monoenfeksiyonlara göre daha şiddetli klinik tablo oluşturabileceği görülmektedir. Bu yönüyle bu araştırma, Siirt ilinde gerçekleştirilen araştırmanın

(Kozat ve Tuncay 2018) sonucunu destekler niteliktedir. Fakat, monoenfeksiyona neden olan etiyolojik etkene göre (örneğin *E. coli*) de bazı koenfeksiyonların (örneğin *Cryptosporidium* spp. + *Giardia* spp.) daha hafif klinik tablolara yol açabileceği gözlenmektedir. Monoenfeksiyon ve koenfeksiyonların oluşturduğu klinik şiddetteki değişimlere neden olabilecek nedenlerden birisi ise koenfeksiyon gruplarındaki olgu sayısının az olması ihtimali de unutulmamalıdır. Bu nedenle, buzağı sağlık skorlaması yönünden etiyolojik ajanlara bağlı değişimlerin belirlenmesi için daha fazla olgu içeren çalışmalara ihtiyaç olduğu düşünülmektedir.

SONUÇ

Sonuç olarak, Aydın ilinde gerçekleştirilen bu çalışma ile bölgede en yaygın olarak *Cryprosporidium* spp. ve *Rotavirus* etkenlerinin hem mono hem de koenfeksiyonlarının bulunduğu belirlenmiştir. Ayrıca, buzağıların sağlık kontrollerinde kullanılan buzağı sağlık skorlaması sistemi, neonatal dönemdeki ishalli buzağıların etiyolojilerine göre araştırılarak literatüre bir katkıda bulunulmuştur. Bu çalışmada sunulan verilerin gelecekte yapılacak araştırmalara kaynak sağlayabileceği ve bölgede çalışan veteriner hekimlerin sağaltım ve profilaksi stratejileri geliştirmelerine destek olabileceği düşünülmektedir.

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Authors' Contributions: CB and MG contributed to the project idea, design and execution of the study. CB, KU, HE, MG and GG contributed to the acquisition of data. CB, HE and MG analysed the data. CB, KU, HE, MG and GG drafted and wrote the manuscript. CB, KU, HE and MG reviewed the manuscript critically. All authors have read and approved the finalized manuscript.

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KAYNAKLAR

Abbasov, V., & Zeynalova, S. (2023). Clinical Signs of Seasonal Disease Dynamics in Calves Caused by *Rotavirus* and Coronavirus Infections. Annual Research & Review in Biology, 38(1), 1-11. https://doi.org/10.9734/arrb/2023/v38i130563

Abikoğlu, R., & Özgünlük, İ. (2022). Şanlıurfa İlindeki İshalli Buzağılarda Bovine *Coronavirus* Varlığının ELISA Yöntemi ile Araştırılması. Harran Üniversitesi Veteriner Fakültesi Dergisi, 11(1), 120-127. https://doi.org/10.31196/huvfd.1118140

Al, M., & Balıkçı, E. (2012). Neonatal İshalli Buzağılarda *Rotavirus*, *Coronavirus*, *E. coli* K99 ve *Cryptosporidium parvum*'un Hızlı Test Kitleri ile Teşhisi ve Enteropatojen ile Maternal İmmünite İlişkisi. Fırat Üniversitesi Sağlık Bilimleri Veteriner Dergisi 26(2), 73-78.

Atcalı, T., & Yıldız, R. (2020). Neonatal Buzağı İshallerinde Farklı Etiyolojik Faktörlerin Hemogram Parametreleri Üzerine Etkisi. Mehmet Akif Ersoy Üniversitesi Sağlık Bilimleri Enstitüsü Dergisi, 8(3), 119-127. https://doi.org/10.24998/maeusabed.823819

Ates, O., & Yesilbag, K. (2023). Characterization of bovine rotavirus isolates from diarrheic calves in Türkiye. Molecular Biology Reports, 50(4), 3063-3071. https://doi.org/10.1007/s11033-022-08169-4

Ayan, A., Alıç Ural, D., Erdoğan, H., Orunç Kılınç, Ö., Gültekin, M., & Ural, K. (2019). Prevalance And Molecular Characterızatıon Of *Giardıa duodenalis* In Lıvestock In Van, Turkey. International Journal of Ecosystems and Ecology Science (IJEES) 9(2), 289-296. https://doi.org/10.31407/ijees

Aydin, O., Ulas, N., Genc, A., Baysal, S., Kandemir, O., & Aktas, M. S. (2022). Investigation of hemogram, oxidative stress, and some inflammatory marker levels in neonatal calves with *Escherichia coli* and *Coronavirus* diarrhea. Microbial Pathogenesis, 173, 105802. https://doi.org/10.1016/j.micpath.2022.105802

Aydoğdu, U., & Güzelbekteş, H. (2018). Effect of colostrum composition on passive calf immunity in primiparous and multiparous dairy cows. Veterinarni Medicina 63, 1-11. https://doi.org/10.17221/40/2017-VETMED

Aydoğdu, U., Gülersoy, E., & Şen, İ. (2018b). Buzağı ishalleri ve oral sıvı takviyeleri. Türkiye Klinikleri Animal Nutrition and Nutritional Diseases-Special Topics 4, 56-64.

Aydoğdu, U., Işık, N., Ekici, Ö. D., Yıldız, R., Şen, İ., & Coşkun, A. (2018a). Comparison of the effectiveness of halofuginone lactate and paromomycin in the treatment of calves naturally ınfected with *Cryptosporidium parvum*. Acta Scientiae Veterinariae 46, 1-9. https://doi.org/10.22456/1679-9216.81809

Aygün, O., & Yıldız, R. (2018). Evaluation of thrombomodulin and pentraxin-3 as diagnostic biomarkers in calves with

sepsis. Veterinární medicína, 63, 313-320. https://doi.org/10.17221/159/2017-VETMED

Bendali, F., Bichet, H., Schelcher, F., & Sana, M. (1999). Pattern of diarrhoe in newborn calves in South-West France. Veterinary Research 30, 61-74.

Björkman, C., Svensson, C., Christensson, B., & De Verdier, K. (2003). *Cryptosporidium parvum* and *Giardia intestinalis* in calf diarrhoea in Sweden. Acta Veterinaria Scandinavica, 44(3), 145.

Blanchard, P. C. (2012). Diagnostics of dairy and beef cattle diarrhea. Veterinary Clinics of North America Food Animal Practice 28, 443-464. https://doi.org/10.1016/j.cvfa.2012.07.002

Brunauer, M., Roch, F. F., & Conrady, B. (2021). Prevalence of worldwide neonatal calf diarrhoea caused by bovine rotavirus in combination with Bovine *Coronavirus*, *Escherichia coli* K99 and *Cryptosporidium* spp.: a meta-analysis. Animals, 11(4), 1014. https://doi.org/10.3390/ani11041014

Chen, Y., Huang, J., Qin, H., Wang, L., Li, J., & Zhang, L. (2023). *Cryptosporidium parvum* and gp60 genotype prevalence in dairy calves worldwide: a systematic review and meta-analysis. Acta Tropica, 106843. https://doi.org/10.1016/j.actatropica.2023.106843

Cho, Y. I., & Yoon, K. J. (2014). An overview of calf diarrhea-infectious etiology, diagnosis, and intervention. Journal of veterinary science, 15(1), 1-17. https://doi.org/10.4142/jvs.2014.15.1.1

Constable, P.D., Hinchcliff, K. W., Done, S.H., Grünberg, W., (2017). Veterinary medicine a textbook of the diseases of cattle, horses, sheep, pigs, and goats. Elsevier, St. Louis Missouri.

Coşkun, A., & Kaya, K (2018). Tokat Bölgesindeki Neonatal Buzağı İshallerinin Etiyolojisinin Belirlenmesi. Manas Journal of Agriculture Veterinary and Life Sciences, 8(1), 75-80.

Değerli, S., Çeliksöz, A., Kalkan, K., & Özçelik, S. (2005). Prevalence of *Cryptosporidium* spp. and *Giardia* spp. in cows and calves in Sivas. Turkish Journal of Veterinary & Animal Sciences, 29(4), 995-999.

Değirmençay, Ş., Eroğlu, M. S., & Eren, E. (2023). Differential Diagnostic Value of Serum Procalcitonin and Iron Levels in Diarrheic Neonatal Calves Caused by Escherichia coli and Rotavirus. Kocatepe Veterinary Journal, 16(1), 77-85. https://doi.org/10.30607/kvj.1205080

Du, W., Wang, X., Hu, M., Hou, J., Du, Y., Si, W., Yang, L., Xu, L. & Xu, Q. (2023). Modulating gastrointestinal microbiota to alleviate diarrhea in calves. Frontiers in Microbiology, 14, 1181545. https://doi.org/10.3389/fmicb.2023.1181545

Gao, H., Liang, G., Su, N., Li, Q., Wang, D., Wang, J., Zhao, L., Kang, X., & Guo, K. (2023). Prevalence and Molecular Characterization of *Cryptosporidium* spp., *Giardia duodenalis*, and *Enterocytozoon bieneusi* in Diarrheic and Non-Diarrheic Calves from Ningxia, Northwestern China. Animals, 13(12), 1983. https://doi.org/10.3390/ani13121983

Geng, H. L., Meng, X. Z., Yan, W. L., Li, X. M., Jiang, J., Ni, H. B., & Liu, W. H. (2023). Prevalence of bovine coronavirus in cattle in China: A systematic review and meta-analysis. Microbial Pathogenesis, 106009. https://doi.org/10.1016/j.micpath.2023.106009

Gültekin, M., Ural, K., Aysul, N., Ayan, A., Balıkçı, C., Toplu, S. & Akyıldız, G. (2017). Prevalence and Molecular Characterization of *Giardia duodenalis* in Calves in Turkey. Acta Scientiae Veterinariae, 45, 1450. https://doi.org/10.22456/1679-9216.80032

Güzelbekteş, H., Coşkun, A., & Şen, İ., (2007). Relationship between the degree of dehydration and the balance of acid-based changes in dehydrated calves with diarrhoea. Bulletin of the Veterinary Institute in Pulawy 51, 83-87.

Higgs, C., Breiitenbuecher, J., Pablo Gomez, J., Samah, F., Fausak, E. D., Van Noord, M., & Ute Maier, G. (2023). Efficacy of dam vaccination for the prevention of neonatal diarrhea caused by enterotoxigenic *E. coli* in calves: protocol for a systematic review and meta-analysis. California Digital Library University of California. https://escholarship.org/uc/item/71d3x0tt

Izzo, M., Gunn, A. A., & House, J. K. (2015). Neonatal Diarrhea. In: Large Animal Internal Medicine, 5th Edition. Editör: Smith, B.P. Mosby, Elsevier.

İçen, H., Arserim, N. B., Işık, N., Özkan, C., & Kaya, A. (2013). Prevalence of Four Enteropathogens with Immunochromatographic Rapid Test in the Feces of Diarrheic Calves in East and Southeast of Turkey. Pakistan Veterinary Journal, 33(4).

Kabir, M. H. B., Ceylan, O., Ceylan, C., Shehata, A. A., Bando, H., Essa, M. I., Xuan, X., Sevinç, F., & Kato, K. (2020). Molecular detection of genotypes and subtypes of *Cryptosporidium* infection in diarrheic calves, lambs, and goat kids from Turkey. Parasitology international, 79, 102163. https://doi.org/10.1016/j.parint.2020.102163

Kalınbacak, A. 2003. İshalli Buzağıların Sıvı Sağaltımında Hipertonik Salin-Dextran ve Oral Elektrolit Solüsyonunun Kullanımı. Ankara Üniversitesi Veteriner Fakültesi Dergisi 50, 113-118.

Kaske, M., & Kunz, H. J., (2003). Manual of diarrheal diseases in calves. Kamlage Werlag, Auflage Stuttgart, pp: 15-140.

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

Kuliğ, C. C., & Coşkun, A. (2019). Sivas ve ilçelerindeki neonatal ishalli buzağılarda *E. coli*, *Cryptosporidium*, *Clostridium perfringens*, *Rotavirus* ve *Coronavirus* Prevalansı. Turkish Veterinary Journal, 1(2), 69-73.

Kuraa, H. M., & Malek, S. S. (2023). The role of the *Cryptosporidium parvum* in diarrhea in calves and lambs. Veterinary Integrative Sciences, 21(3), 735-749. https://doi. org/10.12982/VIS. 2023.053.

McGuirk, S. (2013). Calf Health Scoring Chart. University of Wisconsin, School of Veterinary Medicine.https://fyi.extension.wisc.edu/heifermgmt/files/2015/02/calf_health_scoring_chart.pdf [Erişim Tarihi: 10.06.2023]

Özer, E., Erdoğmuş, S. Z., & Köroğlu, E. (1990). Elazığ çevresinde buzağı ve kuzularda bulunan *Cryptosporidium*'un yaygınlığı üzerinde araştırmalar. Turkish Journal of Veterinary Animal Science, 14, 439-445.

Öztürkler, O. (2023). Ozturkler, O. Investigation of some microbial and protozoon factors with rapid test kits in neonatal diarrheas in calves in Kars province: Neonatal diarrheas in calves in Kars province. Journal of One Health Research, 1(1), 1-6. https://doi.org/10.5281/zenodo.7513438

Park, Y. J., Cho, H. C., Jang, D. H., Park, J., & Choi, K. S. (2023). Multilocus genotyping of Giardia duodenalis in pre-weaned calves with diarrhea in the Republic of Korea. Plos one, 18(1), e0279533. https://doi.org/10.1371/journal.pone.0279533

Pesavento, J. B., Billingsley, A. M., Roberts, E. J., Ramig, R. F., & Prasad, B. V. V. (2003). Structures of *Rotavirus* reassortants demonstrate correlation of altered conformation of the VP4 spike and expression of unexpected VP4-associated phenotypes. Journal of Virology, 77(5), 3291-3296. https://doi.org/10.1128/jvi.77.5.3291-3296.2003

Sezer, S., & Akgul, G. (2022). Rapid etiological diagnosis of neonatal calf diarrhea with immunochromatographic test kits in Esme district of Usak. Assiut Veterinary Medical Journal, 68(173), 10-15. https://doi.org/10.21608/avmj.2022.118605.1049

Taghipour, A., Sharbatkhori, M., Tohidi, F., Ghanbari, M. R., Karanis, P., Olfatifar, M., Majidiandi, H., Khazaei, S., Bahadory, S., & Javanmard, E. (2022). Global prevalence of *Giardia duodenalis* in cattle: A systematic review and meta-analysis. Preventive Veterinary Medicine, 203, 105632. https://doi.org/10.1016/j.prevetmed.2022.105632

Uhde, F.L., Kaufmann, T., Sager, H., Albini, S., Zanoni, R., Schelling, E., & Meylan, M. (2008). Prevalence of four enteropathogens in the faeces of young diarrhoeic dairy calves in Switzerland. The Veterinary record, 163, 362-366. https://doi.org/10.1136/vr.163.12.362

Yıldız, R., Beslek, M., Beydilli, Y., Özçelik, M. M., & Biçici, Ö. (2018). Evaluation of platelet activating factor in neonatal calves with sepsis. Veteriner Hekimler Derneği Dergisi 89, 66-73.

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RESEARCH ARTICLE

Exploring *Apis mellifera* L. Venom's Antioxidant Power in Various Solvents: Unveiling its *In Vitro* Potential

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ABSTRACT

Apis mellifera L. venom contains bioactive components with antioxidant properties. Diluted in various polar solvents, the venom is utilized for therapeutic purposes. This study aims to determine the *in vitro* antioxidant activities (AOA) of standard crude venom (SV) and venom from breeders (BV) by dissolving them in distilled water, saline, and PBS at concentrations of 1.95-500 µg.ml^{-1}. Radical scavenging activity (DPPH) and metal chelating activity (MCA) assays were employed for AOA assessment. SV dissolved in distilled water exhibited higher RSA (73.26±11.24%) than BV (34.60±21.08%), with no difference between SV, ascorbic acid (AA), and Trolox RSA's. BV's RSA was lower than AA (75.07±15.59%) and Trolox (84.02±1.63%). BV's MCA (30.31±24.06%) exceeded AA (8.93±16.08%). SV in saline showed higher RSA (63.83±9.73%) than BV (46.99±18.31%), lower than AA (71.63±4.14%) and Trolox (79.01±6.94%). MCAs of SV (85.42±4.65%) and BV (85.53±7.19%) surpassed Trolox (55.06±30.92%). No difference existed between RSA's of SV (37.16±16.54%) and BV (38.47±17.24%) in PBS, both lower than AA (71.48±3.66%) and Trolox (72.87±6.05%). Optimal RSA and MCA were observed at different solvents and concentrations, indicating the use of 500 µg.ml^{-1} (1.95 µg.ml^{-1} BV for RSA) venom dissolved in saline for optimal AOA. PBS or distilled water usage resulted in decreased AOA.

Keywords: Antioxidant activity, Apitoxin, Bee venom, Metal chelating activity, Radical scavenging activity, Venom solubility

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Apis mellifera L. Zehirinin Çeşitli Solventlerdeki Antioksidan Gücü: *In Vitro* Potansiyelin Ortaya Çıkarılması

ÖZ

Apis mellifera L. zehiri, antioksidan özelliklere sahip biyoaktif bileşenler içermektedir. Çeşitli polar çözücülerde seyreltilen zehir, terapötik amaçlar için kullanılmaktadır. Bu çalışma, standart ham zehir (SV) ve üreticiden temin edilen zehir (BV) örneklerinin *in vitro* antioksidan aktivitelerini (AOA) belirlemeyi amaçlamaktadır. Bu amaçla örnekler, 1.95-500 µg.ml^{-1} konsantrasyon aralığında distile su, fizyolojik tuzlu su ve PBS içinde çözülmüştür. Antioksidan aktivitelerin değerlendirilmesi için serbest radikal giderme aktivitesi (DPPH) ve metal şelasyon aktivitesi (MCA) analizleri kullanılmıştır. SV, distile suda çözündüğünde BV'ye göre daha yüksek RSA (73.26±11.24%) sergilemiş, SV, askorbik asit (AA) ve Trolox RSA'ları arasında fark bulunmamıştır. BV'nin RSA'sı, AA (75.07±15.59%) ve Trolox (84.02±1.63%) RSA'larından düşük bulunmuştur. BV'nin MCA'sı (30.31±24.06%), AA (8.93±16.08%) değerini aşmıştır. SV, tuzlu su içinde çözüldüğünde BV'ye göre daha yüksek RSA (63.83±9.73%) sergilemiş, AA (71.63±4.14%) ve Trolox (79.01±6.94%) RSA'larından düşük bulunmuştur. SV (85.42±4.65%) ve BV (85.53±7.19%) örneklerinin MCA değerleri, Trolox (55.06±30.92%) değerini aşmıştır. SV (37.16±16.54%) ve BV (38.47±17.24%) örneklerinin PBS içindeki RSA değerleri arasında fark bulunmamış, her ikisi de AA (71.48±3.66%) ve Trolox (72.87±6.05%) değerlerinin altında kalmıştır. Optimal RSA ve MCA değerleri farklı çözücü ve konsantrasyonlarda gözlemlenmiş, bu durum 500 µg.ml^{-1} (BV için RSA'da 1.95 µg.ml^{-1}) konsantrasyonda fizyolojik tuzlu su içinde çözünen zehirin optimal AOA için kullanılmasına işaret etmektedir. PBS veya distile su kullanımı ise AOA değerlerinde azalmaya neden olmuştur.

Anahtar kelimeler: Antioksidan aktivite, Apitoksin, Arı zehiri, Metal şelasyon aktivitesi, Serbest radikal giderme aktivitesi, Zehir çözünürlüğü

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INTRODUCTION

The European honeybee, *Apis mellifera* L., stands as a focal point of extensive research, specifically within the realm of apitherapy, and holds the distinction of being the most investigated subspecies. Renowned for its utilization in apitherapeutic products, this species has a longstanding tradition of being harnessed for its diverse array of healing elements, including honey, pollen, propolis, royal jelly, and venom (Eze et al., 2016; Şenel and Demir 2018). These natural substances have been employed for therapeutic purposes for millennia, rooted in empirical knowledge, and have transcended into contemporary practice under the guidance of certain clinicians. Notably, applications targeting chronic inflammatory ailments such as arthritis have been prevalent. Moreover, the attention garnered by the species extends to its applications in antimicrobial, anti-inflammatory, anti-cancer, and wound healing contexts (Han et al. 2012; Gupta and Stangaciu 2014).

Within the diverse matrix of bee products, the venom of the European honeybee has emerged with heightened prominence, propelled by its biologically active constituents, particularly peptides (Mehdi et al. 2022). The increasing recognition of these bioactive components underscores their potential in the treatment of various ailments (Zhang et al. 2018). However, the transformative journey from traditional bee-product therapies to their acceptance within evidence-based medicine hinges upon the establishment of rigorous scientific foundations and empirical validation of venom's role in disease treatment and prevention (Hwang et al. 2015; Denk and Fidan 2021).

This study bridges this gap by delving into the *in vitro* antioxidant activity (AOA) of *Apis mellifera* L. venom. We explore the optimal AOA attributes of the venom using three commonly employed solvents—distilled water, physiological saline, and phosphate-buffered saline (PBS). These solvents were chosen due to their prevalence in scientific research.

Within the context of this study, we conduct a comparative statistical analysis of the antioxidant capacity of venom samples sourced from standard *Apis mellifera* L. venom (SV) and venom obtained directly from beekeepers (BV), employing well-established *in vitro* AOA markers such as the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay and metal chelating activity (MCA) assay. The investigation further encompasses a diverse range of venom concentrations, from 1.95 to 500 $\mu g.ml^{-1}$, for each solvent, including both venom samples and standard preparations. The outcomes are meticulously analyzed and compared, shedding light on the venom's potential antioxidant properties.

By unraveling the intricate properties of *Apis mellifera* L. venom and investigating its antioxidant potential, this study takes strides towards advancing our comprehension of the therapeutic prowess of bee-derived substances. Furthermore, it endeavors to provide the scientific substantiation needed to foster the integration of venom into evidence-based medical practices for disease management and prevention.

MATERIAL and METHODS

Venom Acquisition and Preparation:

The SV was obtained as a commercially available HPLC-grade preparation from Sigma (Sigma-Aldrich, Darmstadt, Germany). The BV was sourced from a local beekeeper engaged in beekeeping activities in the İzmir/Foça region (38.6704° N, 26.7579° E). Venom collection from the beekeeper was carried out mid-April 2021 using a venom collection device. Both SV

and BV were stored in crude and powdered forms at -80 °C until further use.

Stock Solution Preparation:

To create stock solutions, 5 mg of each venom was weighed and dissolved in distilled water, physiological saline (0.9% NaCl solution), and PBS (pH:7.4). Subsequently, 8 dilution solutions were prepared from each stock solution at a 1:2 ratio, yielding concentrations ranging from 1.95 to 500 $\mu g.ml^{-1}$. The same dilution methodology was applied to prepare dilution solutions of two standard antioxidants, ascorbic acid (AA) and polar vitamin E analogue ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid; Trolox), which are commonly used for AOA measurements.

Antioxidant Activity Analysis:

The study encompassed the comparative analysis of AOA in SV and BV using DPPH assay and MCA assay. AOA measurements were also conducted for AA and Trolox as reference antioxidants. The experimental design aimed to elucidate the potential variations in antioxidant capacities across different venom samples and reference compounds.

The DPPH assay is a widely employed method for assessing antioxidant activity. DPPH is a stable free radical molecule at room temperature, displaying a characteristic purple color in the solution. It serves as an electron acceptor, reflecting its capacity to capture free electrons from suitable antioxidants (Munteanu and Apetrei 2021). The principle underlying the DPPH assay lies in the conversion of DPPH to its reduced form, DPPH-H, through the transfer of a hydrogen ion (H^+) from antioxidants possessing hydrogen-donating (H-donating) capabilities. This reduction process leads to a color change in the solution from deep purple to a lighter shade of yellow, a reaction that can be quantified spectrophotometrically at 520 nm. The degree of color change inversely correlates with the concentration of the reduced DPPH radical, forming the basis for evaluating the scavenging potential of antioxidants (Pinto et al. 2021).

To perform the DPPH assay, a DPPH working solution is prepared with an optical density of 0.968 at 520 nm. The procedure is carried out in a dark and standard room temperature environment. For each sample (SV, BV, AA, Trolox), 50 µl of the sample is mixed with 450 µl of the DPPH working solution. The samples are then incubated for 30 minutes to allow the interaction between antioxidants and DPPH. As a control solution, DPPH alone is used. After the incubation period, the absorbance of all solutions, including the control, is measured at 520 nm using a spectrophotometer (Shimadzu Corp. Kyoto, Japan). The term radical scavenging activity (RSA) is used in the context of DPPH assay to describe the ability of an antioxidant to neutralize or scavenge free radicals present in the solution (Dontha 2016; Pinto et al. 2021).

The RSA percentage, indicative of the antioxidant efficacy, is calculated using the following formula:

$$RSA(\%)=[(Abs_{control}-Abs_{sample})\times(Abs_{control})^{-1}]\times 100$$

Where:

$Abs_{control}$ represents the absorbance of the control solution (containing only DPPH),

Abs_{sample} represents the absorbance of the sample solution (containing DPPH, the SV, the BV, and the standard antioxidant dilutions).

Antioxidants play a crucial role in terminating or delaying oxidative processes by chelating catalytic metal ions (Dontha 2016). Due to the functional groups capable of metal binding, antioxidants have been reported to exhibit effective iron-chelating capabilities (Gulcin and Alwasel 2022).

The MCA assay involves the competition between antioxidants and 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p'-disulfonic acid monosodium salt hydrate (FerroZine™, Sigma-Aldrich, Darmstadt, Germany) for binding with Fe^{2+} ions. This assay sheds light on the capacity of antioxidants to form complexes with metal ions, inhibiting their participation in oxidative reactions. The principle behind this assay centers on the fact that as antioxidants vie for binding to Fe^{2+} ions, a decrease occurs in the formation of a reddish-colored complex, resulting in a measurable change in absorbance (Dontha 2016; Gulcin and Alwasel 2022).

To conduct the MCA assay, 100 µl of each sample (SV, BV, AA, Trolox, EDTA) is mixed with 50 µl of a 2 mM $FeCl_2.4H_2O$ solution. The mixture is incubated at room temperature for 5 minutes. Following this incubation, 100 µl of a 5 mM FerroZine solution is added to the mixture. The final volume is adjusted to 3 ml using distilled water. The mixture is then incubated for an additional 10 minutes at room temperature. Subsequently, the absorbance of the solution is measured at 562 nm using a spectrophotometer (Dontha 2016; Gulcin and Alwasel 2022).

The MCA is quantified as the percentage of metal-chelating activity, which can be calculated using the formula:

$$MCA(\%)=[(Abs_{control}-Abs_{sample})\times(Abs_{control})^{-1}]\times 100$$

Where:

$Abs_{control}$ represents the absorbance of the control solution (containing only Fe^{2+} and FerroZine),

Abs_{sample} signifies the absorbance of the sample solution (containing the SV, the BV, and the standard antioxidant dilutions).

Statistical Analysis:

After verifying the fulfillment of the normality assumption through both the Shapiro-Wilk and Kolmogorov-Smirnov tests, we conducted a two-tailed unpaired t-test on the dataset. This approach enabled us to evaluate the statistical significance of distinctions between the two groups while confirming the conditions required for parametric testing. We employed the two-tailed unpaired t-test method, executed via the SPSS program (v20, IBM Corp., New York, United States), to assess the statistical significance among different samples. To support our analysis, we calculated the group means and standard deviations.

RESULTS

The findings related to the RSA assay are presented in Figure 1, Figure 2, and Figure 3.

DPPH Assay Findings for Standard Venom:

The SV exhibited a remarkable RSA with the highest percentage recorded at 91.63%. The solvent and concentration combination that yielded the most effective antioxidative response was distilled water at 500 $\mu g.ml^{-1}$, indicating optimal solubility. Conversely, the lowest RSA percentage of 3.10% was observed, attributed to the solvent and concentration combination of PBS at 7.81 $\mu g.ml^{-1}$.

DPPH Assay Findings for Venom from Beekeeper:

The venom obtained from the beekeeper demonstrated a noteworthy RSA percentage of 73.86%.The solvent and concentration combination that resulted in the highest RSA was distilled water at 1.95 $\mu g.ml^{-1}$, reflecting optimal solubility and potent radical scavenging ability. Conversely, the lowest RSA value of 15.50% was observed for the solvent and concentration combination of PBS at 250 $\mu g.ml^{-1}$.

The RSA percentages for Different Solvents:

The percentages of RSA for various solvents are presented in Table 1. When comparing the average RSA percentages of venom dilutions in distilled water, it was observed that the RSA of SV dissolved in this solvent was higher than that of BV ($p<0.001$). No significant difference was noted between the RSA of SV and the RSA of AA and Trolox ($p<0.05$). However, the RSA of BV was notably lower than that of both AA and Trolox ($p<0.001$).

In the case of venom dissolved in physiological saline solution, the RSA of SV was observed to be higher than that of BV ($p<0.05$). However, both SV ($p<0.05$) and BV ($p<0.05$, compared to AA; $p<0.001$, compared to Trolox) exhibited lower RSA values compared to AA and Trolox.

For venom dissolved in PBS, there was no significant difference between the RSA of SV and BV ($p<0.05$). However, the RSA values for both venoms were lower than those of AA and Trolox ($p<0.001$).

The findings related to the MCA assay are presented in Figure 4, Figure 5, and Figure 6.

MCA Assay Findings for Standard Venom:

In the MCA assay conducted on the SV, the highest MCA percentage recorded was 91.63%. The solvent and concentration combination that exhibited the highest MCA was saline at 500 $\mu g.ml^{-1}$. On the other hand, the lowest MCA percentage of 1.04% was observed. This limited activity was linked to the solvent and concentration combination of distilled water at 15.63 $\mu g.ml^{-1}$.

MCA Assay Findings for Venom from Beekeeper:

The highest MCA percentage was recorded at 93.63%. The solvent and concentration combination that resulted in the highest MCA was similar to the SV, saline at 500 $\mu g.ml^{-1}$. However, the lowest MCA percentage of 8.67% was observed, attributed to the solvent and concentration combination of distilled water at 15.63 $\mu g.ml^{-1}$.

The MCA Percentages for Different Solvents:

The percentages of MCA for different solvents are displayed in Table 2. When comparing the average MCA percentages of venom dilutions in distilled water, distinct observations were made. The MCA of SV dissolved in distilled water did not exhibit significant statistical differences from the MCA of BV ($p<0.05$). However, the MCA of both venoms was notably lower than that of Trolox ($p<0.05$). Furthermore, the MCA of BV surpassed that of AA ($p<0.05$).

For venoms dissolved in physiological saline solution, no significant statistical differences were observed in the MCA between SV and BV ($p<0.05$). Notably, the MCA values of both venoms exceeded that of Trolox ($p<0.05$).

When venom and standards were dissolved in PBS, no significant statistical differences were found among the MCA values ($p<0.05$).

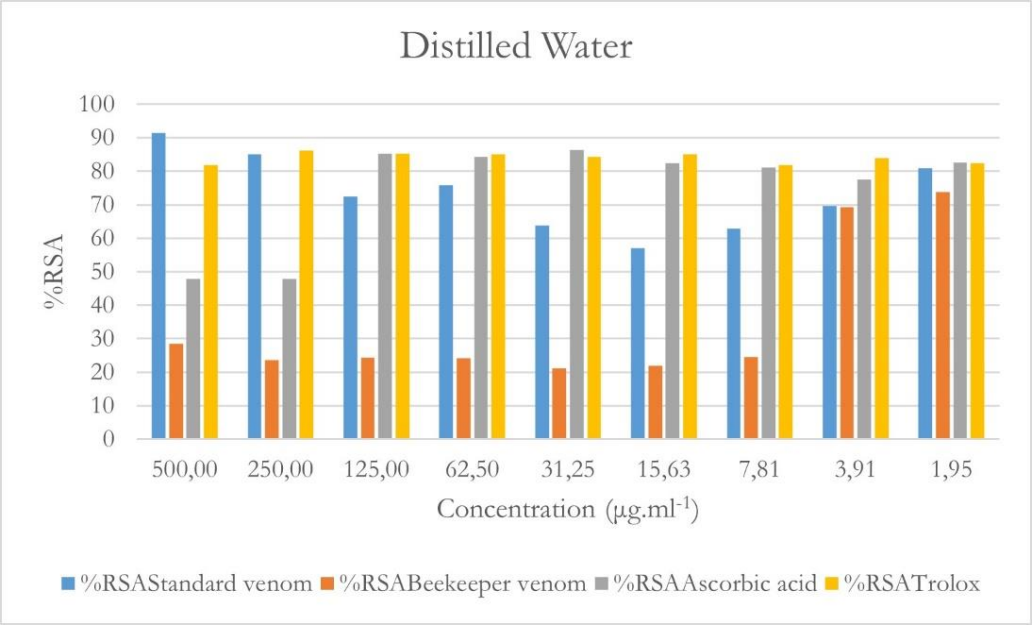

Figure 1. Concentration-RSA Percentage Graph of Venom Samples and Standard Antioxidants diluted in Distilled Water

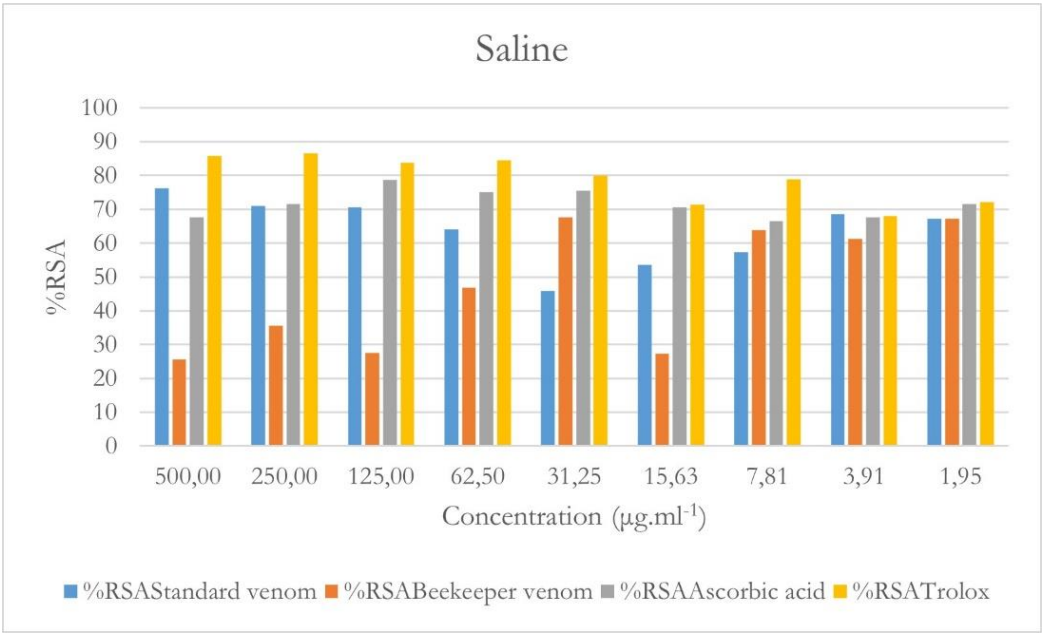

Figure 2. Concentration-RSA Percentage Graph of Venom Samples and Standard Antioxidants diluted in Saline

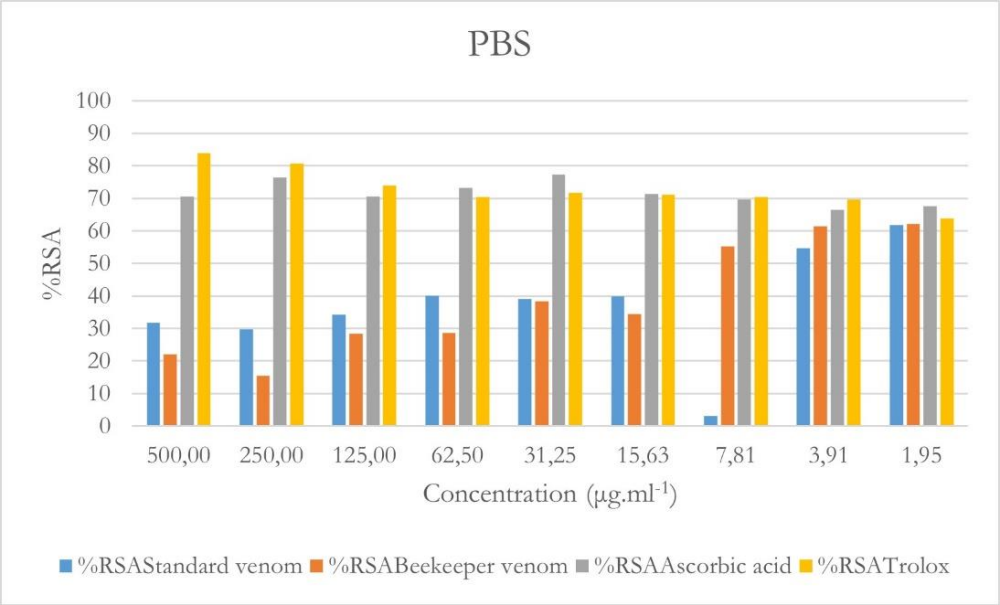

Figure 3. Concentration-RSA Percentage Graph of Venom Samples and Standard Antioxidants diluted in PBS

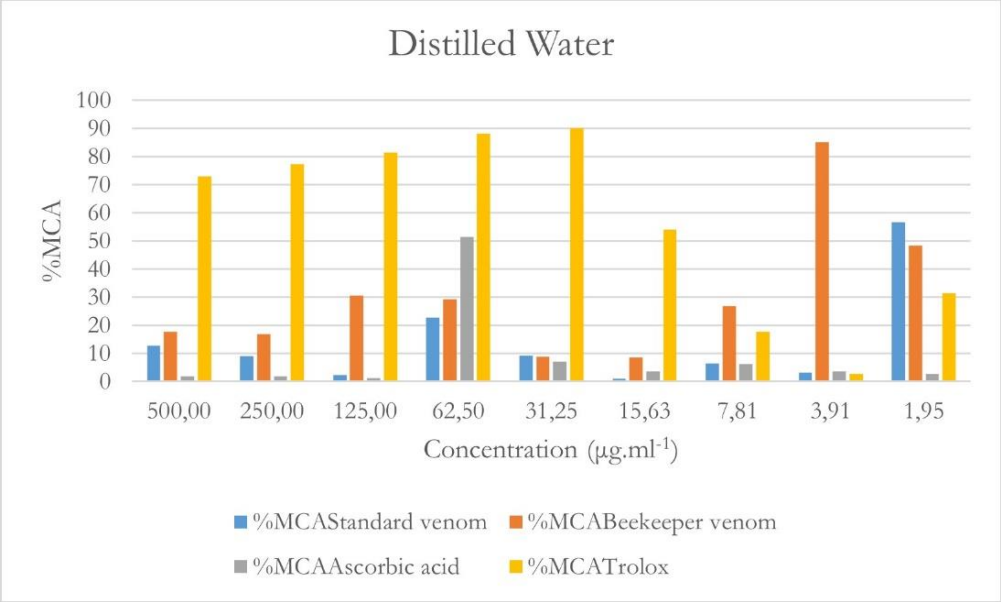

Figure 4. Concentration-MCA Percentage Graph of Venom Samples and Standard Antioxidants diluted in Distilled Water

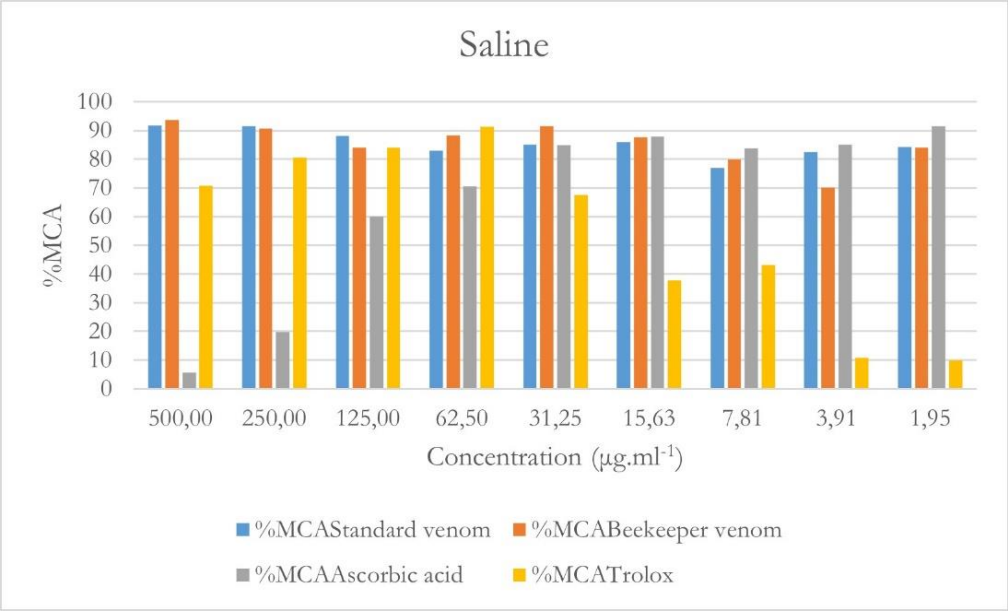

Figure 5. Concentration-MCA Percentage Graph of Venom Samples and Standard Antioxidants diluted in Saline

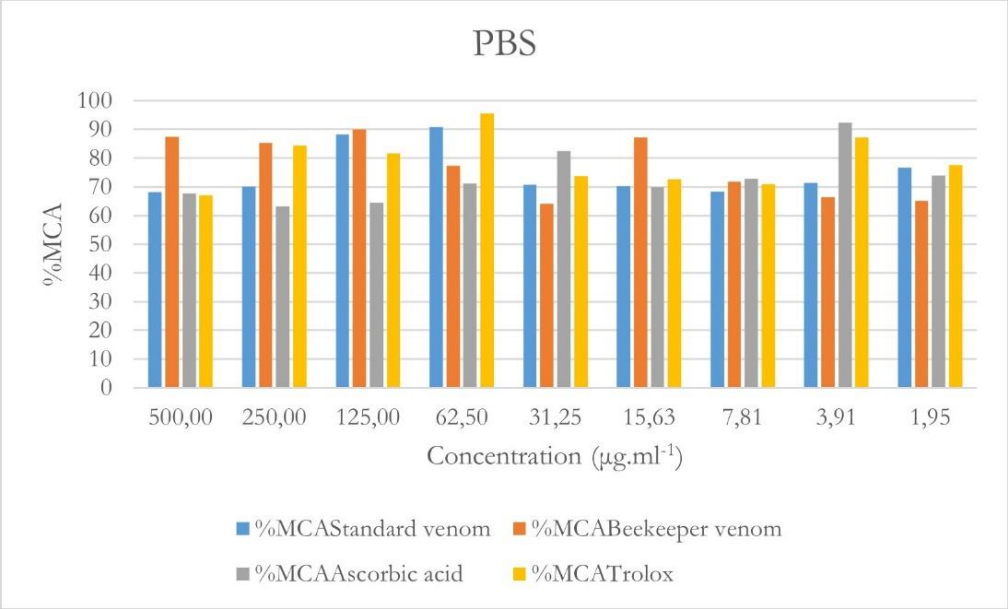

Figure 6. Concentration-MCA Percentage Graph of Venom Samples and Standard Antioxidants diluted in PBS

Table 1. Average RSA percentages of venom dilutions and standard antioxidants in different solvents

	Distilled water	**Saline**	**PBS**
Standard venom (SV)	73.26±11.24[a]	63.83±9.73[a]	37.16±16.54[a]
Beekeeper venom (BV)	34.60±21.08[b]	46.99±18.31[b]	38.47±17.24[a]
Ascorbic acid (AA)	75.07±15.59[a]	71.63±4.14[c]	71.48±3.66[b]
Trolox	84.02±1.63[a]	79.01±6.94[c]	72.87±6.05[b]

The letters "a", "b", and "c" in the respective columns of the table represent significant statistical differences between groups ($p<0.05$).

Table 2. Average MCA percentages of venom dilutions and standard antioxidants in different solvents

	Distilled water	Saline	PBS
Standard venom (SV)	13.75±17.36[a, b]	85.42±4.65[a]	74.95±8.65
Beekeeper venom (BV)	30.31±24.06[a]	85.53±7.19[a]	77.16±10.58
Ascorbic acid (AA)	8.93±16.08[b]	65.45±31.67[a, b]	73.09±9.22
Trolox	57.35±32.55[c]	55.06±30.92[b]	78.98±9.05

The letters "a", "b", and "c" in the respective columns of the table represent significant statistical differences between groups ($p<0.05$).

DISCUSSION

Bee products are chemically complex, and the dissolution using solvents of varying polarity can influence the composition of the analyzed extract. Hydrophilic components tend to dissolve more readily in polar solvents such as alcohols, while hydrophobic ones, like hydrocarbons, show a greater affinity for non-polar solvents. This phenomenon has been observed in various studies (Martinello and Mutinelli 2021). The components of venom also possess hydrophilic and hydrophobic characteristics; certain enzymes like melittin, apamin, and phospholipase A2 are recognized as amphipathic polycationic peptides (Lee et al. 2016). Hence, variations in AOA based on the solvent medium are likely to be observed.

In our study, the DPPH assay results provided insights into the distinctive antioxidant capacities of the SV and BV. The SV exhibited a noteworthy RSA with the highest percentage, revealing its potent antioxidant capability. Conversely, the lowest RSA percentage was attributed to the solvent and concentration combination of PBS at 7.81 $\mu g.ml^{-1}$, indicative of reduced solubility and a corresponding reduction in antioxidant potential. Similarly, BV demonstrated a significant RSA percentage, underscoring its substantial antioxidant effectiveness. The lowest RSA value observed for BV was linked to the solvent and concentration combination of PBS at 250 $\mu g.ml^{-1}$, suggesting compromised solubility and diminished radical scavenging potential within this specific context.

In line with our findings, a study by Frangieh et al. (2019) evaluated AOA using DPPH assay on *Apis mellifera syriaca* crude venom. They demonstrated dose-dependent AOA of the venom, with a lower RSA compared to ascorbic acid, their standard antioxidant (Frangieh et al. 2019). Similarly, Sobral et al. (2016) employed DPPH assay to assess AOA on five bee venom samples obtained from *Apis mellifera iberiensis*. They found comparable antioxidant effects of diluted venom in distilled water, along with AA and Trolox, standard antioxidants (Sobral et al. 2016). Our results aligned with these studies, as we observed a similar pattern of RSA for SV diluted in distilled water across the range of 15.63-500 $\mu g.ml^{-1}$, showing antioxidant effects statistically indistinguishable ($p<0.05$) from AA and Trolox, though this trend was not evident in BV. Notably, the highest RSA for BV was observed at the lowest concentration (1.95 $\mu g.ml^{-1}$).

Contrasting observations were highlighted in a study by Somwongin et al. (2018), which assessed AOA using DPPH assay on venom samples diluted in PBS from *Apis cerena*, *Apis florea*, *Apis dorsata*, and *Apis mellifera*. Extracts from *Apis dorsata* exhibited the highest AOA, even comparable to AA, a recognized antioxidant compound (Somwongin et al. 2018). In contrast, our results indicated that both SV and BV diluted in PBS displayed lower RSA compared to standard antioxidants. Furthermore, it can be noted that the points at which the RSA-related AOA of SV and BV samples diluted in PBS were observed to be

the highest are at the lowest doses (1.95 μg.ml^{-1}).The results obtained from the MCA assay shed light on the distinct metal chelation capacities of the SV and BV. In the MCA assay conducted on SV, the highest MCA percentage emphasized its robust metal-chelating capability. Conversely, the lowest MCA percentage was linked to the solvent and concentration combination of distilled water at 15.63 μg.ml^{-1}, indicating reduced solubility and subsequent compromised metal chelation potential. Similarly, BV exhibited a notable MCA percentage, consistent with its effective metal-chelating efficacy. The solvent and concentration combination of saline at 500 μg.ml^{-1} demonstrated optimal solubility for efficient metal chelation, while the solvent and concentration combination of distilled water at 15.63 μg.ml^{-1} resulted in diminished activity due to solubility-related limitations.

In general, the MCA, reducing power (RP), and Ferric reducing/antioxidant power (FRAP) methods employ different fundamental principles to measure antioxidant capacity, focusing on different compounds. While MCA is based on the assumption that metal ions are chelated to prevent metal-catalyzed oxidation (Gulcin and Alwasel 2022), RP and FRAP methods assess AOA through reducing capacity (Sobral et al. 2016; Somwongin et al. 2018). Although scientific literature on MCA measurement for *Apis mellifera* venom is scarce, the AOA of the venom has been evaluated using RP (Sobral et al. 2016) and FRAP (Somwongin et al. 2018) assays, measuring their ferric ion reducing activities. It can be concluded that all three methods suggest that *Apis mellifera* venoms exhibit AOA by chelating and/or reducing metal ions.

The impact of solvent choice and concentration on the manifestation of antioxidant and metal chelation activities is a pivotal observation. While SV and BV demonstrated diverse responses across various solvents, a consistent pattern was discernible. Optimal AOA was consistently achieved within a physiological saline solution, as evidenced by both the DPPH and MCA assays. This study further delineated distinct trends in the AOA of Apis mellifera venom across different solvent systems and concentrations. Notably, the use of 500 μg/mL (equivalent to 1.95 μg/mL BV in terms of RSA) of venom dissolved in saline emerged as the prime strategy for maximizing RSA and MCA. Conversely, the employment of PBS or distilled water resulted in diminished AOA outcomes. This underscores the pivotal roles of solvent selection and concentration in harnessing the venom's complete antioxidative potential.

In the broader context of research, it is well-established that the powdered form of bee venom is often dissolved in distilled water, physiological saline solution, or PBS for both *in vitro* and *in vivo* applications. These findings underscore the significance of solvent and concentration optimization when assessing and harnessing the antioxidative and metal chelation potential of bee venoms. Overall, this study contributes to the understanding of the nuanced interactions between bee venom, different solvents, and their antioxidative and metal chelation capacities.

CONCLUSION

Drawing insights from the findings of our study, several important conclusions can be drawn:

1. Polar solvents have a notable impact on the *in vitro* AOA levels of bee venom. Our results emphasize the significance of solvent selection in evaluating the antioxidative potential of bee venom.
2. When considering application methods for assessing the optimal AOA of bee venom in an *in vivo* context, both topical and other parenteral applications may benefit from dissolving bee venom in physiological saline

solution. This solvent choice could potentially enhance the antioxidative efficacy of the venom.

3. Our investigation has highlighted the variability in solvent and concentration preferences for different AOA measurements, suggesting that these factors play a key role in biological applications. Future research endeavors could explore additional AOA measurement methods in conjunction with the two methods evaluated in this study.

In conclusion, our study sheds light on the intricate relationship between solvent selection, concentration, and the antioxidative potential of bee venom. These findings have implications for the development of therapeutic applications involving bee venom and underscore the importance of tailoring solvent and concentration choices based on the desired outcomes. Further investigations into other AOA measurement methods and their responses to various solvents could provide a more comprehensive understanding of bee venom's antioxidative capabilities.

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

Authors' Contributions: BD contributed to the project idea, acquisition of data, data analysis, and writing the original draft, as well as designing and executing the study. All authors have read and approved the finalized manuscript.

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

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REFERENCES

Denk, B., & Fidan, A. F. (2021). Effects of honeybee (apis mellifera) venom on redox balance, biochemical andhematological profile in diabetic rats: A preliminary study. Turkish Journal of Veterinary & Animal Sciences, 45(2), 257-265.

Dontha, S. (2016). A review on antioxidant methods. Asian J. Pharm. Clin. Res, 9(2), 14-32.

El Mehdi, I., Falcão, S. I., Boujraf, S., Mustapha, H., Campos, M. G., & Vilas-Boas, M. (2022). Analytical methods for honeybee venom characterization. Journal of Advanced Pharmaceutical Technology & Research, 13(3), 154.

Eze, O. B., Nwodo, O. F., & Ogugua, V. N. (2016). Therapeutic effect of honey bee venom. Proteins (enzymes), 1(2).

Frangieh, J., Salma, Y., Haddad, K., Mattei, C., Legros, C., Fajloun, Z., & El Obeid, D. (2019). First characterization of the
venom from apis mellifera syriaca, a honeybee from the middle east region. Toxins, 11(4), 191.

Gulcin, İ., & Alwasel, S. H. (2022). Metal ions, metal chelators and metal chelating assay as antioxidant method. Processes, 10(1), 132.

Gupta, R. K., & Stangaciu, S. (2014). Apitherapy: holistic healing through the honeybee and bee products in countries with poor healthcare system. In Beekeeping for poverty alleviation and livelihood security (pp. 413-446). Springer, Dordrecht.

Han, S. M., Lee, G. G., & Park, K. K. (2012). Acute dermal toxicity study of bee venom (Apis mellifera L.) in rats. Toxicological research, 28, 99-102.

Hwang, D. S., Kim, S. K., & Bae, H. (2015). Therapeutic effects of bee venom on immunological and neurological diseases. Toxins, 7(7), 2413-2421.

Lee, K. S., Kim, B. Y., Yoon, H. J., Choi, Y. S., & Jin, B. R. (2016). Secapin, a bee venom peptide, exhibits anti-fibrinolytic, anti-elastolytic, and anti-microbial activities. Developmental & Comparative Immunology, 63, 27-35.

Martinello, M., & Mutinelli, F. (2021). Antioxidant activity in bee products: A review. Antioxidants, 10(1), 71.

Munteanu, I. G., & Apetrei, C. (2021). Analytical methods used in determining antioxidant activity: A review. International Journal of Molecular Sciences, 22(7), 3380.

Pinto, D., Vieira, E. F., Peixoto, A. F., Freire, C., Freitas, V., Costa, P., ... & Rodrigues, F. (2021). Optimizing the extraction of phenolic antioxidants from chestnut shells by subcritical water extraction using response surface methodology. Food Chemistry, 334, 127521.

Sobral, F., Sampaio, A., Falcão, S., Queiroz, M. J. R., Calhelha, R. C., Vilas-Boas, M., & Ferreira, I. C. (2016). Chemical characterization, antioxidant, anti-inflammatory and cytotoxic properties of bee venom

collected in Northeast Portugal. Food and Chemical Toxicology, 94, 172-177.

Somwongin, S., Chantawannakul, P., & Chaiyana, W. (2018). Antioxidant activity and irritation property of venoms from Apis species. Toxicon, 145, 32-39.

Şenel, E., & Demir, E. (2018). Bibliometric analysis of apitherapy in complementary medicine literature between 1980 and 2016. Complementary therapies in clinical practice, 31, 47-52.

Zhang, S., Liu, Y., Ye, Y., Wang, X. R., Lin, L. T., Xiao, L. Y., ... & Liu, C. Z. (2018). Bee venom therapy: Potential mechanisms and therapeutic applications. Toxicon, 148, 64-73.

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RESEARCH ARTICLE

Effect of Cryopreservation on DNA Damage and Various Sperm Parameters in the Post-Mortem Obtained Buffalo Sperm

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ABSTRACT

This study aimed to investigate the effects of freezing on DNA damage and some sperm parameters in epididymal sperm obtained from the buffalo testicles after slaughtering. Epididymal semen examination of 50 male Anatolian buffaloes (three years old or older) was performed after being obtained from slaughterhouses. The semen samples were divided into two groups: the control group (the fresh semen samples) and the research group (frozen-thawed semen samples). Sperm progressive motility (%), viability (%), abnormal sperm (%), and sperm DNA damage (%) were analyzed. Significant differences ($p<0.001$) between fresh and frozen semen on the progressive motility, viability, and mid-piece, tail, and total abnormality rates were found in the study. It was determined that the difference between the DNA damage values in fresh and thawed semen was significant ($p<0.0001$). Progressive motility rates of fresh and thawed semen were consistent with the studies conducted. It is thought that epididymal buffalo semen obtained post-mortem can be used in biotechnological methods. This research will contribute to further studies to increase the Anatolian buffalo population. More comprehensive studies should be performed to determine the impact of fertility due to the semen DNA damage and minimize the DNA damage in the freezing-thawing semen samples.

Keywords: Anatolian buffalo, DNA damage, epididymal, semen freezing, semen parameters

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Mandalardan Postmortem Elde Edilen Spermalarda Dondurmanın DNA Hasarı ve Bazı Spermatolojik Parametreler Üzerine Etkisi

ÖZ

Bu çalışmada, kesim sonrası manda testislerinden elde edilen epididimal spermada dondurma işleminin DNA hasarı ve bazı spermatolojik parametreler üzerindeki etkisinin araştırılması amaçlanmıştır. 50 adet erkek Anadolu mandasının (3 ve üzeri) testisleri mezbahanelerden kesim sonrası temin edildikten sonra elde edilen epididimal sperma progresif motilite (%), canlılık (%), anormal spermatozoa oranı (%) ve spermatozoon DNA hasarı (%) yönünden analiz edildi. Sperma örnekleri kontrol grubu (taze sperma örnekleri) ve araştırma grubu (dondurulmuş-çözünmüş sperma örnekleri) olmak üzere iki gruba ayrıldı. Taze ve dondurulmuş sperma arasında progresif motilite, canlılık, orta kısım, kuyruk ve toplam anormallik oranlarında önemli farklılıklar bulundu ($p<0,001$). Taze ve çözdürülmüş spermada arasındaki DNA hasarı sonuçlarının anlamlı derecede farklı olduğu belirlendi ($p<0,0001$). Taze ve çözdürülmüş spermadaki progresif motilite oranları yapılan çalışmalarla uyumluydu. Çalışma sonucunda elde edilen veriler ışığında postmortem olarak elde edilen epididimal manda spermasının biyoteknolojik yöntemlerde kullanılabileceği düşünülmektedir. Bu araştırma, Anadolu manda popülasyonunun artırılmasına yönelik daha sonraki çalışmalara katkı sağlayacaktır. Spermatozoonlarda dondurma işlemi sonucunda şekillenen DNA hasarının belirlenmesi ve en aza indirilmesi için daha kapsamlı çalışmalar yapılmalıdır.

Anahtar Kelimeler: Anadolu mandası, DNA hasarı, epididimal, sperma dondurma, sperma parametreleri

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INTRODUCTION

Anatolian (water) buffalo, including the northern part of Central Anatolia, still exists in Turkey. Although more common on the Black Sea coast, it is also found in Eastern Anatolia. Taxonomically, it is classified as a "*Mediterranean*" type; however, all buffaloes belong to the *'River'* species in Europe and the Near East countries, which have similar phenotypes but varying appearances (Borghese and Mazzi 2005).

Artificial insemination is one of the methods that can be used to increase the pregnancy and fertility rates in buffaloes. On this technique, one of the most important factors is the quality of the frozen semen (Saacke 1984). Buffalo semen has a low freezing capacity compared to cattle semen. It has been reported that the main reason for the difference between these two types of semen can be the difference in lipid ratios of plasma membrane (Tatham 2000).

When comparing cattle and buffalo semen during short-term storage at +4°C, it was shown that buffalo semen was more prone to oxidative stress due to the high lipid peroxidation dependent on the decreased activity of antioxidant enzymes (Nair et al. 2006).

Cooling-freezing-thawing processes lead to physical and chemical stresses, which cause a decrease in semen viability and fertilization capacity (Chatterjee et al. 2001). The most prominent stress is lipid peroxidation. It was shown that the effects of lipid peroxidation led to irreversible motility loss, respiratory inhibition, intracellular enzyme leakage, and sperm deoxyribonucleic acid (DNA) damage (White 1993).

Semen diluents are prepared for ejaculated semen samples; therefore, dilution of epididymal semen with commercial diluents and use of seminal plasma may result in motility loss. In the freezing process, semen samples are mixed with seminal plasma before dilution. Therefore, it may not be right to wash the epididymal semen samples with diluents before mixed with seminal plasma. In this case, adding a few drops of diluent is recommended. Seminal plasma can be added after this step and semen samples should be diluted only with semen diluents (Herold et al. 2004a).

The spermatozoon DNA integrity is vital for the embryo, fetus, normal offspring development, and fertilization success, which can be achieved using natural or assisted reproductive techniques (Morris et al. 2002).

It has been reported that storing frozen samples leads to DNA damage and abnormal embryo development, and infertility in humans, mice, fishes, and oysters. Semen with DNA damage negatively affects embryonic development and increases the genetic disease probability (Zini et al. 2001).

Comet test is used in somatic cells to determine the genotoxic damage and mainly single and double-strand breaks (Singh et al. 1988). This test is a gel electrophoresis method used to visualize and measure the DNA damage in cells using a microscope (Olive 2002). It is both a cheap and sensitive method known as reliable in detecting DNA damage (Morris et al. 2002). It has been shown that the Comet test is a more sensitive method compared to others, such as Sperm chromatin structure assay (SCSA) and Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay (Donnelly et al. 2000).

The purpose of this research was to investigate the effects of freezing on DNA damage and some sperm parameters in epididymal sperm obtained from buffalo testicles taken from slaughterhouses after slaughtering.

MATERIALS and METHODS

Epididymal semen examination of a total of 50 male *Anatolian buffaloes* (three years old or older) was performed after obtained from slaughterhouses.

Obtaining the Testicles

Testicles of buffaloes were cut immediately after the slaughter as Lambrechts et al. (1999) explained. The scrotum was incised with a knife and removed with epididymis by cutting the spermatic cord.

Obtaining Semen Samples and Processing of Semen

The cauda epididymis was incised with a sterile lancet. The sperm in the ductus epididymis was allowed to pull out. Semen was obtained by pulling out with a sterile syringe (Guerrero 2006). The semen samples were divided into two groups: the control group (the fresh semen samples) and the research group (frozen-thawed semen samples). Sperm examinations (progressive motility, viability, and head-acrosome, mid-piece, tail, total abnormalities) were performed for these groups (Tekin 1994). Furthermore, samples of both groups were examined for DNA damage. The control group-fresh semen samples semen samples were diluted with the commercial Bioxcell® extender (IMV Technologies, L"Aigle, France), and then sperm parameters and DNA damage were examined. Samples were frozen according to the instructions of the company to form the research group. Then, samples were stored at least for 24 hours in liquid nitrogen (-196°C). Samples were thawed in the water bath (at 37°C) for 25-30 seconds (Arriola and Foote 1987) and examined in terms of sperm parameters and DNA damage. Dead/live spermatozoa ratios were determined as percentages by staining method (2% Eosin). This process was performed with a slide, coverslip, and 2% Eosin dye adjusted to body temperature. One drop of semen was mixed with two drops of Eosin and a smear was drawn on a slide. The slides were left to dry for 15 seconds and 400 spermatozoa were counted under the microscope at 40X magnification, and the

number of spermatozoa receiving dye (dead cells) was determined as a percentage. The fluid fixation method was used to determine the abnormal spermatozoa ratio. The rate of head-acrosome, middle part, tail anomalies and total spermatozoa anomalies were determined as percent. The sperm was fixed in Hancock solution and a drop of this prepared solution was placed on the slide, the coverslip was covered, and a drop of immersion oil was dropped on the covered coverslip, and their morphology was determined by counting 400 spermatozoons at 100X magnification (Tekin 1994). Epididymal semen, diluted with Bioxcell® diluent, a commercial diluent, to a density of 120-150x10^6 sp/ml (Sansone et al., 2000), was manually drawn into 0.25 ml straws. It was equilibrated at +5°C for 4 hours in accordance with the recommended procedure. After equilibration, the straws were frozen by keeping them in liquid nitrogen vapor at an average temperature of −80°C to −120°C for approximately 20 minutes, and finally they were immersed in liquid nitrogen at -196°C and stored until thawing.

Sperm DNA Damage Detection
Spermatozoon DNA damage was detected by using a gel electrophoresis method named "Comet test" (Single Cell Gel Electrophoresis) (Olive 2002).

Washing Semen Samples
Semen samples were centrifuged two times with Mg^{+2} and Ca^{+2} free phosphate buffer solution (PBS) at 4°C (800 g for 10 minutes) and samples were diluted with PBS (20×10^6 sp/ml) after washing steps (Fraser and Strzezek 2004).

Slide Preparation and Gel Placement
Fully frosted slides were covered with 100µl of 0.75% normal melting point agarose, a coverslip added and the agarose allowed to solidify at room temperature for 5 minutes. The coverslip was removed and approximately 1×10^5 sperm cells in 5µl of PBS were mixed well with 75µl of 0.5% low melting point agarose was used to form the second layer. A coverslip was added again and stored at +4°C for solidification. Then, slides were ready after removing the coverslip (Singh et al. 2003).

Cell Lysis
The cold lysis solution (+4°C) (2.5 M NaCl, 100 mM Na_2 EDTA, 10 mM Tris, 1% Triton X-100, pH: 10) was prepared and slides were put in this solution for 1 hour at +4°C. Then, 40mM of DTT (Dithiothreitol) was added and samples were left for an additional 1 hour at +4°C. In the end, the lysis solution was mixed with Proteinase K (100 µg/ml) and slides were incubated in the solution at 37°C for 15 hours (Singh et al. 2003).

Electrophoresis
Following the lysis step, slides were incubated in freshly prepared and cooled (0-4°C) electrophoresis buffer (300 mM NaOH and 1 mM EDTA, pH: 12.5) for 20 minutes to ensure the separation of the DNA strands from each other. After the incubation, samples were run for 20 minutes in this buffer solution (300mA and 20V) (Singh et al. 2003).

Neutralization
After the electrophoresis step, slides were washed three times with Tris buffer (40 mM Tris HCl, pH: 7.4) to remove the detergents and to change the alkalinity (Singh et al. 2003).

Staining the Samples
Following the neutralization process, slides were stained with fluorescent ethidium bromide (5µg/ml) and DNA structures of samples were evaluated in four hours upon the staining (Hu et al. 2008).

Comet Image Analysis
Camera attachment fluorescence microscope (Olympus BX51) with an excitation wavelength of 580 nm and image analysis system (TriTek CometscoreTM version 1.5 software) were used to evaluate the Comet parameters from 100 DNA images for each sample (Xu et al. 2013).

Thawing of Frozen Semen
Straws were thawed in the water bath (37°C) for 25-30 seconds after 24 hours storage period in liquid nitrogen (-196°C) (Arriola and Foote 1987).

Statistical Analysis
The mean, standard deviation, and mean standard error values were calculated by using the SPSS statistical program (Version 21, IBM Crop., USA). Results were represented as mean±SEM. Fresh and thawed semen samples were examined in terms of sperm parameters and results were analyzed by using a paired t-test. Comet DNA analysis results were compared using Student's t-test.

RESULTS

In this study results showed that progressive motility, viability, and mid-piece, tail, total abnormalities (Tekin 1994) were significantly different between fresh and thawed semen samples ($p<0.001$). Only head-acrosome abnormality was not significantly different between fresh and thawed semen samples (Table 1).

In this study, the percentages of DNA damage in the Comet head and the Comet tail were significantly different in thawed semen samples than in fresh samples ($p<0.0001$) (Table 2).

Singh et al. (2003) described, the tail moment parameter quantified the amount of DNA damage, as it explained the distance the DNA had migrated and

the amount of DNA that had migrated from the head region. The results of this measurement can be seen in Figure 1.

Table 1. Spermatological examination of fresh and frozen semen samples

Semen parameters		Fresh Semen $\overline{X} \pm S_{\overline{X}}$	Frozen Semen $\overline{X} \pm S_{\overline{X}}$	P
Progressive motility (%)		50.10 ± 1.78[a]	27.50 ± 1.41[b]	p<0.001
Viability (%)		16.58 ± 0.48[a]	36.76 ± 0.95[b]	p<0.001
Abnormal spermatozoa (%)	Head-acrosome	3.38 ± 0.26	3.50 ± 0.23	p>0.05
	Mid-piece	15.00 ± 0.84[a]	22.10 ± 1.11[b]	p<0.001
	Tail	13.68 ± 1.10[a]	17.30 ± 1.07[b]	p<0.001
	Total	32.06 ± 1.62[a]	42.90 ± 1.64[b]	p<0.001

a, b: For each trait, means with the different letters in the same row were significantly different.

Table 2. Comet parameters of fresh and frozen semen

Comet Parameters	Fresh Semen $\overline{X} \pm S_{\overline{X}}$	Frozen Semen $\overline{X} \pm S_{\overline{X}}$	P
Comet Head (%)	89.44 ± 1.0[a]	32.47 ± 1.3[b]	p<0.001
Comet Tail (%)	10.58 ± 1.0[a]	67.29 ± 1.3[b]	p<0.001

a, b: For each trait, means with the different letters in the same row were significantly different.

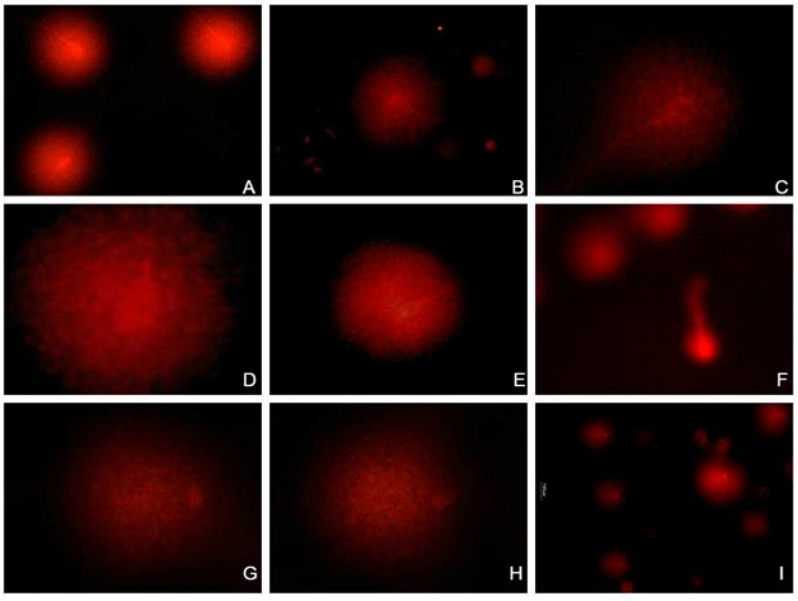

Figure 1: Comet images. (A, B, C: Comet images of the control group the spermatozoon. D, E, F: Spermatozoon with less DNA damage. G, H, I: Spermatozoon with DNA damage)

DISCUSSION

Many researchers have studied fresh Buffalo epididymal sperm parameters. In this research, the progressive motility rate of the fresh semen samples was higher than by Herold et al. (2004b; 2006) and lower than by Hiron et al. (2006). Morphology expresses the physical structure of spermatozoa, and during analysis, the different types of abnormalities in the sample are detected. Abnormalities are categorized according to head, midpiece and tail

abnormalities resulting from either environmental conditions or genetic factors. The ratio of abnormalities in a sample may be affected by the season, gender, frequency of ejaculation, and stress encountered before ejaculation. The sample's capability to be used for AI or IVF or to be used for cryopreservation can drastically impact a high percentage of abnormalities (Vale 1994). Various studies examined the viability and abnormal spermatozoa parameters in fresh epididymal buffalo semen samples. According to this research's results, viability was compatible with the results of studies performed by Kumar et al. (2008) and Singh et al. (2007). On the other hand, the results related to viability were lower compared to the rates detected in the research of Lambrechts et al. (1999). The rates of abnormal spermatozoa were lower than the values in the literature mentioned above.

In this research, the spermatozoa motility rate of frozen semen samples was higher and partially compatible with the rate detected by Herold et al. (2004b). However, it was higher than Herold et al. (2006) and lower than Hiron et al. (2006). The viability and abnormal spermatozoa rates of frozen buffalo semen samples were examined in this research. According to the results, viability was compatible with the results of studies performed by Kumar et al. (2008) and Hiron et al. (2006). On the other hand, the viability results were lower than Herold et al., (2006). The rates of abnormal spermatozoa were lower than the values in the literature mentioned above.

Factors such as breed, age, methods of animal care, nutrition conditions, geographical position of the region in which the research is conducted, and the climate are the reasons for this research's different results related to the sperm parameters. It was detected that the differences between the fresh and the thawed semen samples in terms of the progressive motility, viability, and mid-piece, tail, total abnormality rates ($p<0.001$). Only head-acrosome abnormality rates were not significantly different between fresh and thawed semen samples.

Abnormalities in the male genome are the potential reason for post-fertilization failures and problems (Sakkas et al. 2002; Sergerie et al. 2005). Saleh et al. (2002) showed that DNA damage in the spermatozoon negatively affected fertility. It is believed that the oxidative damage in the spermatozoon DNA leads to problems in the genetic material transfer, infertility, congenital disabilities, genetic diseases, and cancer development in infants by increasing the mutations and abnormalities (Ames et al. 1994; Cummins et al. 1994). Various studies determined that freezing and thawing of the buffalo semen samples prominently increased the DNA damage (Fraser and Strzezek 2004, El-Kon and Darwish 2011). DNA damage results of fresh semen are compatible with the results of Kumar et al. (2011) whereas the results of frozen semen were higher compared to the results of El-Kon and Darwish (2011) and Kumar et al. (2011). The reason for the higher DNA damage rates detected in this research can be that the alkaline Comet assay was used, which detected various damages such as base damages, single-strand breaks, and crosslinks. Furthermore, DNA damage rates of thawed semen samples were also higher than other studies and the reason can be the use of the epididymal semen samples. The diluents used in this research are not prepared for the epididymal semen samples and the lack of antioxidants can be the reason for these higher rates of DNA damage.

Cooling-freezing-thawing processes lead to physical and chemical stresses on the spermatozoon membrane, thus decreasing fertilization ability (Chatterjee et al. 2001). Furthermore, it is commonly accepted that these steps cause a more than 50% decrease in spermatozoon vitality (Watson 1979). Buffalo semen is richer in polyunsaturated fatty acids than bull semen (Nair et al. 2006). The damage is comparatively higher in buffalo sperm during freezing and thawing cycles, and buffalo semen has lower motility and pregnancy rates. Therefore, buffalo semen samples are more prone to oxidative damage. These risks can be minimized by optimizing the cooling and freezing rates and using appropriate diluents for the frozen spermatozoa (Kumar et al., 1992).

Assisted reproductive techniques can be used to minimize the errors during the manipulations and freezing-thawing steps, determine new strategies, take precautions on *in vivo* and *in vitro* conditions, and stabilize the possible DNA damage in the spermatozoon DNA (Türk et al. 2006).

CONCLUSION

Consequently, progressive motility rates of fresh and thawed semen samples are in line with other studies and it is possible to use the postmortem epididymal buffalo semen samples by using biotechnological techniques. Furthermore, in case a male buffalo with a high fertility rate dies because of any reason, epididymal semen samples can be obtained, and its genetic material can be used with the assisted reproductive techniques. This research concluded that the semen freezing-thawing process leads to prominent damage and problems in buffalo epididymal semen samples as it is commonly observed in other animal races. This research reports the spermatozoon DNA damage levels during the freezing-thawing process, which can negatively affect the fertility rates in buffaloes, and it is believed that our results will be efficiently used to increase the buffalo population in Turkey. More comprehensive studies should be performed to determine the impact of fertility due to the semen DNA damage and minimize the DNA damage in the freezing-thawing semen samples.

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

Authors' Contributions: The authors declare that they contributed equally to the article.

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

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REFERENCES

Ames, B. N., Motchnik, P. A., Fraga, C. G., Shigenaga, M. K., & Hagen, T. M. (1994). Antioxidants prevention of birth defects and cancer. In: Male-mediated development toxicology, Reproductive Biology. Springer, Boston. 243-259.

Arriola, J. R., & Foote, H. (1987). Glycerolation and thawing effects on bull spermatozoa frozen in detergent-treated egg yolk and whole egg extenders. J Dairy Sci. 70: 1664-1670.

Borghese, A., & Mazzi, M. (2005). Buffalo population and strategies in the world. In: Buffalo production and research, Reu Technical Series 67. 1-39.

Chatterjee, S., De Lamirande, E., & Gagnon, C. (2001). Cryopreservation alters membrane sulfhydryl status of bull spermatozoa: protection by oxidized glutathione. Mol Reprod Dev. 60: 498–506.

Cummins, J. M., Jequier, A. M., & Kan, R. (1994). Molecular biology of human male infertility: Linked with aging, mitochondrial genetics, and oxidative stress? Mol Reprod Dev. 37: 345-362.

Donnelly, E. T., O'connell, M., Mcclure, N., & Lewis, S. E. (2000). Differences in nuclear DNA fragmentation and mitochondrial integrity of semen and prepared human spermatozoa. *Hum Reprod.* 15(7): 1552-1561.

El-Kon, I., & Darwish, A. S. (2011). Effect of glutathione (GSH) on microscopic parameters, DNA integrity in Egyptian buffalo semen during liquid and frozen storage. J Reprod Infertil. 2(3): 32-40.

Fraser, L., & Strzezek, J. (2004). The use of comet assay to assess DNA integrity of boar spermatozoa following liquid preservation at 5°C and 16°C. Folia Histochem Cytobiol. 42: 49-55.

Guerrero, C. A. (2006). Cryopreservation and intracytoplasmic sperm injection with bovine epididymal spermatozoa. PhD Thesis, Louisiana State University, Louisiana, USA. 216.

Herold, F. C., Aurich, L. E., & Gerber, D. (2004a). Epididymal sperm from the African buffalo (*Syncerus Caffer*) can be frozen successfully with Andromed® and with Triladyl™ but the addition of bovine seminal plasma is detrimental. Theriogenology. 61: 715–724.

Herold, F. C., De Haas, K., Colenbrander, B., & Gerber, D. (2006). Comparison of equilibration times when freezing epididymal sperm from African buffalo (*Syncerus Caffer*) using Triladyl™ or Andromed®. Theriogenology. 66: 1123–1130.

Herold, F. C., De Haas, K., Cooper, D., Colenbrander, B., Nöthling, J. O., Theunisen, W., Spillings, B., & Gerber, D. (2004b). Comparison of three different media for freezing of epididymal sperm from the African buffalo (*Syncerus Caffer*) and influence of equilibration time on the post-thaw sperm quality. Onderstepoort J Vet Res. 71: 203–210.

Hiron, M. H., Singh, L. P., Arangasamy, A., Ansari, M. R., & Kumar, S. (2006). Effect of buffalo seminal plasma heparin binding protein (HBP) on freezability and in vitro fertility of buffalo cauda spermatozoa. Anim Reprod Sci. 93: 124–133.

Hu, J. H , Li, Q. W., Jiang, Z. J., & Li, W. Y. (2008). Effects of different extenders on DNA integrity of boar spermatozoa following freezing–thawing. Cryobiology. 57: 257–262.

Kumar, A., Singh, L. P., Hiron, M. H., & Majumdar, A. C. (2008). Seminal plasma non-heparin binding proteins (NHBP) reduce the cryoinjury to buffalo cauda epididymal spermatozoa induced by heparin binding proteins (HBP). Anim Reprod Sci. 104: 220–226.

Kumar, R., Mohanarao, G. J., Arvind, S., & Atreja, K. (2011). Freeze–thaw induced genotoxicity in buffalo (*Bubalus Bubalis*) spermatozoa in relation to total antioxidant status. Mol Biol Rep. 38: 1499–1506.

Kumar, S., Sahni, K. L., & Mohan, G. (1992). Effect of different levels of glycerol and yolk on freezing and storage of buffalo semen in milk, tris and sodium citrate buffers. Buffalo J. 8: 151–156.

Lambrechts, H., Van Niekerk, F. E., Coetzer, W. A., Cloete, S. W. P., & Van Der Horst, G. (1999). The effect of cryopreservation on the survivability, viability and motility of epididymal African buffalo (*Syncerus Caffer*) spermatozoa. Theriogenology. 52: 1241-1249.

Morris, I. D., Ilott, S., Dixon, L., & Brison, D. R. (2002). The spectrum of DNA damage in human sperm assessed by single cell gel electrophoresis (comet assay) and its relationship to fertilization and embryo development. Hum Reprod. 17(4): 990- 998.

Nair, S. J., Brar, A. S., Ahuja, C. S., Sangha, S. P. S., & Chaudhary, K. C. (2006). A comparative study on lipid peroxidation, activities of antioxidant enzymes and viability of cattle and buffalo bull spermatozoa during storage at refrigeration temperature. Anim Reprod Sci. 96: 21-29.

Olive, P. L. (2002). The comet assay: An overview of techniques. In: In situ detection of DNA damage. Methods and protocols. Methods And Molecular Biology, Humana Press Inc. Totowa. 179-194.

Sansone, G., Nastri, M.J.F., Fabbrocini, A. (2000). Storage of buffalo (Bubalus Bubalis) semen. Anim Reprod Sci. 62: 55–76.

Saacke, R. G. (1984). Semen quality: Importance of and influencing factors. In: Proceedings of 10th National Association of Animal Breeders, Artificial Insemination and Reproduction. Columbia. 12-14.

Sakkas, D., Moffatt, O., Manicardi, G. C., Mariethoz, E., Tarozzi, N., & Bizzaro, D. (2002). Nature of DNA damage in ejaculated human spermatozoa and possible involvement of apoptosis. Biol Reprod. 66: 1061–1070.

Saleh, R. A., Agarwal, A., Nada, E. A., El-Tonsy, M. H., Evenson, D. P., & Larson, K. (2002). Negative effects of sperm nuclear DNA damage on the fertility potential of couples with idiopathic and male-factor infertility. Fertil Steril. 78: S61.

Sergerie, M., Laforest, G., Bujan, L., Bissonnette, F., & Bleau, G. (2005). Sperm DNA fragmentation: Threshold Value in Male Fertility. Hum Reprod. 20: 3446–3451.

Singh, L. P., Hiron, M. R., & Ansari, M. R. (2007). Effect of egg yolk and seminal plasma heparin binding protein interaction on the freezability of buffalo cauda epididymal spermatozoa. Anim Reprod Sci. 99: 395–400.

Singh, N. P., Mccoy, M. T., Tice, R. R., & Schneider, E. L. (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res. 175(1): 184-191.

Singh, N. P., Muller, C. H., & Berger, R. E. (2003). Effects of age on DNA double-strand breaks and apoptosis in human sperm. Fertil Steril. 80(6): 1420-1430.

Tatham, B. (2000). Increasing buffalo production: using reproduction technology: Report for the rural industries research and development corporation, Rural Industries Research & Development Corporation. Australia. 32.

Tekin, N. (1994). Examination and evaluation of sperm. In: Artificial insemination, birth and infertility in domestic animals. Ankara. 69-79.

Türk, G., Aksu, E. H., & Bozkurt, T. (2006). DNA damage of sperm. Fırat Uni Vet J Health Sci. 20(1): 85-95.

Vale, W. G. (1994). Collection, processing and deep freezing of buffalo semen. Buffalo J. 2: 65-72.

Watson, P. F. (1979). The preservation of semen in mammals. In: Oxford reviews of reproductive biology. 1st ed. Oxford. 283–350.

White, I. G. (1993). Lipids and calcium uptake of sperm in relation to cold shock and preservation. Reprod Fertil Dev. 5: 639–658.

Xu, X. R., Zhu, J. Q., Ye, T., Wang, C. L., Zhu, Y. F., Dahms, H. U., Jin, F., & Yang, W. X. (2013). Improvement of single-cell gel electrophoresis (SCGE) alkaline comet assay. Aquat Biol. 18: 293–295.

Zini, A., Bielecki, R., Phang, D., & Zenzes, M. T. (2001). Correlations between two markers of sperm DNA integrity, DNA denaturation and DNA fragmentation, in fertile and infertile men. Fertil Steril. 75: 674-677.

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RESEARCH ARTICLE

Effects of Insulin Lispro on Ram Semen During Cryopreservation

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ABSTRACT

In the present study, it was aimed to evaluate the effects of semen extender supplemented with insulin on ram semen at the post-thaw stage. Semen was collected from 5 Tuj rams five times with an electro-ejaculator. Experimental groups were designated as control group and groups containing 15 IU and 20 IU of insulin lispro supplemented semen extenders. At the post-thaw stage, motility and plasma membrane integrity were evaluated by microscopy. Acrosome integrity (Fitc-Peanut Agglutinin) and mitochondrial membrane potential (Rhodamine 123) were assessed by dual staining with propidium iodide (PI) using flow cytometry. It was found that motility and plasma membrane functional integrity were better preserved in the experimental groups than the control group ($p<0.05$). Acrosome integrity results were similar between the control and 15 IU insulin groups ($p>0.05$), but acrosome integrity was negatively affected in the 20 IU insulin group ($p<0.05$). Compared to the control, mitochondrial membrane potential was found to be higher in the group containing 20 IU insulin ($p<0.05$). As a result, it was thought that energy metabolism was stimulated in ram semen frozen with insulin-supplemented extenders, and the semen was better preserved than the control group.

Keywords: Cryopreservation, insulin, ram semen, tuj

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Insülin Lispro'nun Kriyoprezervasyon Sırasında Koç Sperması Üzerine Etkileri

ÖZ

Sunulan çalışmada, insülin ilave edilen sperma sulandırıcısının koç sperması üzerindeki etkilerininin çözdürme sonrası aşamada değerlendirilmesi amaçlanmıştır. Tuj ırkı 5 adet koçtan beş kez elektro-ejakülatör ile sperma alındı. Deneme grupları, kontrol grubu, 15 IU ve 20 IU insulin lispro ilave edilen sperma sulandırıcıları ile oluşturuldu. Çözdürme sonrası, motilite ve plazma membran bütünlüğü mikroskopi ile değerlendirildi. Akrozom bütünlüğü (Fitc-Peanut Agglutinin) ve mitokondrial membran potansiyeli (Rhodamine 123) akış sitometrisi kullanılarak, propidium iodide (PI) ile ikili boyama yapılarak değerlendirildi. Motilite ve plazma membran fonksiyonel bütünlüğünün, araştırma gruplarında, kontrol grubuna göre daha iyi korunduğu bulundu ($p<0,05$). Akrozom bütünlüğü sonuçlarının kontrol ve 15 IU insülin grupları arasında benzer olduğu ($p>0.05$), ancak 20 IU insulin grubunda akrozom bütünlüğünün olumsuz etkilediği bulundu ($p<0,05$). Mitokondrial membran potansiyeli, 20 IU insulin içeren grupta, kontrol grubuna göre daha yüksek bulundu ($p<0,05$). Sonuç olarak, insulin ilave edilmiş sulandırıcılar ile dondurulan koç spermasında enerji metabolizmasının uyarıldığı ve spermanın kontrol grubuna göre daha iyi korunduğu düşünülmüştür.

Anahtar kelimeler: İnsülin, koç sperması, kriyoprezervasyon, tuj

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INTRODUCTION

Beta cells located in the Islets of Langerhans within the pancreas are responsible for the production of insulin. The hormone insulin, comprises 51 amino acids and possessing a double-chain polypeptide structure, was first isolated by Banting and Best in 1922 (Karamitsos 2011, Koyunlu 2023). The hormone insulin, which plays a crucial role in energy metabolism, also has a role in the regulation of reproductive functions (Dias et al. 2014). The disease known as diabetes mellitus, which is characterized by elevated levels of blood glucose, has been found to have a negative impact on the reproductive functions of males (Dias et al. 2014, Lunze et al. 2013). The negative impact of spermatological parameters, such as decreased motility and morphological disorders in diabetes mellitus can lead to a reduction in male fertility (Dias et al. 2014).

Insulin has been added into semen extenders for ram, human, and pig semen due to its potential involvement in the energy metabolism of spermatozoa. This inclusion is believed to enhance motility and reduce observed morphological disorders (Aquilla et al. 2013, Lampiao and du Plessis 2008, van Tilburg et al. 2021). There is an investigation conducted on the semen of rams focused on the assessment of short term semen preservation (van Tilburg et al. 2021), while the impact of such preservation on the subsequent freezing-thawing procedures was not taken into account. Insulin lispro, an insulin analogue, has a rapid onset of action and a shorter duration compared to endogenous insulin and is administered to humans by subcutaneous injection. According to reports, the peak level is achieved between 30 and 90 minutes after subcutaneous injection, and the duration of the action ends in under 5 hours (Noble et al. 1998). Furthermore, there have been reports indicating that it exhibits comparable sensitivity to insulin receptors, similar to the effects of regular insulin (Holleman and Hoekstra 1997). Insulin lispro was chosen as the optimal treatment strategy in the study due to the fact that the duration between semen dilution and freezing was much less than 5 hours. The current investigation aims to examine the impact of insulin lispro-supplemented semen extender on the reconstitution and cryopreservation of ram spermatozoa.

MATERIAL and METHODS

All issues associated to the experimental setups and evaluation techniques have been approved by The Scientific Ethical Committee at the Kafkas University in Kars, Türkiye (2021–196). Five different Tuj rams of the 3-5 years old were used and maintained at Prof. Dr. Ali Riza Aksoy Education, Research and Application Farm, Kafkas University, Faculty of Veterinary Medicine in Kars, Türkiye.

Chemicals

Except where otherwise noted, all of the chemicals used in the study were purchased from Merck and Sigma.

Experimental Design

The research was designed to include three distinct groups, namely those that received 15 IU and 20 IU of insulin lispro (Humalog, Lilly, Italy) in the extenders, and the control group without insulin lispro. Insulin doses of 15 IU (I-15) and 20 IU (I-20) were administered on a 3 mL of extenders. The extender was composed of 223.7 mM Tris, 55.5 mM fructose, 66.6 mM citric acid, 100.4 mM Trehalose, 4.03 mM EDTA, 4 g/l penicillin G, 3 g/L dihydrostreptomycin, and 20% egg yolk (v/v) in distilled water.

The process of semen collection was conducted five times, with a frequency of every other day, utilizing an electro-ejaculator. Following the collection process, the ejaculates were subsequently transferred to a water bath maintained at a temperature of 37°C. Using a heated (37°C) slide, a phase-contrast microscope (Nikon Eclipse-E400, Tokyo, Japan) was used to assess the rapid wave motion and motility. Semen samples were pooled and exhibiting a motility rate exceeding 70% and containing over 1.5x109 spermatozoon/ml were selected for the purpose of cryopreservation.

The dilution process involved reducing the concentration of each group to 25x106 sperm/ml using a corresponding extender. The groups were subsequently brought down to a temperature of 5°C within a time frame of one hour. After undergoing the cooling process, the sperm samples were allowed to reach equilibrium for a duration of two hours at a temperature of 5°C. The techniques employed for cryopreservation, thawing, and incubation were executed in accordance with the methodology outlined by Yildiz et al. (2015).

Semen Analysis

The subjective evaluation of sperm motility was conducted using a 400x phase-contrast microscope, with a slide warmed to 37°C. The functional integrity of the plasma membrane was assessed through the hypoosmotic swelling test (HOST), as per the method described by Alcay et al. (2016).

Flow Cytometric Analysis

The Attune NxT Acoustic Focusing Cytometer (Invitrogen, USA) was used to conduct flow cytometric analysis. The fluorescence was assessed using a 480 nm excitation wavelength with a 10 nm excitation bandwidth. The emission was filtered using 530/30 nm filter (BL-1) and 695/40 nm filter (BL-3) and connected to Attune NxT software v2.7 (Thermo

Fisher). Upon gating the cell population based on forward and side scatter light signals, the mean fluorescence intensity of the analyzed sperm cells was quantified. The assay contained a quantity of 10,000 sperm cells.

The evaluation of acrosome integrity was conducted using the dual-staining technique of fluorescein isothiocyanate-conjugated peanut agglutinin (PNA) and propidium iodide (PI). The evaluation of mitochondrial membrane potential was carried out using Rhodamine 123/PI. The flow cytometric analysis was conducted following the methodology outlined by Gürler et al. (2016).

Statistical Analysis

Statistical analysis was conducted using IBM SPSS version 28. The normality of the data was evaluated using the Shapiro-Wilk test. The presented information was displayed in the form of mean values accompanied by their corresponding standard errors. The statistical significance of inter-group differences was assessed through the utilization of one-way ANOVA, followed by Tukey's post-hoc test. The statistical analysis employed to investigate data with a non-normal distribution was the Kruskal-Wallis test. Statistical significance was determined for P values that were less than 0.05.

RESULTS

Table 1 provides an extensive overview of motility, plasma membrane functional integrity, acrosome integrity and mitochondrial membrane potential concentration.

Post cryopreservation, the motility percentages were recorded as 44.44%, 50.56%, and 48.33% in the control, I-15, and I-20 groups, respectively. The preservation of motility was observed to be significantly better in groups that were administered with insulin ($P<0.05$).

HOST results of the study were found as 65.00% in control group, 73.22% in I-15 and 70.44% in I-20. The study revealed that semen dilution that included insulin exhibited a superior ability to maintain the integrity of the plasma membrane in comparison to the control group (C) ($P<0.05$).

The study found that there was no significant difference in the total acrosome integrity (C: 79.12%; I-15: 80.91) and the acrosome integrity with PI staining (C: 53.90%; I-15: 53.91%) ratio between the control group and the I-15 group ($P>0.05$). However, the I-20 group had significantly lower acrosome integrity (I-20: 74.67% in PNA and 46.53% in PNA/PI) compared to the other groups ($P<0.05$). The results of the analysis indicate that the mitochondrial membrane potential was found to be significantly higher in I-20 compared to control group (C: 74.08%; I-20: 79.35%) ($P<0.05$). Mitochondrial membrane potential results in Rhodamine 123/PI were found to be similar in all groups ($P>0.05$).

Table 1. Effect of insulin on spermatological parameters

Measurements	Motility (%)	HOST (%)	A (%)	A-P (%)	M (%)	M-P (%)
Groups						
Control	44,44±1,30^{a}	65,00±1,09^{a}	79,12±0,92^{a}	53,90±1,90^{a}	74,08±1,42^{a}	37.84±1.64
I-15	50,56±1,00^{b}	73,22±1,05^{b}	80,91±0,64^{a}	53,91±1,53^{a}	78,41±1,17^{ab}	38.21±2.25
I-20	48,33±0,83^{b}	70,44±0,88^{b}	74,67±1,21^{b}	46,53±1,20^{b}	79,35±1,44^{b}	38.44±1.79

^{a,b}: Values with different superscripts in the same column for each times are significantly different ($P < 0.05$). HOST: Plasma Membrane Functional Integrity, A: Total Acrosome Integrity, A-P: Acrosome integrity with Intact Plasma Membrane, M: Total Mitochondrial Membrane Potential, M-P: Mitochondrial Membrane Potential with Intact Plasma Membrane.

DISCUSSION

The freezing of gamete cells has a number of detrimental impacts on cells. In order to lessen negative effects like reduced viability and motility in semen as well as morphological problems, experiments have been conducted on adding antioxidant substances or other sources to semen extenders (Alcay et al. 2016, Alcay et al. 2021, Alcay et al. 2022). Insulin has a key role in spermatogenesis and spermatozoa's metabolic activity (Aitken et al. 2021, Bruning et al. 2000). Low insulin levels in the blood plasma have a negative impact on spermatological parameters in bulls (Weerakoon et al. 2020). The present study aimed to investigate the effects of insulin supplementation on ram semen extenders after cryopreservation, given the crucial role of insulin in reproductive performance. The assessment of motility is an important component in numerous research endeavors, as the forward movement of spermatozoa within the female reproductive tract is a critical factor in achieving successful fertilization. Studies have reported a positive impact on motility when substances involved in energy metabolism are added into sperm extenders (Aquilla et al. 2013, Lampiao and du Plessis 2008,

Onder et al. 2022). The findings of our research, in conjunction with other studies, demonstrated a significant enhancement in the motility of ram sperm. The findings of van Tilburg et al.'s (2021) study, which involved the addition of insulin to the ram's semen extender during short-term storage, align with the results of our own investigation, as we also observed a positive impact on the motility.

The process of freezing sperm has been found to result in damage to the membrane structure of the spermatozoa, ultimately leading to a decrease in the efficacy of the freezing process. The researchers assess the integrity of the membrane subsequent to the process of freezing and thawing, as the adverse impacts on the structure of the membrane can lead to a reduction in potential fertility (Onder et al. 2022, Vazquez et al. 1997). Based on the results of the HOS test, our study revealed that the insulin-containing groups exhibited a higher rate of membrane integrity. The study conducted by van Tilburg et al. (2021), reported the absence of any noticeable variation in the integrity of the plasma membrane. The dissimilarity noted between the current investigation and the aforementioned study could potentially be attributed to the administration of elevated insulin dosages in our study.

According to reports, the inclusion of insulin in extenders has been found to induce the acrosome reaction in spermatozoa (Lampiao and du Plessis 2008, Silvestroni et al. 1992). The findings of our investigation indicate that the reduction in overall acrosome integrity and acrosome integrity in viable cells at elevated insulin levels aligns with the existing knowledge on this subject. Another study's finding indicated that the addition of insulin during the short-term storage of ram semen did not result in any significant alteration in acrosome integrity (van Tilburg et al. 2021). The researchers in the previously mentioned study have indicated that there may be a correlation between dosage and the stimulation of the acrosome reaction. Specifically, high doses may potentially stimulate this reaction. The results obtained in our research validate the notion suggested by the preceding study.

For the sustenance of spermatozoa motility, it is essential that they maintain their energy production through either the glycolytic pathway or oxidative phosphorylation. The process of cryopreservation induces cellular damage, leading to a reduction in energy generation and a consequent decline in motility. The assessment of energy production and the efficacy of the freezing process involves the consideration of mitochondrial membrane potential as a significant parameter (Alamo et al. 2020, Wang et al., 2003). Upon examination of the findings from our study, it is apparent that the utilization of insulin in elevated quantities results in an increase in the potential of the mitochondrial membrane (in I-20) compared to the control group. Also, the data related to the insulin group administered at a dosage of 15 IU exhibited a numerical escalation. These findings are consistent with the previously mentioned information. The augmentation in motility has been verified to be attributed to the favorable impact on energy metabolism. The study carried out by Onder et al. (2022), used alpha lipoic acid, a compound involved in energy metabolism, and revealed a beneficial impact on spermatological parameters during the process of cryopreservation. Furthermore, a noteworthy reduction in these parameters was observed in comparison to the control group after a 6-hour incubation period. The study's authors posited that the observed phenomenon could potentially be attributed to heightened levels of reactive oxygen species resulting from elevated energy metabolism. The authors posit that it is crucial to consider the prolonged utilization of raising insulin dosages.

CONCLUSION

In conclusion, the administration of insulin lispro at adequate dosages to ram semen extenders was seen to have favorable outcomes in terms of motility and plasma membrane integrity during cryopreservation. The administration of higher doses of insulin lispro resulted in a statistically significant rise in mitochondrial membrane potential. However, this increase had a detrimental impact on the integrity of the acrosome.

Conflict of Interest: The authors declare that they have no conflict of interest.

Author Contribution: NTO designed study and wrote the manuscript. NTO, TG, SY and YO performed spermatological analysis. NTO performed the statistical analysis.

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REFERENCES

Aitken RJ, Curry BJ, Shokri S, Pujianto DA, Gavriliouk D, Gibb Z, Whiting S, Connaughton HS, Nixon B, Salamonsen LA, Baker MA. Evidence that extrapancreatic insulin production is involved in the mediation of sperm survival. Mol Cell Endocrinol. 2021; 526: 111193.

Alamo A, De Luc C, Mongioì LM, Barbagallo F, Cannarella R, La Vignera S, Calogero AE, Condorelli RA. Mitochondrial membrane potential predicts 4-hour sperm motility. Biomedicines. 2020; 8 (7): 196.

Alcay S, Aktar A, Koca D, Kilic MA, Akkasoglu M, Sagirkaya H. Positive effect of autologous platelet rich plasma on Saanen buck semen cryopreservation in non-breeding season. Cryobiology. 2021; 103: 45-48.

Alçay S, Cakmak S, Cakmak I, Aktar A, Ustuner B, Yilmaz M, Duman M, Ozkan H, Akkaya Y, Sağırkaya H, Nur, Z. Honey bee drone (Apis mellifera) sperm cryopreservation with rainbow trout seminal plasma supplemented extenders. J Hellenic Vet Med Soc. 2022; 73 (1): 3751-3756.

Alcay S, Gokce E, Toker MB, Onder NT, Ustuner B, Uzabacı E, Gul Z, Cavus S. Freeze-dried egg yolk based extenders containing various antioxidants improve post-thawing quality and incubation resilience of goat spermatozoa. Cryobiology. 2016; 72 (3): 269-273.

Aquila S, Giordano F, Guido C, Rago V, Carpino A. Insulin affects sperm capacity in pig through nitric oxide. Asian J Androl. 2013; 15 (6): 835-837.

Bruning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, Klein R, Krone W, Müller-Wieland D, Kahn CR. Role of brain insulin receptor in control of body weight and reproduction. Science. 2000; 289 (5487): 2122-2125.

Dias TR, Alves MG, Silva BM, Oliveira PF. Sperm glucose transport and metabolism in diabetic individuals. Mol Cell Endocrinol. 2014; 396 (1-2): 37-45.

Gürler H, Malama E, Heppelmann M, Calisici O, Leiding C, Kastelic JP, Bollwein H. Effects of cryopreservation on sperm viability, synthesis of reactive oxygen species, and DNA damage of bovine sperm. Theriogenology. 2016; 86 (2): 562-571.

Holleman F, Hoekstra JB. Insulin lispro. N Engl J Med, 1997; 337 (3): 176-183.

Karamitsos DT. The story of insulin discovery. Diabetes Res Clin Pract. 2011; 93: 2-8.

Koyunlu A. Spor ve Egzersiz Metabolizmasına Güncel Bakış, In: İnsülin Metabolizması ve Egzersiz İlişkisi, Ed; Pancar Z, 1st Ed., İstanbul, 2023; pp. 73-89.

Lampiao F, Du Plessis SS. Insulin and leptin enhance human sperm motility, acrosome reaction and nitric oxide production. Asian J Androl. 2008; 10 (5): 799-807.

Lunze K, Singh T, Walter M, Brendel MD, Leonhardt S. Blood glucose control algorithms for type 1 diabetic patients: A methodological review. Biomed Signal Process Control. 2013; 8 (2): 107-119.

Noble SL, Johnston E, Walton B. Insulin lispro: a fast-acting insulin analog. American Family Physician, 1998; 57 (2): 279-286.

Onder NT, Alcay S, Nur Z. Effects of alpha-lipoic acid on ram semen cryopreservation and post-thaw life span. Andrologia. 2022; 54 (1): e14249.

Silvestroni L, Modesti A, Sartori C. Insulin-sperm interaction: effects on plasma membrane and binding to acrosome. Arch Androl. 1992; 28 (3): 201-211.

van Tilburg MF, de Oliveira RV, Gadelha CRF, Fagundes B, Dias AJB, Quirino CR, Silva JSF. Effects of insulin on sperm cell quality in ram semen cooled at 5° C. Semina Cien Agrar, 2021; 42 (6): 3803-3812.

Vazquez JM, Martinez EA, Martinez P, Garcia-Artiga C, Roca J. Hypoosmotic swelling of boar spermatozoa compared to other methods for analysing the sperm membrane. Theriogenology. 1997; 47 (4): 913-922.

Wang X, Sharma RK, Gupta A, George V, Thomas Jr AJ, Falcone T, Agarwal A. Alterations in mitochondria membrane potential and oxidative stress in infertile men: a prospective observational study. Fertil Steril. 2003; 80: 844-850.

Weerakoon, W. W. P. N., Sakase, M., Kohama, N., & Kawate, N. Plasma estradiol-17β, cortisol, and insulin concentrations and serum biochemical parameters surrounding puberty in Japanese Black beef bulls with normal and abnormal semen. Theriogenology. 2020; 148: 18-26.

Yildiz S, Öztürkler Y, Ari UC, Lehimcioglu NC, Atakisi E, Kulaksiz R. The effects of l-ergothioneine, n-acetylcystein and cystein on freezing of ram semen. Kafkas Univ Vet Fak Derg. 2015; 21 (1): 81-86.

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RESEARCH ARTICLE

The Evaluation of Fertility Achieved by Modified Different Ovsynch Treatments in Holstein Cows

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ABSTRACT

The aim of this study was to evaluate the effect of modified preovsynch and ovsynch synchronization methods on fertility in dairy cows. A total of 60 Holstein dairy cows were used in the study. Dairy cows were divided into two groups Group I (n = 30) modified preovsynch, and Group II (n = 30) modified ovsynch. In the study, the amount of cervical mucus was recorded from the last GnRH injection until artificial insemination for monitoring of oestrus. Pregnancy examinations were performed by ultrasonography on the 40th day after artificial insemination. At the end of the study, pregnancy rates obtained from modified preovsynch and modified ovsynch were 63.33±0.08% and 56.66±0.09%, respectively. Cervical mucus in 16 dairy cows of modified presynch was light whereas 14 pregnant cows of modified preovsynch had normal cervical mucus. A total of 23 dairy cows in the modified ovsynch had light cervical mucus, while 4 dairy cows had normal cervical mucus. Furthermore, while cervical mucus was intensive in one dairy cow, two dairy cows did not have cervical mucus. In conclusion, the pregnancy results obtained from the modified preovsynch and ovsynch protocols were similar to each other, but many studies conducted in herds with a high number of dairy cows are required to determine the effectiveness of the modified protocols.

Keywords: Dairy cow, fertility, ovsynch, preovsynch

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Holştayn İneklerde Modifiye Farklı Ovsynch Uygulamaları İle Elde Edilen Dölveriminin Değerlendirilmesi

ÖZ

Yapılan bu çalışmanın amacı süt ineklerinde modifiye preovsynch ve ovsynch senkronizasyon yöntemlerinin dölverimi üzerine etkisinin değerlendirilmesidir. Çalışmada Holştayn ırkı toplam 60 baş süt ineği kullanılmıştır. Süt inekleri I. Grup (n=30) modifiye preovsynch, II. Grup (n=30) modifiye ovsynch olmak üzere iki gruba ayrılmıştır. Çalışmada östrus takibi için son GnRH enjeksiyonundan suni tohumlama yapılana kadarki sürede çara miktarı kaydedildi. Suni tohumlama sonrası 40. günde ultrasonografi ile gebelik muayeneleri yapıldı. Araştırma sonunda modifiye preovsynch ve modifiye ovsynchten elde edilen gebelik oranları sırasıyla %63.33±0.08 ve 56.66±0.09 olarak tespit edildi (p>0.05). Modifiye preovsynch grubunda bulunan süt ineklerinin 16 başında hafif, 14 başında normal çara görülmüştür. Modifiye ovsynch grubunda bulunan süt ineklerinin 23 başında hafif, 4 başında normal, 1 başında yoğun çara tespit edilmiş ve 2 başında ise çara görülmemiştir. Sonuç olarak, preovsynch protokolünde elde edilen gebelik sonuçlarının ovsynch protokolünden elde edilen sonuçlarla birbirine benzer olduğu ancak modifiye protokollerin etkinliğinin belirlenmesi için daha fazla sayıda süt ineğine sahip sürülerde yapılacak çalışmalara gereksinim olduğu düşünülmektedir.

Anahtar Kelimeler: Dölverimi ovsynch, preovsynch, süt ineği

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GİRİŞ

Süt yönlü işletmelerde, her inekten yıl başına yaşama gücü yüksek sağlıklı bir buzağının elde edilmesi sürünün reprodüktif verimliliğinin değerlendirilmesinde büyük önem taşır. Bu amaçla öncelikle östrus zamanının doğru tespiti, uygun zamanda tohumlamanın yapılması ve iyi bir kayıt sisteminin tutulması gerekir. Bununla beraber, süt yönlü işletmelerin büyük bir kısmında doğru östrus zamanının belirlenme oranı %50'nin altındadır. Bu nedenle, sürüye uygulanan senkronizasyon yöntemleri, belirli zamanlarda östrusları uyarması ya da östrus zamanının tespit zorunluluğu olmaksızın sabit zamanlı tohumlama için imkan sağlaması nedeniyle tercih edilebilir protokollerdir (Pekçok ve Aksu, 2015).

İneklerde östrus siklusunun senkronizasyonunda en başta PG ya da analogları ve progestagenler kullanılmaktadır. Başlıca progesteron ve/veya PGF2α kullanılan senkronizasyon protokollerinin etkisini artırmak, folliküler gelişmeyi ve CL regresyonunu senkronize etmek için östrojen, GnRH ve agonistleri kullanılmaktadır. Östrus senkronizasyonu programlarında plasental Gonadotropinden sağlanan Equine Chorionic Gonadotropin (eCG) (FSH aktivitesi fazla olan) ve human Chorionic Gonadotropin (hCG) (LH aktivitesi fazla olan) de kullanılmaktadır (Adataş, 2006).

Senkronizasyon programlarında yaşanan sorunları çözmek ve östrus tespitinin yetersizliğinden kaynaklanan gebeliklerin gerçekleşmeme durumunun azaltılmasında 1995'te Wisconsin-Madison Üniversitesinde geliştirilen Ovsynch programı östrus yerine ovulasyonu senkronize etmektedir (Pursley, ve ark., 1995).

Ovsynch protokolü, tohumlamadan önce GnRH, PG hormonlarının ard arda enjekte edilmesi ile yapılır. GnRH hipofizden LH'nın salınımına sebep olurken, GnRH uygulamasından yedi saat sonra LH hormonu pik yapar. GnRH, östrus siklusunun evresine ya da folliküler gelişim dönemine göre (folliküler dalgalar), genç follikküllerin gelişimini hızlandırır, östrojenin baskın olduğu folliküllerde ovulasyonu gerçekleştirir ve büyük veya yaşlı follikülleri luteinize eder. PG ise CL'nin lizisini kontrol eder (Dinç, 2006).

Östrusları doğru belirlenen ve boğa ile çiftleştirilen inekler ile ovsynch programına göre tohumlanan ineklerden benzer gebelik oranları elde edilmektedir. Özellikle laktasyondaki yüksek verimli sütçü ineklerde ovsynch'in reproduktif performansı geliştirmek için yüksek etkili ve ekonomik bir sistem olduğu bildirilmektedir. Östrus siklusunun herhangi bir döneminde uygulanan birinci GnRH enjeksiyonu %65 oranında ovulasyonu uyarır ve ineklerin %100'de yeni bir folliküler dalganın oluşmasına neden olur. GnRH uygulamasından 7 gün sonra uygulanan $PGF_{2\alpha}$ enjeksiyonu kendiliğinden oluşan veya GnRH'nın uyardığı CL'nin regresyonunu sağlar. PGF2α uygulamasından 1 gün sonra uygulanan ikinci $PGF_{2\alpha}$ enjeksiyonu birinci GnRH enjeksiyonundan sonra oluşan folliküler dalgadaki dominant follikülün ovulasyonunu senkronize eder. Son yapılan GnRH enjeksiyonunu takiben 16. saattede tohumlama yapılır (Burke, ve ark.,1996).

Yapılan bu çalışmada, östrus takibinin doğru yapılamadığı sürülerde östrus yerine ovulasyonun senkronize edilerek gebelik oranlarının artırılabileceği varsayımıyla, ineklerde modifiye iki farklı senkronizasyon yönteminin dölverimi sonuçları üzerine etkisinin değerlendirilmesi amaçlanmıştır.

MATERYAL ve METOT

Materyal

Çalışmada Amasya İlinde faaliyet gösteren bir süt yönlü işletmede yetiştirilen, yaşları 2-6 arasında, 2. ve 4. laktasyon aralıklarında olan, en az postpartum 45. günde bulunan ve reprodüktif açıdan sağlıklı olan toplam 60 baş Holştayn ırkı inek kullanıldı.

Metot

Çalışmaya alınacak hayvanlar iki gruba ayrıldı. I. Gruba (n=30) modifiye preovsynch, II. Gruba (n=30) modifiye ovsynch protokolü aşağıda açıklandığı şekilde uygulandı.

Seçilen ineklere yapılan suni tohumlama uygulamalarında; Artıvet veterinerlik firmasının ithal ettiği 076HO00721 küpe numaralı Magnavox adlı holştayn ırkı boğaya ait aynı tarihli spermalar kullanıldı.

Grup I (Modifiye Preovsynch)

0.gün: 5 ml PGF2α (Kas içi) / inek
14.gün: 5 ml PGF2α (Kas içi) / inek
25.gün: 2 ml GnRH (Kas içi) / inek
32.gün: 5 ml PGF2α (Kas içi) / inek
34.gün: 2 ml GnRH (Kas içi) / inek
16 saat sonra Suni Tohumlama

Grup II (Modifiye Ovsynch)

0. gün: 2 ml GnRH (Kas içi) / inek
7.gün: 5 ml PGF2α (Kas içi) / inek
8.gün: 5 ml PGF2α (Kas içi) / inek
9.gün: 2 ml GnRH (Kas içi) / inek
16 saat sonra Suni Tohumlama

İnekler son GnRH enjeksiyonundan suni tohumlama yapılana kadar geçen süre içerisinde üç kez (her biri yarım saat) östrus takibi için kontrol edildi. Bu kontrollerde çara miktarları (yok, hafif, normal, yoğun) kaydedildi. Suni tohumlama sonrası 40. günde ultrasonografi (The Mindray DP-6600 digital ultrasound) ile 7.5 Mhz Lineer Prob kullanılarak gebelik muayeneleri yapıldı. Gebelik muayene sonuçları gebelik (+) ya da gebelik (-) olarak kaydedildi.

İstatistik Analiz

Çalışmada, ortalama, standart hata gibi tanıtıcı istatistikler hesaplandı. Gebelik ile çara arasındaki ilişkilerde Spearman korelasyon analizi uygulandı. Gruplar arası farklılıklar ki-kare bağımsızlık (independance) testi ile belirlendi. Tüm analiz ve hesaplamalarda SAS (2009) istatistik programı kullanıldı.

BULGULAR

İki farklı modifiye senkronizasyon protokolü uygulanan bu çalışmada gruplara ait gebelik oranları Tablo 1'de sunulmuştur. Gebelik oranları açısından modifiye preovsynch grubunda modifiye ovsynch grubuna oranla rakamsal bir artış ($p>0.05$) gözlenmiştir.

Çalışmada son GnRH enjeksiyonunun yapıldığı gün ve suni tohumlama yapılana kadar geçen süre içerisinde gruplardaki ineklere yapılan kontroller sonucu belirlenen çara miktarları ile her iki grupta gebelik (+) ve gebelik (-) olan ineklere ait çara miktarları Tablo 2 ve 3'de sunulmuştur. Araştırmanın I. grubunda (modifiye preovsynch) yer alan toplam 30 ineğin 16'sında hafif, 14'ünde normal çara akıntısının olduğu belirlenmiştir. Bu grupta gebelik (-) olan 11 ineğin tamamında hafif çara akıntısı görülürken, gebelik (+) olan 19 ineğin 5'inde hafif, 14'ünde normal miktarda çara akıntısı tespit edilmiştir (Tablo 2). Çalışmanın II. grubunda (modifiye ovsynch) bulunan toplam 30 ineğin 23'ünde hafif, 4'ünde normal, sadece bir tanesinde yoğun çara akıntısı olduğu belirlenirken, 2 inekte çara akıntısı saptanmamıştır. Bu grupta gebelik (-) olan 13 ineğin tamamında çara miktarı hafif, gebelik (+) olan 17 ineğin 10'unda hafif, 4'ünde normal, bir tanesinde yoğun çara tespit edilirken, iki inekte çara görülmemiştir (Tablo 3).

Tablo 1. Gruplar arası gebe kalma oranları
Table 1. Pregnancy rates between groups

Gebelik	Grup I $\overline{X} \pm S_{\overline{X}}$	Grup II $\overline{X} \pm S_{\overline{X}}$	P
(%)	63.33±0.08	56.66±0.09	P>0.05

Tablo 2. Kızgınlık takibinde çara değerlendirmeleri (Grup I)
Table 2. Vaginal mucus evaluations during estrus follow-up (Group I)

Gebelik		Çara			
		Yok	Hafif	Normal	Yoğun
(-)	n	0	11	0	0
	%	0.00	36.67	0.00	0.00
(+)	n	0	5	14	0
	%	0.00	16.67	46.67	0.00
Toplam	n	0	16	14	0
	%	0.00	53.33	46.67	0.00

Tablo 3. Kızgınlık takibinde çara değerlendirmeleri (Grup II**)**
Table 3. Vaginal mucus evaluations during estrus follow-up (Group II)

Gebelik		Çara			
		Yok	Hafif	Normal	Yoğun
(-)	n	0	13	0	0
	%	0.00	43.33	0.00	0.00
(+)	n	2	10	4	1
	%	6.67	33.33	13.33	3.33
Toplam	n	2	23	4	1
	%	6.67	76.67	13.33	3.33

TARTIŞMA

Süt yönlü işletmelerde östrus zamanının doğru tespiti sürünün üreme performansının yönetiminde büyük önem taşımaktadır. Özellikle modern süt işletmelerinde süt ineklerinin bireysel takibinin zor ve çoğunlukla subjektif olması östrusun tespitinde ciddi bir sorundur. Bu sorun çalışan personel ve süt inekleri arasındaki etkileşimi en aza indiren sağımda robotik sistemlerin kullanıldığı sürülerde de görülebilmektedir. Bununla beraber yüksek verime sahip süt ineklerinin östrus semptomları düşük verimlilere göre daha zayıftır. Bunun nedenleri arasında üreme hormonlarının (östradiol gibi) daha yüksek metabolik klirens oranına bağlı olarak uygulanan yoğun besleme programı ve sağımın yapıldığı barınak sistemleri, doğum sonrası uzayan anöstrus ve üreme sistemi patolojileri prevalansının artması sayılabilir (Jaśkowski, ve ark. 2006; Macmillan, 2010; Wankhade, ve ark. 2017; Jaśkowski, ve ark. 2018). Yapılan çalışmalar (Martinez, ve ark. 2000; Bartolome, ve ark. 2005), süt ineklerinde folliküler ve luteal dinamikleri manipüle etmenin mümkün olduğunu, suni tohumlama ve embriyo transferi için östrus tespitine olan gereksinimin ortadan kaldırılabileceği gösterilmiştir. Bu amaçla GnRH ve PGF2α hormonları uygulanarak ovulasyon zamanını belirleyen ve suni tohumlamayı içeren protokoller mevcuttur. Bu protokollerden ilki olan ovsynch, ovulasyonun senkronizasyonu protokolüdür (Pursley, ve ark.,1995). Uygulanan diğer tüm protokoller temelde ovsynch protokolünün varyasyonlarıdır (Ax, ve ark.,2005). Bu protokollerin temel amacının, zamanı belirlenmiş bir ovulasyonu gerçekleştirmek amacıyla üreme fizyolojisi için ihtiyaç duyulan olguları uygun bir şekilde kurgulamak ve süt ineklerinde reprodüktif sistemi maksimum düzeyde kontrol etmek olduğu belirtilmektedir (Whittier ve Geary, 2000). Yapılan bu çalışmada, süt ineklerinde ovsynch ve preovsynch protokollerinde farklı zamanlarda GnRH ve PGF2α uygulamaları sonucu GnRH tarafından stimüle edilen yeni bir foliküler dalga oluşumu ve ovulasyon zamanının kontrolü ile gebelik oranlarının artırılması ön görülmüştür.

Ovsynch protokolü sırasında üreme hormonlarının optimizasyonu ile ilk tohumlama başına düşen gebelik oranının artırdığı ifade edilmektedir (Souza ve ark., 2008; Carvalho ve ark., 2014). Bleach ve ark. (2004), ineklerde folikül gelişiminin başlangıcından östrusa kadar geçen sürenin 1 gün kısaltılmasının gebe kalma olasılığını artırdığını ifade etmiştir. Yapılan bir çalışmada (Yılmazbaş-Mecitoğlu ve ark. 2013), 920 baş süt ineğinden oluşan bir sürüde modifiye ovsynch (1. gün GnRH + 6 gün sonra PGF2α + 56 saat sonra 2. GnRH + 16-18 saat sonra suni tohumlama) ve ovsynch (1. GnRH + 7 gün sonra PGF2α + 56 saat sonra 2. GnRH + 16-18 saat sonra suni tohumlama) protokollerinin gebelik oranına etkisi araştırılmıştır. Çalışmada modifiye ovsynch uygulaması ile elde edilen gebelik oranının %40,90, ovsynch uygulamasında ise bu değerin %43,80 olduğu saptanmıştır. Kara vd. (2011) tarafından 48 baş Holstein ırkı inekte uygulanan ovsynch senkronizasyon yöntemi sonucu gebelik oranının %50 olduğu belirlenmiştir. Postpartum 50-75. günler arasında bulunan Holstein ırkı süt ineği (60 baş) kullanılarak yapılan diğer bir araştırmada (Elibol, vd., 2009), doğal östrus, ovsynch ve ovsynch + 12. gün GnRH protokolünü takiben yapılan suni tohumlama

sonucunda gebelik oranları sırasıyla %75, %55 ve %65 bulunmuştur. İlk ve üçüncü laktasyonda bulunan süt ineklerinde ovsynch protokolünün uygulandığı bir başka çalışmada (Peters ve Pursley, 2002), gebelik oranının ilk laktasyonda bulunan süt ineklerinde (%48,2) üçüncü laktasyondakilere (%33,9) göre daha yüksek olduğu tespit edilmiştir.

GnRH enjeksiyonu sonrasında ovulasyonu optimize eden stratejiler ve uygulanan son GnRH enjeksiyonundan sonra ovulasyonu stimüle eden stratejiler Ovsynch protokolünün etkinliğini artırabilir. Nitekim bu stratejiler birçok çalışmaya araştırma konusu olmuştur (Galvão ve Santos, 2010; Nowicki ve ark., 2017; Jaśkowski ve ark., 2018). Ovsynch protokolünün etkinliğini artırmanın en kolay yolu, iki prostaglandin enjeksiyonu içeren preovsynch protokolüdür (Jaśkowski ve ark., 2018). Bazı araştırıcılar (Marquezini ve ark., 2011; Carvalho ve ark. 2015a ve b) böyle bir protokolün ovsynch etkinliğinde artış ile sonuçlandığını ifade etmektedir.

Nak vd., (2005) tarafından 331 baş süt ineğinde ovsynch, PRID+ PGF2α+PMSG ve kulak implantı+ PGF2α +PMSG uygulamaları yapılarak yürütülen çalışmada, gebelik oranları sırasıyla %42,20, %39,63 ve %45,94 belirlenmiştir. Navanukraw ve ark., (2004) tarafından yapılan bir diğer çalışmada, ovsynch ve preovsynch protokolleri uygulanan süt ineklerinde gebelik oranları sırasıyla %43,40 ve %53,70 olarak belirlemiştir. Herlihy ve ark., (2012) tarafından yapılan bir araştırmada double ovsynch ve presynch-ovsynch protokolleri uygulanmıştır. Çalışma sonunda double ovsynch ve presynch-ovsynch protokolleri için gebelik oranları sırasıyla %40,30 ve 34,30 olarak saptanmıştır. Holstein ırkı süt ineklerinde yürütülen bir diğer araştırmada (Alkan ve Alkan, 2020) presynch-ovsynch uygulaması sonrasında gebelik oranı %32 bulunmuştur.

Yapılan bu araştırmada, uygulanan modifiye ovsynch protokolü sonrası elde edilen gebelik oranı (%56,66), Peters ve Pursley (2002), Nak vd. (2005), Kara vd., (2011) ve Yılmazbaş-Mecitoğlu ve ark., (2013)'nın bildirdiklerinden daha yüksek, Elibol vd., (2009)'un sonuçlarına benzer bulunmuştur. Yürütülen bu araştırmada diğer bir protokol olan preovsynch uygulaması sonucu elde edilen gebelik oranı (%63,33), birçok araştırıcının bildirdiğinden (Navanukraw ve ark., 2004; Herlihy ve ark., 2012; Alkan ve Alkan, 2020) daha yüksektir. Yapılan bu çalışma ile daha önceki araştırmalar arasındaki farklılıkların, ineklerin tohumlama sonrası gebe kalma oranlarının genel sağlık durumu (geçiş döneminde meydana gelebilen metabolik ve enfeksiyöz hastalıklar gibi), vücut kondisyon skoru, beslenme, laktasyon sayısı ve dönemi, yaş, süt verimi ve çevresel koşullar (sıcaklık, nem, mevsim gibi) gibi birçok faktörle ilişkili olmasına ve bu faktörlerin ülkeden ülkeye hatta işletmeden işletmeye göre oldukça değişken olmasından kaynaklanabileceği düşünülmektedir.

Süt ineklerinde östrus zamanının tespit yöntemlerinden biri olan çaranın takibi, sürünün reprodüktif performansı, buzağılama aralığı ve yavru verimi bakımından önem taşımasına rağmen, ineklerin bireysel izlenmesinin güç olduğu yüksek süt verimine sahip süt ineklerinden oluşan büyük ölçekli sürülerde, hafif çara gibi zayıf östrus belirtilerinin varlığından dolayı uygun tohumlama zamanının belirlenememesi mümkündür. Yapılan bu çalışmada, modifiye ovsynch ve preovsynch protokollerinde östrus takibi için izlenen çaranın sürünün %65,00'ünde hafif, %30'unda normal, %1,67'sinde yoğun olduğu, ancak %3,33'ünde ise çara görülmemesine karşın ovsynch protokolü kapsamında sabit zamanlı yapılan tohumlama sonrası gebe kalma sonucu dikkate alındığında, sürü yönetiminde ovsynch ve preovsynch gibi sekronizasyon protokollerinin uygulanması ile sabit zamanlı tohumlamanın yapılması gebelik oranının artırılmasında, reprodüktif performansın iyileştirilmesinde ve dolayısıyla reprodüktif sistemin kontrol altında tutulmasında önem taşıyabililir.

SONUÇ

Süt yönlü modern işletmelerin en büyük problemlerinden biri östrusun tespitindeki sorunlar ve tohumlamaların zamanında yapılamaması sonucu her yıl bir buzağının alınamaması ve buna bağlı ekonomik kayıplardır. Karlı bir işletmede doğum gebe kalma aralığının uzamaması, gebelik için gerekli tohumlama sayısının artmaması istenmektedir.

Sonuç olarak, östrus takibinin doğru yapılamadığı sürülerde östrus yerine ovulasyonun senkronize edilerek gebelik oranlarının artırılabileceği varsayımıyla, östrus siklusunun evreleri belirlenmeden, modifiye preovsynch ve modifiye ovsynch protokollerinin uygulandığı ineklerin gebe kalma oranlarının ve bu oranda etkili olabilen çara miktarının değerlendirildiği bu çalışmada, modifiye her iki protokolden elde edilen gebelik oranlarının birbirine benzer olması her iki protokolün de ineklerde başarılı olarak kullanılabileceği sonucuna varılmıştır. Bununla beraber, senkronizasyon yöntemlerinden elde edilen gebelik oranlarının farklılık gösterebileceği, hatta aynı ırk içinde aynı senkronizasyon yönteminin uygulandığı inek ve düvelerde dahi farklı başarılar ile sonuçlanabileceği göz önünde bulundurulmalıdır. Bu nedenle modifiye protokollerin etkinliğinin belirlenmesinde daha büyük sürüler ile yapılacak ileri düzeydeki çalışmalara gereksinim duyulmaktadır.

Çıkar çatışması: Yazarlar çıkar çatışması olmadığını beyan ederler.

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KAYNAKLAR

Adataş, T. (2006). İneklerde Ovsynch Ve Co-Synch Yöntemleri İle Ovulasyonun Senkronizasyonu. . A. Ü. Sağlık Bilimleri Enstitüsü Ankara, Yüksek Lisans Tezi

Alkan KK & Alkan H. (2020). Holstein ırkı ineklerde target breeding ve presynch-ovsynch senkronizasyonyöntemlerinin postpartum ilk tohumlamada gebe kalma oranı üzerine etkisi. Eurasian J Vet Sci. 36 (1): 36-41.

Ax R, Pollard B, McCauley T, Fish D & Faber S. (2005). Hormone options to increase pregnancy rates. 108th Annual Meeting, Minnesota Veterinary Medical Association, 04 February 2005, Minnesota, USA

Bartolome JA, Silvestre FT, Kamimura S, Arteche ACM, Melendez P, Kelbert D, McHale J, Swift K, Archbald LF & Thatcher WW (2005). Resynchronization of ovulation and timed insemination in lactating dairy cows I: Use of the Ovsynch and Heatsynch protocols after non-pregnancy diagnosis by ultrasonography. Theriogenology. 63: 1617-1627.

Bleach, E. C. L., R. G. Glencross, & P. G. Knight. (2004) Associa-tion between ovarian follicle development and pregnancy rates indairy cows undergoing spontaneous oestrous cycles. Reproduction. 127:621-629.

Burke, J.M., R.L. De La Sota, Rısco, C.A., Staple, C.R., Schmıtt, E.J.P.&Thatcher, W.W. (1996). Evaluation of time insemination using agonadotropin-releasing hormone agonist in lactating dairy cows. J Dairy Sci. 79:1385-1393.

Carvalho, P. D., J. N. Guenther, M. J. Fuenzalida, M. C. Amundson, M. C. Wiltbank, & P. M. Fricke. (2014). Presynchronization using a modified Ovsynch protocol or a single gonadotropin-releasing hormone injection 7 d before an Ovsynch-56 protocol for submission of lactating dairy cows to first timed artificial insemination. J. Dairy Sci. 97:6305-6315.

Carvalho PD, Fuenzalida MJ, Ricci AH, Souza RV, Barletta MC, Wiltbank MC, &Fricke PM. (2015a) Modifications to Ovsynch improve fertility during resynchronization: Evaluation of presynchronization with GnRH 6 days before Ovsynch and addition of a second prostaglandin F2α treatment. J Dairy Sci, 98: 8741–8752

Carvalho PD, Wiltbank MC & Fricke PM. (2015b) Manipulation of progesterone to increase ovulatory response to the first GnRH treatment of an Ovsynch protocol in lactating dairy cows receiving first timed artificial insemination. J Dairy Sci, 98: 8800-8813

Dinç, D.A. (2006). İneklerde Reprodüktif Verimliliği Artırma Programları. Vet Hek Dern Derg. 77, 50-64

Elibol E., Uçar M. & Yılmaz O., (2009). Ovsynch uygulanan ineklerde sun'i tohumlama sonrası 12. günde yapılan GnRH enjeksiyonunun gebelik oranına etkisi. Kocatepe Veteriner Dergisi. 2, 13- 18.

Galvão KN & Santos JE. (2010). Factors affecting synchronization and conception rate after the Ovsynch protocol in lactating Holstein cows. Reprod Domest Anim. 45: 439-446.

Herlihy M.M., Giordano J.O., Souza A.H., Ayres H., Ferreira R M., Keskin A., Nascimento A.B., Guenther J.N., Gaska J. M., Kacuba S.J., Crowe M.A., Butler S.T. & Wiltbank M.C. (2012). Presynchronization with Double- Ovsynch improves fertility at first postpartum artificial insemination in lactating dairy cows. J Dairy Sci. 95(12), 7003-7014.

Jaśkowski JM, Olechnowicz J & Nowak W. (2006). Some causes of declining fertility in dairy cows. Med Weter. 62: 385-389

Jaśkowski JM, Herudzinska M, Kulus J, Brüssow KP & Gehrke M. (2018). OvSynch program, its modifications and alternative hormonal programs in cow reproduction. Med Weter. 74: 626-633

Kara U., Ayaşan T., Hizli H. & Gök K., (2011). Ovsynch protokolünün inek ve düvelerin gebelik oranı üzerine etkisi. Erciyes Üniversitesi Veteriner Fakültesi Dergisi. 8, 1-8

Macmillan K.L. (2010). Recent advances in the synchronization of estrus and ovulation in dairy cows. J Reprod Dev. 56: 42-47.

Martinez M.F., Adams G.P., Kastelic J.P., Bergfelt D.R. & Mapletoft R.J. (2000). Induction of follicular wave emergence for estrus synchronization and artificial insemination in heifers. Theriogenology. 54: 757-769.

Marquezini G.H., Dahlen C.R., Bird S.L. & Lamb G.C. (2011). Administration of human chorionic gonadotropin to suckled beef cows before ovulation synchronization and fixed-time insemination: replacement of gonadotropin-releasing hormone with human chorionic gonadotropin. J Anim Sci. 89: 3030-3039

Nak Y., Nak D., Seyrek İntaş K., Tek HB., Keskin A. & Tuna B. (2005). Ovsynch, PRD + PGF2α + PMSG ve norgestomet içeren kulak implantı + PGF2α PMSG ile sağıtılan siklik ve asiklik sütçü ineklerde kızgınlık ve gebelik oranlarının karşılaştırılması. Uludag Universitesi Veteriner Fakültesi Dergisi, 24, 33-39**.**

Navanukraw C., Redmer D.A., Reynolds L.P., Kirsch J.D., Grazul-Bilska A.T. & Fricke P.M. (2004). A modified presynchronization protocol improves fertility to timed artificial insemination in lactating dairy cows. J Dairy Sci. 87(5), 1551-1557.

Nowicki A, Barański W, Baryczka A & Janowski T. (2017). OvSynch protocol and its modifications in the reproduction management of dairy cattle herds. J Vet Res. 61: 329-336

Pekçok D. & Aksu E.H. (2015). Sığırlarda Östrus Senkronizasyonu ile Birlikte Kullanılan Döl Tutma Oranını Etkileyen Faktörler. Atatürk Üniversitesi Vet. Bil. Derg. 10(3): 205-210.

Peters MW & Pursley JR (2002). Fertility of lactating dairy cows treated with ovsynch after presynchronization injections of PGF2 and GnRH. J Dairy Sci. 85, 2403-2406.

Pursley, J.R., Mee, M.O. & Wıltbank (1995). M.C. Synchronization of ovulation in dairy cows using PGF2α and GnRH. Theriogenology. 44: 915.

SAS. (2009). SAS Stat Sofware. SAS Campus Drive Cary, NC. 27513, USA.

Souza A.H., Ayres H., Ferreira R.M. & Wiltbank M.C. (2008): A new presynchronization system (Double-Ovsynch) increases fertility at first postpartum timed AI lactating dairy cows. Theriogenology. 70, 208-215, 2008.

Wankhade, P.R., Manimaran, A., Kumaresan, A., Jeyakumar, S., Ramesha, K.P., Sejian, V., Rajendran, D. & Varghese, M.R. (2017). Metabolic and immunological changes transition dairy cows: A review. Vet World. 10: 1367-1377

Whittier, J.C. & Geary, T.W. (2000). Frequently asked questions about synchronizing estrus and ovulation in beef cattle with GnRH. Prepared for Iowa Cooperative Extension CHIPS Beef Breeding Management Seminar, 29 January 2000 Ames, Iowa, USA

Yılmazbaş-Mecitoğlu G, Karakaya E., Keskin A., Alkan A. & Gumen A. (2013). Reducing the duration between gonadotropin-releasing hormone (GnRH) and prostaglandin F2α treatment in the ovsynch protocol to 6 days improved ovulation to second GnRH treatment, but inclined to reduce fertility. Journal of Dairy Science. 96, 3817-3824

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RESEARCH ARTICLE

What Is the Latest Status of Cats' Health in Hatay (Türkiye) Province Following the SARS-COV 2 Pandemic?

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ABSTRACT

The current study focused on the latest status of gastrointestinal, urinary tract, and hepatobiliary diseases, which might be induced by stress in cats following the SARS-CoV-2 pandemic in Hatay province in Türkiye. The study looked at post-pandemic (June 2021–June 2022) diagnosis data of cats (n:147) with gastrointestinal, urinary tract, and hepatobiliary diseases, admitted to Hatay Mustafa Kemal University Veterinary Health, Practice, and Research Center. The average age of the cats admitted to the clinic was 33.04±2.77 months in post-pandemic period. The ages of the cats diagnosed with gastrointestinal, urinary tract and hepatobiliary diseases were 27.78±4.33, 38.04±3.95, and 40.12±6.27, respectively. The cat breeds commonly brought to the clinic following the pandemic were Tabby, Mix, British shorthair, Persian, and Scottish fold-ear, respectively. There were no breed and sex difference for diseases diagnosed in post-pandemic duration. Distributions of gastrointestinal, urinary, and hepatobiliary diseases were determined as 51.0% (n=75), 37.4% (n=55), and 11.6% (n=17), respectively. Gastroenteritis (n=30, 40%), gastritis (n=13, 17.33%), and unconfirmed and suspected Feline inflammatory bowel disease (n=10, 13.33%) are more diagnosed digestive system diseases. Cystitis (n=30, 54.54%) and hemorrhagic cystitis (n=18, 32.72%) were commonly diagnosed in urinary diseases. Besides, cholangiohepatitis was commonly diagnosed either alone (n=7, 41.17%) or accompanied by hepatic lipidosis (n=4, 23.52%) or cholecystitis (n=3, 17.65%) in cats following the pandemic. Consequentially, although the restrictions implemented during the pandemic and causing stress on cats were ended, it was concluded that the chronic impacts of stress on cats, especially in terms of gastrointestinal system diseases, may continue following the pandemic.

Keywords: Cat, gastrointestinal disorders, post-pandemic, SARS-CoV-2, stress

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SARS-COV 2 Pandemisi Sonrası Hatay (Türkiye) İlinde Kedilerin Son Sağlık Durumu Nedir?

ÖZ

Bu çalışma, Türkiye'nin Hatay ilinde SARS-CoV-2 pandemisi sonrası kedilerde stresin tetikleyebileceği gastrointestinal, idrar yolu ve hepatobiliyer hastalıkların son durumuna odaklanmıştır. Çalışmada Hatay Mustafa Kemal Üniversitesi Veteriner Sağlık, Uygulama ve Araştırma Merkezi'ne başvuran gastrointestinal, idrar yolları ve hepatobilier hastalıkları olan kedilerin post-pandemik (Haziran 2021–Haziran 2022) tanı verilerine (n:147) bakılmıştır. Pandemi sonrası kliniğe başvuran kedilerin ortalama yaşı 33.04±2.77 aydı. Gastrointestinal, idrar yolu ve hepatobiliyer hastalık tanısı konulan kedilerin yaşları sırasıyla 27.78±4.33, 38.04±3.95 ve 40.12±6.27 idi. Pandeminin ardından kliniğe yaygın olarak getirilen kedi ırkları sırasıyla Tekir, Melez, British shorthair, İran ve Scottish fold-ear idi. Pandemi sonrası dönemde teşhis edilen hastalıklar için ırk ve cinsiyet farkı yoktu. Pandemi sonrası gastrointestinal, üriner ve hepatobiliyer hastalıkların dağılımları sırasıyla %51,0 (n=75), %37,4 (n=55) ve %11,6 (n=17) olarak belirlendi. Gastroenterit (n=30, %40), gastrit (n=13, %17,33) ve Feline yangısal barsak hastalığı şüpheli (n=10, %13,33) hastalıklar en yaygın teşhis edilen sindirim sistemi hastalıkları idi. Sistit (n=30, %54.54) ve hemorajik sistit (n=18, %32.72) yaygın olarak teşhis edilen hastalıklar idi. Ayrıca, kolanjiyohepatit, pandemi sonrasında kedilerde sıklıkla tek başına (n=7, %41,17) veya hepatik lipidoz (n=4, %23,52) ya da kolesistit (n=3, %17,65) ile birlikte teşhis edilmiş olgulardı. Sonuç olarak, pandemi döneminde uygulanan ve kediler üzerinde strese neden olan kısıtlamalara son verilmiş olsa da, pandemi sonrasında özellikle gastrointestinal sistem hastalıkları açısından stresin kediler üzerindeki kronik etkilerinin devam ediyor olabileceği kanısına varıldı.

Anahtar Kelimeler: Gastrointestinal Bozukluklar; Kedi; Post-Pandemi; SARS-CoV-2; Stres

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INTRODUCTION

Cats have gained popularity as pets because they are considered "low-maintenance" or "independent" animals (Lue et al. 2008, Rodan 2016). In today's lifestyle, generally, people have a long working time as opposed to time spent at home and live in cramped buildings (Lue et al. 2008, Horwitz and Rodan 2018). Therefore, cats, being easy to care for, seem to be a suitable candidate as a companion animal in this lifestyle (Lue et al. 2008, Rodan 2016). Because of environmental factors, cat-cat interactions or cat-human interactions, some cats may be sensitive to an indoor lifestyle (Buffington 2002, Adamelli et al. 2005, Rodan and Heath 2016). And this lifestyle for cats may cause some health problems (Buffington, 2002). The behavior of cats is affected by individual variations between cats during socialization with uncommon people in unfamiliar surroundings (Bernstein 2007). Cats usually prefer to communicate with their owners by meowing, rubbing, purring, and wagging their tails to demand food, but they spend the majority of their time post-meal grooming and less time interacting with their owners (Bradshaw and Cook 1996, Bernstein 2007). Cats prefer to spend time alone after meals. However, owners may interact more with their cats during the post-meal period. Owners, unaware of this cats' behavior, may need to increase own mental health outcomes, such as alleviating stress, enhancing social communication, and overcoming difficulties (Shoesmith et al. 2021).

Stress is used to describe deleterious or unpleasant stimuli that bring about behavioral and psychological changes (Moberg and Mench 2000). Stressors in domestic animals include physical stressors, social stressors caused by interactions with other members of the same species, and stressors that occur as a result of human interaction (Moberg and Mench 2000, Amat et al. 2016). If the animal is subjected to long-term stress that exceeds its capacity for adaptation, its welfare will suffer (Broom and Johnson 1993, Moberg and Mench 2000, Amat et al. 2016). Owners, on the other hand, may complain of stress-related behavioral changes in their animals, and finally, relinquishment for cats and dogs may be involved (Edney 1998, Amat et al. 2016). Sharing a house with other animals and the change in the owner's environment are shown to be important causes of behavioral changes in cats that result in relinquishment (Salman et al. 2000, Casey et al. 2009). Crowded life, sudden and unexpected changes (Keeling and Jensen 2002), environmental changes, inadequate or excessive human-cat relations (Stella et al. 2013, Amat et al. 2016), new individual participation in the environment, and changes in daily routine all come to the fore as stress factors in cats (Stella et al. 2011, Amat et al. 2016). Sickness behavior in cats exposed to stressful conditions is possible (Stella et al. 2011). Gastrointestinal symptoms (diarrhea, lack of appetite, low-high water intake), physical symptoms (reduced activity, lethargy, somnolence, increased pain-like behaviors, reduced grooming), and mental symptoms (decreased social interactions, aggression) may be observed as sickness behaviors (Buffington 2011, Stella et al. 2011, Stella et al. 2013, Ropski et al., 2023). Sickness behaviors have been reported to be responses to adverse environmental events, such as the physical and behavioral response to infection, that are well established in the animal species studied (Stella et al. 2011). The production of pro-inflammatory cytokines, immunological activation, emotional state alterations, and pathological pain have been related to psychological stressors (Stella et al. 2011, Stella et al. 2013). Thus, sickness behaviors can be generated by the body's central (psychological) and peripheral (infection) channels (Keeling and Jensen 2002, Stella et al. 2011). The stress reaction may result in the formation of a new infection or the reactivation of an existing one as a result of a decrease in immune system function (Keeling and Jensen 2002, Amat et al. 2016). Consequently, stress seems to be a critical connection between the environment, behavioral problems, and disease (Keeling and Jensen 2002). Herewith, although behavioral issues in cats are assumed to be individual, stress from the owner and the surroundings is frequently disregarded (Keeling and Jensen 2002, Jensen et al. 2020).

The severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2), which broke out approximately four years ago in late December 2019 and was named Coronavirus disease 2019 (COVID-19), caused a new pandemic all over the world (Wang et al. 2020, World Health Organization 2020). In order to limit the spread of the disease during the pandemic process, various restrictions and measures (such as social isolation, travel restrictions, closure of educational institutions, and remote work) were implemented all over the world (Şeker et al. 2020, Islam et al. 2021). Following the first case notification on March 11, 2020, quarantine measures (such as flexible working hours and curfews) were strictly implemented in Türkiye as well as all over the world (Maden 2020, Şeker et al. 2020). It was obvious that both cats and humans were exposed to stress thanks to quarantine measures during the pandemic (Shoesmith et al. 2021, Köse et al. 2022). When the stressful situation ends, induction of the sympathetic nervous system by the adrenal medulla disappears, but the endocrine effect of the stress response may be continued (Buffington 2002, Keser 2018). In this study, considering the endocrine effect of the stress response, it was hypothesized that stress-related diseases may continue following the pandemic. Based on this, in this study, the post-pandemic status of gastrointestinal system

diseases, which were observed to increase especially during the pandemic (Köse et al. 2022), and hepatobiliary and urinary system diseases, which may also be stress-related, were aimed to analyze.

MATERIALS and METHODS

Materials

The data from July 2021, the date when restrictions were ended in Türkiye (Republic of Türkiye Ministry of Interior 2021), to July 2022 were examined. Herewith, a total of 147 diagnosis data of cats admitted to the Department of Internal Medicine Clinic of Hatay Mustafa Kemal University Veterinary Health, Practice, and Research Center comprised the materials of this study. These data included gastrointestinal, hepatobiliary, and urinary system diseases in cats that could be caused by stress (Rodan 2016, Rodan and Heath 2016).

Methods

Initial admission of cats with urinary disorders, complete blood count (CBC) (MS4S, Melet Schloesing Laboratoires, Osny, France) and blood chemistry analysis (BC) (Chem200, Gesan Production S.R.L, Campobello Di Mazara, Italy), ultrasound (USG) (ESAOTE Pie Medical Aquila, Czech Republic) and radiographic imaging (RI) (Regius ∑II, Konica Minolta, Tokyo, Japan) were used for diagnosis. The diagnosis of cystitis, hemorrhagic cystitis, feline urinary syndrome, and urolithiasis was performed in cats as described previously (Köse et al. 2022). Diagnosis of cystitis with urolithiasis was performed with together evaluation of ultrasound imaging (UI) (thickening of the bladder wall, the bladder's fullness, existence of urolith in bladder), stick test of urine (MEDPA, Türkiye) (observation of reaction for leukocyte, protein, or nitrite in addition to pH and SpG levels), microscopic examination of urine sediment (casts, crystalluria, and the presence of WBC), and blood works (CBC and BC). In the urine works (stick and microscopy) of cats with urinary symptoms, hemorrhagic cystitis with urolithiasis was diagnosed if RBC and erythrocyte were present in addition to evidence of cystitis and urolithiasis. The diagnosis of urethral obstruction was made by determining the strain during urinary catheterization. Acute renal failure was diagnosed according to clinical signalments (anuria, vomiting, weakness), laboratory analysis (blood works and urinalysis), and especially UI (presence renal enlargement). In order to diagnose gastrointestinal and hepatobiliary problems in cats, blood works (CBC and BC), imaging techniques (UI and RI), and stool examination (including microscopy and rapid tests, etc.) were carried out as described by authors (Köse et al. 2022). Besides, for the diagnosis of hepatobiliary disorders, serum chemistry (alteration in enzyme, blood urea nitrogen and creatinine levels), and liver ultrasound imaging to determine structural and parenchymal changings were especially used as described previously (Köse et al. 2022).

Evaluation of data

Patient registries and patient monitoring software (EVET, Hasvet, Türkiye) were investigated for the purpose of gathering data. The data belonging to the period between July 2021, when normalization was announced (Republic of Türkiye Ministry of Interior 2021) to July 2022 were accepted as post-pandemic period.

Statistical Analysis

For categorical variables, descriptive statistics were presented as frequencies and percentages, and for continuous variables, as arithmetic means and standard errors. Fisher's Exact Test was used to compare the distributions of categorical variables among subgroups due to the existence of zero cells and a small number of observed and predicted values in cells. To evaluate the difference in the average age of the cats diagnosed in the post-pandemic period, one-way ANOVA test was used. All statistical analyses were performed using SPSS 26.0 (IBM Corp., Armonk, NY), and $p<0.05$ was considered statistically significant.

RESULTS

The average age (mean±SEM) of the cats was 33.04±2.77 months in the post-pandemic period. In this period, gastrointestinal, urinary, and hepatobiliary diseases were diagnosed in cats at 51.0% (n=75), 37.4% (n=55), and 11.6% (n=17), respectively (Table 1). The monthly ages of the cats diagnosed with gastrointestinal, urinary, and hepatobiliary diseases were 27.78±4.33, 38.04±3.95, and 40.12±6.27, respectively. The difference was not detected in the average ages of cats diagnosed in the post-pandemic period ($F=1.925$ (df:2), $p=0.150$). There was no sex difference in urinary ($X2=8.214$, $p=0.252$), gastrointestinal ($X2=4.766$, $p=0.596$), or hepatobiliary ($X2=4.995$, $p=0.484$) diseases diagnosed in the cats admitted to internal medicine clinic following the pandemic (Table 1). It was determined that the cat breeds commonly brought to the clinic as patient and diagnosed in the post-pandemic period were tabby, mixed, British shorthair, Persian, and Scottish fold-ear, respectively. It was also found that there was no statistical difference ($X2=28.732$ (df:26), $p=0.181$) between the cat breeds that presented to the clinic in terms of gastrointestinal, urinary, and hepatobiliary diseases (Table 2). Commonly diagnosed digestive system diseases in cats admitted with gastrointestinal symptoms were gastroenteritis (n=30, 40%), gastritis (n=13, 17.33%), and unconfirmed and suspected Feline inflammatory bowel disease (n=10, 13.33%), respectively (Table 1). On the other hand, in urinary system diseases, cystitis (n=30, 54.54%) and hemorrhagic cystitis (n=18, 32.72%) were commonly

diagnosed (Table 1). Interestingly, in hepatobiliary diseases, it was determined that cholangiohepatitis was commonly diagnosed either alone (n=7, 41.17%) or accompanied by hepatic lipidosis (n=4, 23.52%) or cholecystitis (n=3, 17.65%) in the cats admitted to internal medicine clinic following the pandemic (Table 1).

Table 1. Gender difference in gastrointestinal, urinary and hepatobiliary diseases in cats diagnosed following pandemic.

		Gender (n)		Total
		Female	Male	
Gastrointestinal Diagnosis	Gastritis	5	8	13
	Gastroenteritis	15	15	30
	Enteritis	5	3	8
	Hemorrhagic Gastroenteritis	2	3	5
	Colitis	1	4	5
	Constipation	2	2	4
	FIBD*	7	3	10
	P value	0.596		75
Urinary Diagnosis	Hemorrhagic Cystitis	3	15	18
	Cystitis	11	19	30
	FUS	1	0	1
	Urolithiasis	0	2	2
	Cystitis with Urolithiasis	1	0	1
	Acute Renal Failure	0	1	1
	Hemorrhagic Cystitis with Urolithiasis	0	1	1
	Urethral Obstruction	0	1	1
	P value	0.252		55
Hepatobiliary Diagnosis	Hepatic Lipidosis	1	0	1
	Cholangiohepatitis	2	5	7
	Cholangiohepatitis & Cholecystitis	1	2	3
	Cholangiohepatitis & Hepatic Lipidosis	1	3	4
	Cholestasis	1	0	1
	Cholecystitis	1	0	1
	P value	0.484		17

FIBD*: Feline inflammatory bowel disease suspected; FUS: Feline urologic syndrome

Table 2. Difference* in breed prevalence in disease diagnosis.

		Diagnosis			
		Urinary	Gastrointestinal	Hepatobiliary	Total
Breeds	Tabby	25	40	6	71
	Mix	6	11	3	20
	British Shorthair	6	9	1	16
	Scottish Fold Ear	2	6	1	9
	Tuxedo	2	0	1	3
	Ankara	1	3	0	4
	Bombay	3	0	0	3
	Siamese	1	1	1	3
	Van	2	0	1	3
	Persian	3	4	3	10
	Exotic Shorthair	1	0	0	1
	Norwegian Forest	1	0	0	1
	Russian Blue	2	0	0	2
	Ragdoll	0	1	0	1
Total		55	75	17	147

*($X2 = 28.732$ (df:26), $p=0.181$)

DISCUSSIONS

All over the world, various pandemics, such as the Black Death, cholera, swine flu, and Middle East respiratory syndrome coronavirus, have been recorded (Huremović 2019, Piret and Boivin 2021). The SARS-COV-2 pandemic, which was effective all over the world and was first reported in Wuhan, China in 2019 (Wang et al. 2020, World Health Organization 2020), has been effective in Türkiye for more than a year (Maden 2020, Şeker et al. 2020, Republic of Türkiye Ministry of Interior 2021). With the goal of stopping the spread of the disease throughout the pandemic phase, various limitations and procedures (such as social separation, limitations on travel, closing of educational organizations, and faraway employment) have been put in place in Türkiye as well as all over the world (Maden 2020, Şeker et al. 2020, Islam et al. 2021).

In the studies on human-animal interactions (Wood et al. 2015, Bowen et al. 2020), it is informed that companion animals provide social support for people in overcoming challenges, especially during a time when the majority of people are facing similar environmental and social issues like the SARS-COV-2 pandemic. Pet animals can nonetheless suffer the unfavorable effects of a duration of home restriction; the quality of life of cats and dogs is greatly affected by the features of their physical and social surroundings, as well as the owners' habits and lifestyle, all of which would be significantly altered during quarantine measures (Bowen et al. 2020, Fatjó and Bowen 2020).

Cats are the most popular pets in many countries, and Türkiye is among the top 10 of these (Batson 2008, Rodan and Heath 2016). But, during the pandemic, interest in pets and spending time with cats at home indirectly decreased or increased, contrary to normal days, because of quarantine measures and restrictions implemented in Türkiye. So, it may be stressful for cats to have their regular routines changed (Moberg and Mench 2000, Amat et al. 2016, Horwitz and Rodan 2018, Köse et al. 2022). Under stressful conditions, the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS) are stimulated by environmental stressors. After this stimulation, corticosteroids are released from the adrenal cortex, which interact with ACTH released from the pituitary gland, into the blood stream (Buffington 2011, Amat et al. 2016, Keser 2018, Köse et al. 2022). The HPA axis has an impact that lasts longer in the body due to its endocrine effects (Buffington 2002, Keser 2018, Köse et al. 2022). Chronic distress may occur due to strong, prolonged, or severe stressors (Hargrave 2015, Horwitz and Rodan 2018). Thereby, stress may cause a variety of behavioral and mental issues, as well as new diseases or the recurrence of old infections in cats (Enck and Holtmann 1992, Tanaka et al. 2012, Amat et al. 2016, Köse et al. 2022). A number of gastrointestinal issues (vomiting, diarrhea, etc.), urinary problems like idiopathic cystitis, and some hepatobiliary diseases (hepatic lipidosis, cholangitis, etc.) may occur in cats due to stress (Enck and Holtmann 1992, Buffington 2011, Tanaka et al. 2012, Amat et al. 2016).

Gastrointestinal disease may be brought on by chronic distress (Horwitz and Rodan 2018, Köse et al. 2022). Compared to the previous study of the authors (Köse et al. 2022), the present study showed that gastrointestinal diseases, especially gastroenteritis, enteritis, and hemorrhagic gastroenteritis, were continuing to relatively increase in also post-pandemic season, except gastritis. Different from the previous study of the authors (Köse et al. 2022), feline inflammatory bowel disease (suspected but unconfirmed), colitis, and constipation stand out as new diagnoses (Figure 1). Mast cell activation, degranulation, and colonic mucin depletion can all be caused by stress. Additionally, stress increases one's vulnerability to colonic inflammation (Rakesh et al. 2005). On the other hands, intestinal activities may be affected by stress factors (external or intestinal-related), which may lead to an alteration in the intestinal microbiota and a deterioration in the intestinal barrier (Enck and Holtmann 1992, Köse et al. 2022). The onset of gastrointestinal diseases, especially colitis, constipation, and inflammatory bowel disease in cats in the post-pandemic period, might be related to colonic mucin depletion, increasing vulnerability to colonic inflammation, an alteration in the intestinal microbiota, and a deterioration in the intestinal barrier.

Acute or chronic stress are both possible (Amat et al. 2016, Horwitz and Rodan 2018, Köse et al. 2022). And cats may experience urinary disorders under prolonged distress as opposed to acute stress (Horwitz and Rodan 2018, Köse et al. 2022). In the previous study of the authors (Köse et al. 2022), they did not detect a statistical difference between the number of pre-pandemic and pandemic period urinary diagnoses in cats. On the other hand, in the present study, the number of major urinary disease diagnoses (Figure 2), which are cystitis and hemorrhagic cystitis, is seen approximately equal to sum of the number of diagnosed in pre-pandemic and pandemic period reported in the previous study of authors (Köse et al. 2022). In addition, the other diagnoses, which are the same as those diagnosed in the pre-pandemic and pandemic periods, were also observed at similar levels following the pandemic. In light of these findings, it is suspected that this relative increase in cystitis and hemorrhagic cystitis following the pandemic may be resulted from the endocrine effects of stress in cats.

Under stressful conditions, cats may tend to develop hepatic disease, including hepatic lipidosis and cholangiohepatitis, but the mechanism of this process cannot be definitively explained (Amat et al. 2016, Kuzi et al. 2017, Köse et al. 2022). One approach to this issue suggests that a decrease in appetite or anorexia may develop due to chronic distress, and rapid weight loss resulting in hepatic disorders including cholangitis, cholangiohepatitis, hepatitis, and hepatic lipidosis may easily occur following this process (Rutgers 1998, Köse et al. 2022). Lack of glucuronyl transferase, inability to synthesize arginine, and a propensity for hepatic fat buildup, bacterial overload, small intestinal shortness, and anatomical characteristics all predispose to the development of liver disease in cats (Rutgers 1998, Černá et al. 2020, Köse et al. 2022). In the previous study of the authors (Köse et al. 2022), it was reported that the number of cholangiohepatitis cases observed in the pandemic was relatively higher than in the pre-pandemic. But this increase in cholangiohepatitis was not found to be significant. In the present study, the total number of cholangiohepatitis cases observed (Figure 3) was closely similar to the previous study's data related to the pandemic (Köse et al. 2022). But, in the present study, cholangiohepatitis was diagnosed either alone (n:7) or accompanied by hepatic lipidosis (n:4) or cholecystitis (n:3) (Figure 3). These comorbidities in hepatobiliary disease may be related to the recurrence of previous diseases due to chronic distress in addition to a new one following the pandemic.

The pandemic is reported as a serious stress factor for both people and cats (Wood et al. 2015, Bowen et al. 2020, Fatjó and Bowen 2020, Shoesmith et al. 2021). Before and during the pandemic, diseases that may be caused by stress were examined in the previous study (Köse et al., 2022), and it was determined that there was an increase in gastroenteritis in cats during the pandemic. In the presented study, it was hypothesized that stress-related diseases in cats may continue after the pandemic period. Since the diagnoses made in cats after the pandemic period were only examined and analyzed retrospectively in this study, the factors regarding stress (blood cortisol level, clinical observations of stress, etc.) are not present. This may be the limiting factor of the study.

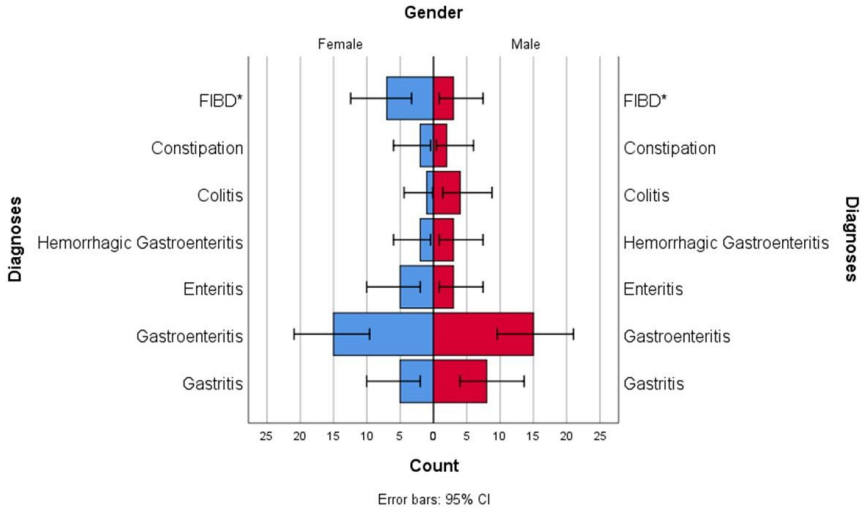

Figure 1: Gastrointestinal diagnosis distributions

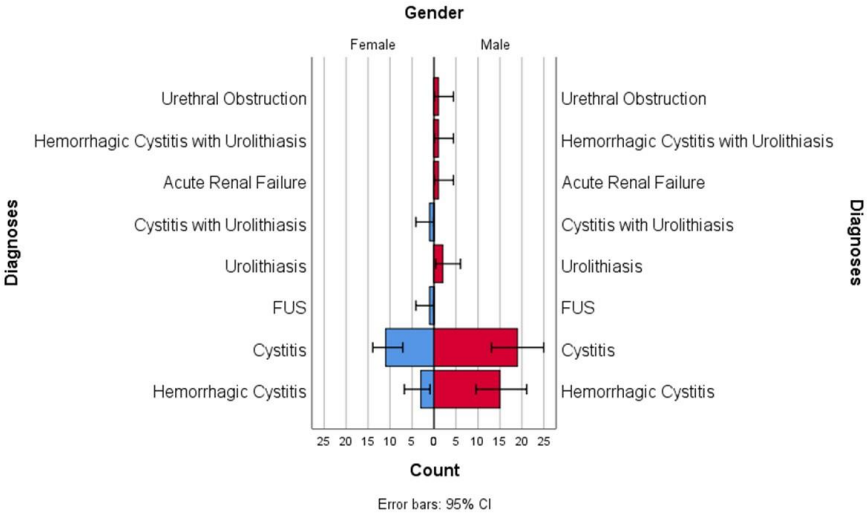

Figure 2: Urinary diagnosis distributions

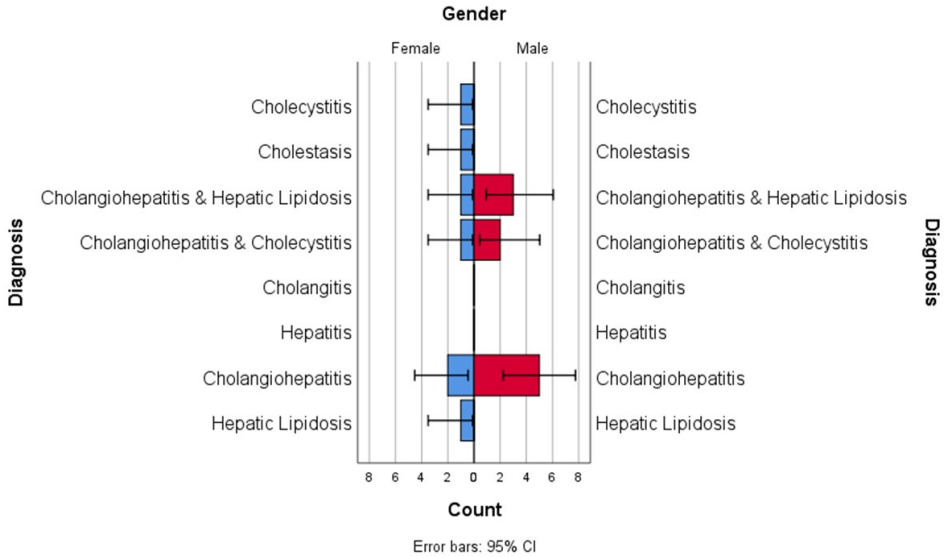

Figure 3: Hepatobiliary diagnosis distributions

CONCLUSION

It is obvious that the daily routine may change due to official quarantine measures, and this may result in stress for both cats and people. Although it is not certain, it is concluded that the cats may have suffered from chronic distress, lasting since the days of the pandemic in Hatay (Türkiye) province. Starting at this point, it is not underestimated that acute stress or chronic distress may have negative effects on cat welfare. Measures, such as environmental enrichment, to relieve stress affecting cats may be implemented. In addition, further comprehensive case-controlling studies, including cat-human and cat-environment relationships, should be conducted for the evaluation of stress-related diseases in cats.

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Authors' Contributions: SİK contributed to the project idea, design and execution of the study. GU and AE contributed to the acquisition of data. SİK analyzed the data. SİK drafted and wrote the manuscript. ASÖ and RD reviewed the manuscript critically. All authors have read and approved the finalized manuscript.

Ethical Approval: Retrospective data from patient registries and client-monitoring software were used in this project. So, this study is not subject to the permission of HADYEK in accordance with the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees" 8 (k). The data, information and documents presented in this article were obtained within the framework of academic and ethical rules.

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REFERENCES

Adamelli, S., Marinelli, L., Normando, S., & Bono, G. (2005). Owner and cat features influence the quality of life of the cat. Applied Animal Behaviour Science, 94(1–2), 89–98. https://doi.org/10.1016/j.applanim.2005.02.003

Amat, M., Camps, T., & Manteca, X. (2016). Stress in owned cats: behavioural changes and welfare implications. Journal of Feline Medicine and Surgery, 18(8), 577–586. https://doi.org/10.1177/1098612X15590867

Batson, A. (2008). Global Companion Animal Ownership and Trade: Project Summary. World Society for the Protection of Companion Animals (WSPCA). https://cupdf.com/document/global-reports-tcm.html?page=1/ [Accessed Date: 20.07.2023]

Bernstein, P. L. (2007). The Human-Cat Relationship. In: Rochlitz, I. (editor). The Welfare of Cats, Springer Netherlands. pp. 47–89. https://doi.org/10.1007/978-1-4020-3227-1_3

Bowen, J., García, E., Darder, P., Argüelles, J., & Fatjó, J. (2020). The effects of the Spanish COVID-19 lockdown on people, their pets, and the human-animal bond. Journal of Veterinary Behavior, 40, 75–91. https://doi.org/10.1016/j.jveb.2020.05.013

Bradshaw, J. W. S., & Cook, S. E. (1996). Patterns of pet cat behaviour at feeding occasions. Applied Animal Behaviour Science, 47(1–2), 61–74. https://doi.org/10.1016/0168-1591(95)01011-4

Broom, D. M., & Johnson, K. G. (1993). Stress and Animal Welfare. Springer Netherlands. https://doi.org/10.1007/978-94-024-0980-2

Buffington, C. A. T. (2002). External and internal influences on disease risk in cats. Journal of the American Veterinary Medical Association, 220(7), 994–1002. https://doi.org/10.2460/javma.2002.220.994

Buffington, C. A. T. (2011). Idiopathic cystitis in domestic cats-beyond the lower urinary tract. Journal of Veterinary Internal Medicine, 25(4), 784–796. https://doi.org/10.1111/j.1939-1676.2011.0732.x

Casey, R. A., Vandenbussche, S., Bradshaw, J. W. S., & Roberts, M. A. (2009). Reasons for relinquishment and return of domestic cats (felis silvestris catus) to rescue shelters in the UK. Anthrozoos, 22(4), 347-358. https://doi.org/10.2752/089279309X12538695316185

Černá, P., Kilpatrick, S., & Gunn-Moore, D. A. (2020). Feline comorbidities: What do we really know about feline triaditis? Journal of Feline Medicine and Surgery, 22(11), 1047–1067. https://doi.org/10.1177/1098612X20965831

Edney, A. T. B. (1998). Reasons for the euthanasia of dogs and cats. Veterinary Record, 143(4), 114. https://doi.org/10.1136/vr.143.4.114

Enck, P., & Holtmann, G. (1992). Stress and gastrointestinal motility in animals: a review of the literature. Neurogastroenterology & Motility, 4(2), 83–90. https://doi.org/10.1111/j.1365-2982.1992.tb00084.x

Fatjó, J., & Bowen, J. (2020). Making the case for multi-axis assessment of behavioural problems. Animals, 10(3), 383. https://doi.org/10.3390/ani10030383

Hargrave, C. (2015). Anxiety, fear, frustration and stress in cats and dogs — Implications for the welfare of companion animals and practice finances. Companion Animal, 20(3), 136-141. https://doi.org/10.12968/coan.2015.20.3.136

Horwitz, D. F., & Rodan, I. (2018). Behavioral awareness in the feline consultation: Understanding physical and emotional health. Journal of Feline Medicine and Surgery, 20(5), 423–436. https://doi.org/10.1177/1098612X18771204

Huremović, D. (2019). Brief History of Pandemics (Pandemics Throughout History). In: Huremović, D. (editor). Psychiatry of Pandemics, Springer International Publishing. pp. 7–35. https://doi.org/10.1007/978-3-030-15346-5_2

Islam, M. S., Potenza, M. N., & van Os, J. (2021). Posttraumatic stress disorder during the COVID-19 pandemic: Upcoming challenges in Bangladesh and preventive strategies. International Journal of Social Psychiatry, 67(2), 205–206. https://doi.org/10.1177/0020764020954469

Jensen, J. B. H., Sandøe, P., & Nielsen, S. S. (2020). Owner-Related Reasons Matter more than Behavioural Problems—A Study of Why Owners Relinquished Dogs and Cats to a Danish Animal Shelter from 1996 to 2017. Animals, 10(6), 1064. https://doi.org/10.3390/ani10061064

Keeling, L., & Jensen, P. (2002). Behavioural Disturbances, Stress and Welfare. In: Jensen, P. (editor). The ethology of domestic animals: an introductory text, CABI Pub. pp. 79–98.

Keser, G. Ö. (2018). İşitsel, koku uyaranlarının ve insan temasının barinak köpeklerinin stres seviyeleri üzerine etkilerinin araştırılması. Uludağ Üniversitesi Sağlık Bilimleri Enstitüsü, Doktora Tezi. https://acikerisim.uludag.edu.tr/bitstream/11452/10200/3/G%c3%96KHAN%20%c3%96NDER%20KESER-K%c4%b1s%c4%b1tl%c4%b1%206%20ay.pdf

Köse, S. İ., Sağkan Öztürk, A., & Uyanık, G. (2022). How did SARS-CoV-2 pandemic affect the cats' health in Hatay province? A retrospective study. Topics in Companion Animal Medicine, 50, 100696. https://doi.org/https://doi.org/10.1016/j.tcam.2022.100696

Kuzi, S., Segev, G., Kedar, S., Yas, E., & Aroch, I. (2017). Prognostic markers in feline hepatic lipidosis: A retrospective study of 71 cats. Veterinary Record, 181(19). https://doi.org/10.1136/vr.104252

Lue, T. W., Pantenburg, D. P., & Crawford, P. M. (2008). Impact of the owner-pet and client-veterinarian bond on the care that pets receive. Journal of the American Veterinary Medical Association, 232(4), 531–540. https://doi.org/10.2460/javma.232.4.531

Maden, M. (2020). COVID-19 Pandemic: Health communication, one health and biosecurity. Turkiye Klinikleri Journal of Veterinary Sciences, 11(2), 81–91. https://doi.org/10.5336/vetsci.2020-76210

Moberg, G., & Mench, J. (2000). The biology of animal stress: basic principles and implications for animal welfare. CABI Publishing.

Piret, J., & Boivin, G. (2021). Pandemics throughout history. Frontiers in Microbiology, 11, 631736. https://doi.org/10.3389/fmicb.2020.631736

Rakesh, D., Tandon, K., Bhatia, V., & Tandon, R. K. (2005). Stress and the gastrointestinal tract. Journal of Gastroenterology and Hepatology, 20, 332–339. https://doi.org/10.1111/j.1400-1746.2004.03508.x

Republic of Türkiye Ministry of Interior. (2021). 81 İl Valiliğine Kademeli Normalleşme Tedbirleri Genelgesi Gönderildi. https://www.icisleri.gov.tr/81-il-valiligine-kademeli-normallesme-tedbirleri-genelgesi-gonderildi [Accessed Date: 18.07.2023]

Rodan, I. (2016). Importance of Feline Behavior in Veterinary Practice. In: Rodan, I. & Heath, S. (editors). Feline Behavioral Health and Welfare, Elsevier. pp. 2–11. https://doi.org/10.1016/B978-1-4557-7401-2.00001-5

Rodan, I., & Heath, S. (2016). Feline Behavior and Welfare. In: Rodan, I. & Heath, S. (editors). Feline Behavioral Health and Welfare, Elsevier. pp. 12–22. https://doi.org/10.1016/B978-1-4557-7401-2.00002-7

Ropski, M. K., Pike, A. L., & Ramezani, N. (2023). Analysis of illness and length of stay for cats in a foster-based rescue organization compared with cats housed in a cat café. Journal of Veterinary Behavior, 62, 1–11. https://doi.org/10.1016/j.jveb.2023.02.002

Rutgers, C. (1998). Feline liver disease. In Practice, 20(1), 16-25. https://doi.org/10.1136/inpract.20.1.16

Salman, M. D., Hutchison, J., Ruch-Gallie, R., Kogan, L., New, J. C., Kass, P. H., & Scarlett, J. M. (2000). Behavioral reasons for relinquishment of dogs and cats to 12 shelters. Journal of Applied Animal Welfare Science, 3(2), 93-106. https://doi.org/10.1207/s15327604jaws0302_2

Şeker, M., Özer, A., Tosun, Z., Korkut, C., & Doğrul, M. (2020). Covid-19 Pandemi Değerlendirme Raporu. Turkish Academy of Sciences. http://www.tuba.gov.tr/files/yayinlar/raporlar/1. Versiyon Covid-19 Pandemi Değerlendirme Raporu.pdf

Shoesmith, E., Shahab, L., Kale, D., Mills, D. S., Reeve, C., Toner, P., de Assis, L. S., & Ratschen, E. (2021). The influence of human–animal interactions on mental and physical health during the first COVID-19 lockdown phase in the U.K.: A qualitative exploration. International Journal of Environmental Research and Public Health, 18(3), 976. https://doi.org/10.3390/ijerph18030976

Stella, J., Croney, C., & Buffington, T. (2013). Effects of stressors on the behavior and physiology of domestic cats. Applied Animal Behaviour Science, 143(2–4), 157-163. **https://doi.org/10.1016/j.applanim.2012.10.014**

Stella, J. L., Lord, L. K., & Buffington, C. A. T. (2011). Sickness behaviors in response to unusual external events in healthy cats and cats with feline interstitial cystitis. Journal of the American Veterinary Medical Association, 238(1), 67-73. https://doi.org/10.2460/javma.238.1.67

Tanaka, A., Wagner, D. C., Kass, P. H., & Hurley, K. F. (2012). Associations among weight loss, stress, and upper respiratory tract infection in shelter cats. Journal of the American Veterinary Medical Association, 240(5), 570–576. https://doi.org/10.2460/javma.240.5.570

Wang, C., Horby, P. W., Hayden, F. G., & Gao, G. F. (2020). A novel coronavirus outbreak of global health concern. The Lancet, 395(10223), 470–473. https://doi.org/10.1016/S0140-6736(20)30185-9

Wood, L., Martin, K., Christian, H., Nathan, A., Lauritsen, C., Houghton, S., Kawachi, I., & McCune, S. (2015). The pet factor - companion animals as a conduit for getting to know people, friendship formation and social support. PLOS ONE, 10(4), e0122085. https://doi.org/10.1371/journal.pone.0122085

World Health Organization. (2020). Who statement regarding cluster of pneumonia cases in wuhan, China. https://www.who.int/china/news/detail/09-01-2020-who-statement-regarding-cluster-of-pneumonia-cases-in-wuhan-china [Accessed Date: 19.07.2023]

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CASE REPORT

Uterine Leiomyosarcoma and Bilateral Ovarian Cysts Subsequent to Mammary Tumor in a Guinea Pig (cavia porcellus)

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ABSTRACT

A 5-year-old, intact female guinea pig was brought to our clinic with a complaint of a large mass in the right mammary lobe. According to ultrasonographic examination, the mass was encapsulated, had a diameter of 8.1 cm, exhibited a polycystic structure, mixed echogenicity, and mixed vascularization. The patient underwent mastectomy. Histopathological analysis of the mass revealed a diagnosis of solid carcinoma (Grade-III). Approximately 2.5 months after mastectomy, the guinea pig developed abdominal swelling. Upon laparotomy, cysts were identified in both ovaries, and a mass containing leiomyosarcoma on the corpus uteri was detected. In conclusion, guinea pigs with a history of mammary tumors should be evaluated for genital organ pathologies as a precaution against the possibility of metastasis at the earliest opportunity.

Keywords: Guinea pig, Mammary tumor, Ovarian cysts, Uterine leiomyosarcoma

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Bir Kobayda (cavia porcellus) Meme Tümörü Sonrası Uterus Leiomyosarkomu ve Bilateral Over Kistleri

ÖZ

Beş yaşında, kısırlaştırılmamış, dişi kobay sağ meme lobunda bir kitle şikayeti ile kliniğimize getirildi. Ultrasonografik muayeneye göre kitle; kapsüllü, 8,1 cm çapında, polikistik yapıda, miks ekojenite ve miks vaskülarizasyona sahipti. Hastaya mastektomi uygulandı. Kitlenin histopatolojisi sonucunda solid karsinom teşhisi konuldu (Grade-III). Mastektomiden ortalama 2,5 ay sonra kobayda karın şişliği meydana geldi. Laparotomi sonrasında her iki overde de kist ve korpus uteri üzerinde leiomyosarkom içeren kitle saptandı. Sonuç olarak, meme tümörü öyküsü olan gine piglerde metastas ihtimaline karşı genital organ patolojileri klinik açıdan mümkün olan en kısa süre içerisinde değerlendirilmelidir.

Anahtar kelimeler: Kobay, Meme tümörü, Ovaryan kist, Uterus leiomyosarkomu.

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INTRODUCTION

Guinea pigs are hystricognath rodents whose lifespan are 6-8 years. Multiple cysts in the ovaries of guinea pigs are usually appeared after 1 year old of age (Donnelly 2021). Cystic changes in the ovaries cause infertility in guinea pigs (Keller et al. 1987). Cysts can be unilateral or bilateral and clinical diagnosis should be made by radiography and ultrasonography (Kohutova et al. 2018).

Types of cystic structures which are serous cysts (cystic rete ovarii), follicular cysts, and paraovarian cysts, can only differentiate by histopathological examination (Pilny 2014). Tumors of the reproductive tract constitute 25% of spontaneous tumors in guinea pigs. The incidence of spontaneous tumors increases in guinea pigs older than 3 years old (Greenacre 2004). The most common guinea pig uterine neoplasms were uterine leiomyomas (46%), followed by adenomas (23%) and leiomyosarcomas (0.7%) (Veiga-Parga et al. 2016). Leiomyosarcoma is a malignant neoplasm with uncertain pathogenesis that originate from smooth muscle cells (Rivas et al. 2014). Mammary tumours are not common, but can occur in both males and females and are most often found in older guinea pigs. Most lesions are benign fibroadenomas and approximately 30% are adenocarcinomas (Amorim et al 2009). Regarding mammary neoplasms, tubuler carcinoma (Oliveira et al. 2023), simple solid carcinomas and simple tubulopapillary carcinomas (Suárez-Bonnet et al. 2010) have been reported. The aim of the present case report is to exhibit the clinical and surgical approach to multiple reproductive pathologies in a female guinea pig.

CASE HISTORY

A five year old, intact, female guinea pig, weighing 1 kg was admitted to the Obstetrics and Gynecology clinic with a huge mass on the right mammary lobe. In the anamnesis, it was learned that the mass had been small for a year but had grown rapidly in the last 3 weeks. In clinical examination, the mass was not adhered to the abdominal muscles. On B-mode ultrasonography, the mass was capsulated, and 8.1 cm in diameter. It had polycystic structure and mixed echogenicity (Fig. 1A). Mix vascularization (both peripheral and central) was observed on color Doppler imaging (Fig. 1B). The examination was carried out with pulsed-wave Doppler USG to characterize the waveform of the vessels on the mammary mass (Fig. 1C).

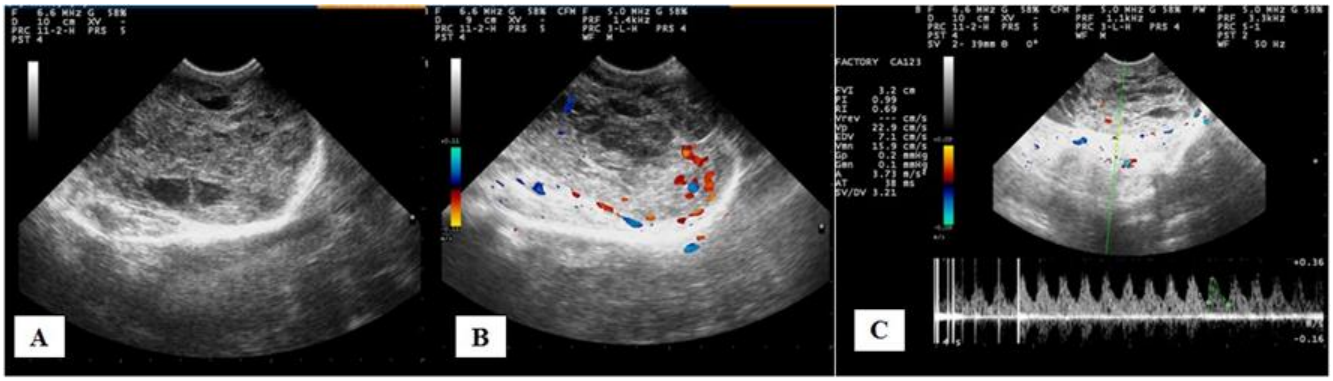

Figure 1. Ultrasonographic evaluation of a mass on the right mammary lobe. **A**. B-mode ultrasonography of the mass. **B**. Color Doppler ultrasonography and vascularization of the mass, **C**. Spectral Doppler ultrasonograhpy and hemodynamics of the mass.

Pulsatility index and resistance index were 0.99 and 0.69, respectively. A radiographic examination of the thorax was requested to investigate possible lung metastasis but was not authorized by the owner. To detect the abdominal metastases, abdominal ultrasonography could not be performed successfully due to the size of the mass in the mammary lobe. Lobectomy was decided for treatment. For general anesthesia, ketamine (50 mg/kg, im) (Alfamine 10%, EgeVet, Turkey) and xylazine (5 mg/kg, im) (Alfazyne 2%, EgeVet, Turkey) were used (Fish et al. 2008). The anesthesia was maintained with isoflurane (1-2%) (Forane, Abbott Laboratories, USA) and oxygen (1-1.5%). In order to prevent the decrease in body temperature due to anesthesia, a warm bed was prepared to put under the guinea pig during the surgical intervention. Because the large mass extended to the proximal part of the right leg (Fig. 2A), a Y-shaped incision was performed (Fig. 2B). Absorbable suture material (Monocryl No: 2/0, Medeks, Turkey) was used for all sutures. Enrofloxacin (Baytril 2.5% oral suspension, 10 mg/kg, BID for a week), meloxicam (0.3 mg/kg, sc) (Melox, Nobel, Turkey) and multivitamin (1 drop to each ounce of drinking water for 5 days) (Oasis Vita-Drops, USA) were prescribed for postoperative care. Wound healing was achieved in 10 days. Also, ovariohysterectomy was recommended to avoid the occurrence of the metastasis. In the macroscopic examination, no deterioration was observed in the

skin of the extirpated mass (Fig. 2C). On the cross-sectional surface of the mass, many vacuolar areas and tissue growths were detected (Fig. 2D).

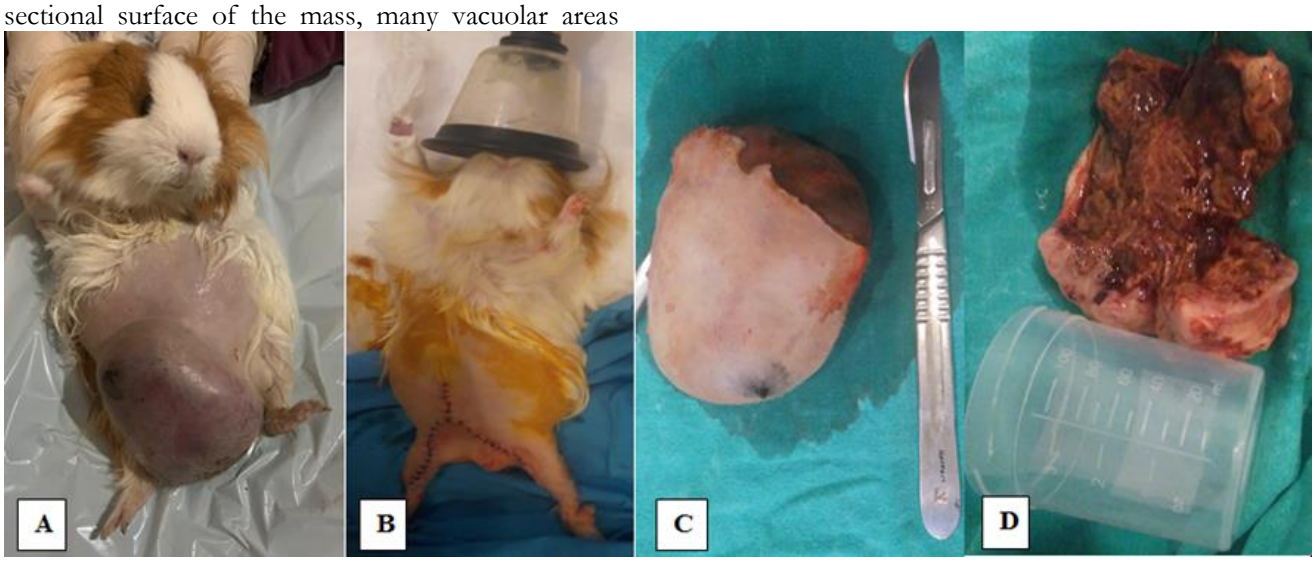

Figure 2. Guinea pig with mammary mass. **A**. Localization of a mass on the right mammary lobe. **B**. Appearrance of caudoabdominal area after mastectomy **C**. Macroscopic view of the excised mammary mass. **D**. Cross-sectional surface of the mass.

Tissue samples were fixed in 10% neutral buffered formalin, routinely processed, and stained with hematoxylin and eosin (HE) to be examined by light microscopy. The mammary tumor in the right lobe was histologically diagnosed as a simple solid carcinoma (Grade-III). The arrangement of the neoplastic epithelial cells in nests with high cellularity and a high mitotic rate was remarkable (Fig. 4 C-D). The female guinea pig was presented to the clinic with a complaint of abdominal swelling on 2.5 months after the lobectomy. According to the abdominal ultrasonography, cysts in both ovaries and a mass on corpus uteri were determined. On the right ovary, there were 2 cystic structures which were measured as 8.1 mm and 21 mm in diameter, respectively (Fig. 3A). On the left ovary, the cyst was 9 mm in diameter. Ovariohysterectomy (Fig. 3B) was performed as a treatment, with the same anesthesia protocol of the lobectomy section. Absorbable suture material (Monocryl No: 2/0, Medeks, Turkey) was used for all sutures. A mass on the corpus uteri was capsulated, solid and 9 cm in diameter.

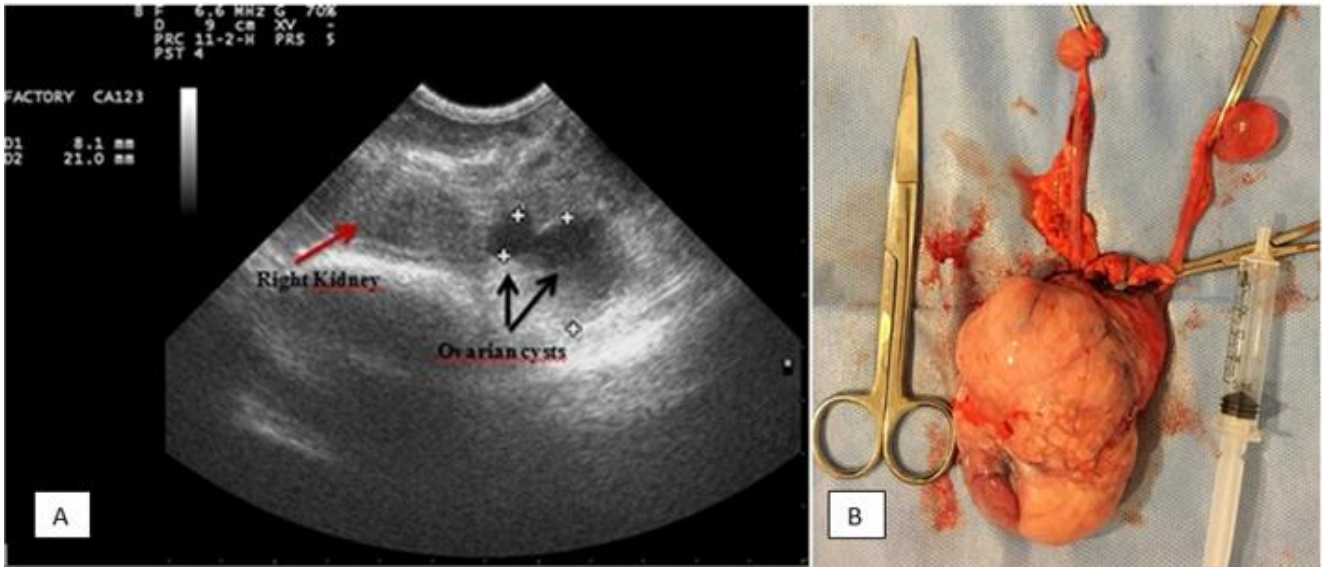

Figure 3. A. B-mode ultrasonography on right ovary **B**. Macroscopic view of the ovariohysterectomy material.

Histologic examination of the uterus revealed leiomyosarcoma characterized by interwoven fascicles of spindle cells with cigar-shaped nuclei that showed moderate pleomorphism (Fig. 4 A-B). Eight months after the last surgical intervention, the guinea pig died at the age of 6 years. It was thought to be due to the old age.

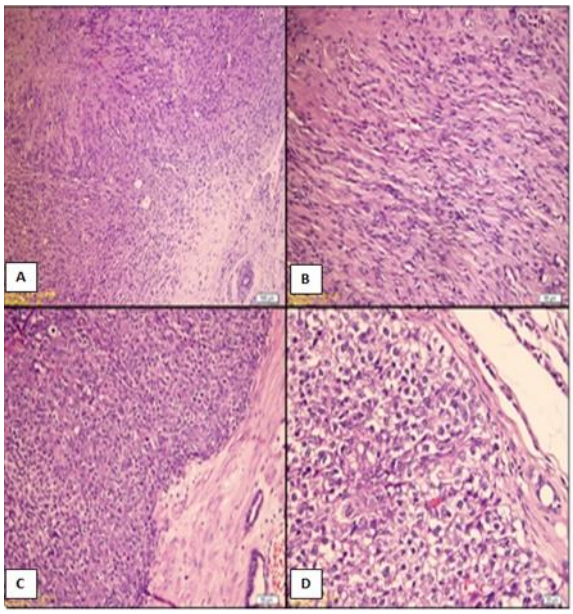

Figure 4. Histopathological examination. **A, B:** Uterus; guinea pig. Leiomyosarcoma. Interwoven fascicles of spindled cells with blunt-ended nuclei. HE. **C, D:** Mammary gland; guinea pig. Simple solid carcinoma(grade-III). HE.

DISCUSSION AND CONCLUSION

The lifespan of the guinea pigs are 6-8 years and spontaneous tumors usually occur in older than 3 years old while the ovarian cysts appear after 1 year old of age (Greenacre 2004; Donnelly 2021). In this case, gynecologic pathologies were diagnosed in an elderly guinea pig and its lifespan was 6 years, as previously reported. The prevalence of mammary tumors in male guinea pig is higher than in other species (Suarez-Bonnet et al. 2010). In contrast with the previous report, mammary tumor occurred in a female guinea pig in this case.

The vascularization of tumoral tissue can be assessed using noninvasive methods like power Doppler and color Doppler by performing the direct measurement of the distribution and flow of intratumoral blood vessels (Abma et al. 2019). The mixed pattern was frequently observed in malignant mammary tumors (Mantziaras and Luvani 2020). In accordance with the previous reports (Abma et al. 2019; Mantziaras and Luvani 2020), mixed vascularization was observed at the tumoral mass on the mammary gland by color Doppler ultrasonography in this case. There are few reports on mammary tumors in guinea pigs. Mammary tumors are generally locally invasive and rarely metastasize (Suarez-Bonnet et al. 2010). The researchers (Suarez-Bonnet et al. 2010) detected mammary carcinosarcoma in one case which was highly vascularized. Although simple solid carcinoma was detected in this case, the mammary tumor had high vascularization as Suarez-Bonnet et al. (2010) reported.

The researchers reported glandular cystic hyperplasia of the uterus and large polycystic ovaries complicated with adenoma in a 4 years old guinea pig with the sign of bilateral alopecia (Uyguner et al. 2021). In contrast with the researchers, glandular cystic hyperplasia of the uterus and bilateral alopecia were not observed in this case but large and polycystic ovaries were detected at bilaterally similar to the previously reported. The most common uterine neoplasms in guinea pigs are leiomyomas and followed by adenomas, leiomyosarcomas, anaplastic tumors of unknown origin and choriocarcinomas (Veiga-Parga et al. 2016). The researchers reported spontaneous reproductive tract leiomyomas usually in uterine body or horn of the aged guinea pigs. In contrast with the previos reports (Veiga-Parga 2016), leiomyosarcoma which was not the most common uterine pathology, was determined at corpus uteri in this case. A relationship exists between polycystic ovarian syndrome and gynaecological cancers in humans (Chittenden et al. 2009). The increased malignancy in the uterine pathology of the guinea pig in this case can be explained by the relationship between polycystic ovaries and gynecological pathologies as previously reported. Sua´rez-Bonnet et al. (2010) analyzed 10 spontaneous mammary gland tumors affecting guinea pigs histologically and immunohistochemically and they reported that all tumors were positive for type a estrogen and progesterone receptors, suggesting a role for steroid hormones in the development of these neoplasias in guinea pigs. Veiga-Parga et al. (2016) indicated that estrogen receptor and progesterone receptor expression was nearly 100% in uterine neoplasms of the guinea pigs. According to the previous reports, it is clear that there is a link between mammary cancer, ovarian cysts, and uterine neoplasia. Because of that reason, ovariohysterectomy was recommended to avoid the occurrence of the metastasis in this case.

It was concluded that guinea pigs with a history of mammary tumors should also be evaluated clinically in terms of internal genital tract pathologies. Surgical method is essential in the treatment of gynecological pathologies in guinea pigs and it is thought to be necessary to ensure the life welfare of these females.

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

Authors Contribution Rate: The authors declared that they contributed equally to the article.

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

REFERENCES

Abma, E., Stock, E., De Spiegelaere, W., Van Brantegem, L., Vanderperren, K., Ni, Y., Vynck, M., Daminet, S., De Clercq, S.,& De Rooster, H. (2019). Power Doppler ultrasound and contrast-enhanced ultrasound demonstrate non-invasive tumour vascular response to anti-vascular therapy in canine cancer patients. Scientific Reports, 9(1), 1-12. https://doi.org/10.1038/s41598-019-45682-2

Amorim, I., Faria, F., Rema, A., & Gärtner, F. (2009). Immunohistochemical Characterization of a Mammary Carcinoma in a Male Guinea Pig (Cavia porcellus). Journal of Comparative Pathology, 141(4), 279. https://doi.org/10.1016/j.jcpa.2009.08.008

Chittenden, B. G., Fullerton, G., Maheshwari, A., & Bhattacharya, S. (2009). Polycystic ovary syndrome and the risk of gynaecological cancer: a systematic review. Reproductive Biomedicine Online, 19(3), 398-405. https://doi.org/10.1016/S1472-6483(10)60175-7

Donnelly, T.M. (2021). Guinea pigs. Available at https://www.merckvetmanual.com/all-other-pets/guinea-pigs/disorders-and-diseases-of-guinea-pigs#v3227529 (Accessed Jan 06, 2021).

Greenacre, C. B. (2004). Spontaneous tumors of small mammals. Veterinary Clinics: Exotic Animal Practice, 7(3),627-651. https://doi.org/10.1016/j.cvex.2004.04.009

Fish, R.E., Brown, M.J., Danneman, P.J. & Karas, A.Z. (2008) Anesthesia and Analgesia in Laboratory Animals (2nd ed) Elsevier, San Diego, CA, U.S.A.

Keller, L. S. F., Griffith, J. W., & Lang, C. M. (1987). Reproductive failure associated with cystic rete ovarii in guinea pigs. Veterinary pathology, 24(4), 335-339. https://doi.org/10.1177/030098588702400408

Kohutova, S., Paninarova, M., Škorič, M., Jekl, V., Knotek, Z., & Hauptman, K. (2018). Cystic endometrial hyperplasia and bacterial endometritis associated with an intrauterine foreign body in a guinea pig with ovarian cystic disease. Journal of Exotic Pet Medicine, 27(1), 41-45. https://doi.org/10.1053/j.jepm.2017.10.001

Mantziaras, G., & Luvoni, G. C. (2020). Advanced ultrasound techniques in small animal reproduction imaging. Reproduction in Domestic Animals, 55, 17-25. https://doi.org/10.1111/rda.13587

Oliveira, R.E.M., Oliveira, R.K.M., Dalmagro, M.J., Silva, D.N., Gurgel, J.V.O., Sousa, A.C.F.C., Attademo, F.L.N., Soares, M.K.F., Arcoverde, K.N., Olinda, R.G., Santos, P.R.S., Torres, D.C.B., Rego, R.O., Diniz, J.A.R., Moura, C.E.B., Batista, J.S., Oliveira, M.F. (2023). Mammary tubular carcinoma in a guinea pig (Cavia porcellus): case report. Brazilian Journal of Case Reports, 3(4), 18-23. https://doi.org/10.52600/2763-583X.bjcr.2023.3.4.18-23

Pilny, A. (2014). Ovarian cystic disease in guinea pigs. Veterinary Clinics: Exotic Animal Practice, 17(1), 69-75. https://doi.org/10.1016/j.cvex.2013.09.003

Rivas, G., Bonilla, C., Rubiano, J., & Arango, N. (2014). Primary leiomyosarcoma of the ovary: A case report. Case Reports in Clinical Medicine, 3, 192-196. https://doi.org/10.4236/crcm.2014.34045

Suárez-Bonnet, A., Martín de Las Mulas, J., Millan, M. Y., Herráez, P., Rodríguez, F., & Espinosa de los Monteros, A. (2010). Morphological and immunohistochemical characterization of spontaneous mammary gland tumors in the guinea pig (Cavia porcellus). Veterinary pathology, 47(2), 298-305. https://doi.org/10.1177/0300985809358426

Uyguner, K., Enginler, S. Ö., Yıldar, E., Öztürk, G. Y., & Gülçubuk, A. (2021). Serous multicystic papillary adenoma of the ovaries and glandular cystic hyperplasia of uterus in a Guinea Pig (Cavia porcellus). Journal of Istanbul Veterinary Sciences, 5(3), 144-148. https://doi.org/10.30704/http-www-jivs-net.999648

Veiga-Parga, T., La Perle, K. M., & Newman, S. J. (2016). Spontaneous reproductive pathology in female guinea pigs. Journal of Veterinary Diagnostic Investigation, 28(6),656-661.https://doi.org/10.1177/1040638716665429