ERCİYES ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ
Journal of Faculty of Veterinary Medicine, Erciyes University

İÇİNDEKİLER / CONTENTS **21(3): 143-208, 2024**

Sayfa / Page

ARAŞTIRMA MAKALELERİ / RESEARCH ARTICLES

DERLEMELER / REVIEW ARTICLES

Journal of Faculty of Veterinary Medicine, Erciyes University
21(3): 143- 208, 2024
Erciyes Üniversitesi Veteriner Fakültesi Dergisi

ERCİYES ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Journal of Faculty of Veterinary Medicine, Erciyes University

ISSN-1304-7280

Yıl/Year: 2024
Cilt/Volume: 21
Sayı/Number: 3

Yılda 3 sayı yayımlanır / Published 3 issues per year
http://ercivet.erciyes.edu.tr
E-posta: ercvet@gmail.com

ISSN-1304-7280

Erciyes Üniversitesi Veteriner Fakültesi Dergisi

Journal of Faculty of Veterinary Medicine, Erciyes University

Yılda 3 sayı yayımlanır
Published 3 issues per year

Bu dergi Web of Science- Zoological Records, EBSCO Host, CABI Abstracts, World Agricultural Economicsand Rural Sociology Abstract, Global Health, Tübitak-Ulakbim TR Dizin ve Türkiye Atıf Dizini tarafından dizinlenmektedir.

This journal is reviewed by Web of Science- Zoological Records, EBSCO Host, CABI Abstracts, World Agricultural Economicsand Rural Sociology Abstract, Global Health, Tubitak-Ulakbim TR Dizin and Turkey Citation Index.

Yıl / Year : 2024
Cilt / Volume : 21
Sayı / Number : 3

http://ercivet.erciyes.edu.tr
E-posta: ercvet@gmail.com

Baskı Tarihi: Aralık 2024

Erciyes Üniversitesi Veteriner Fakültesi Dergisi
Journal of Faculty of Veterinary Medicine, Erciyes University
Yılda 3 sayı yayımlanır
Published 3 issues per year

Sahibi / Owner
Erciyes Üniversitesi Veteriner Fakültesi Adına
Prof. Dr. Murat KANBUR (Dekan)

Yazışma Adresi / Correspondence
Erciyes Üniversitesi, Veteriner Fakültesi
Dergisi Editörlüğü
38039-Kayseri / TÜRKİYE

http://ercivet.erciyes.edu.tr
E-posta : ercvet@gmail.com
Tel : 0 352 339 94 84
Fax : 0 352 337 27 40

Yayın Türü / Publication Type: Yaygın süreli ve hakemli/ Common term and peer reviewed
Mizanpaj / Designer: Erhan GÜMÜŞ
Basım / Print: Erciyes Üniversitesi Matbaası, Melikgazi/KAYSERİ
ISSN-1304-728

İÇİNDEKİLER / CONTENTS

Sayfa / Page

ARAŞTIRMA MAKALELERİ / RESEARCH ARTICLES

DERLEMELER / REVIEW ARTICLES

ERCİYES ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ
Journal of Faculty of Veterinary Medicine, Erciyes University
Araştırma Makalesi / Research Article
21(3), 143-147, 2024
DOI: 10.32707/ercivet.1514585

Hesperidin Mikroemülsiyonunun Bazı Standart (ATCC) Gram Negatif Bakterilere Karşı Antimikrobiyal Etkinliğinin Tespiti

Mukaddes Barel[1,a]

[1]Erciyes Üniversitesi, Veteriner Fakültesi, Veteriner Halk Sağlığı Anabilim Dalı, Kayseri-TÜRKİYE
ORCID: [a]0000-0002-1170-8632

Sorumlu yazar: Mukaddes BAREL; E-posta: mukaddesbarel@erciyes.edu.tr
Atıf yapmak için: Barel M. Hesperidin mikroemülsiyonunun bazı standart (ATCC) Gram negatif bakterilere karşı antimikrobiyal etkinliğinin tespiti. Erciyes Univ Vet Fak Derg 2024; 21(3):143-147

Öz: Sentetik antibiyotiklere karşı artan antimikrobiyal direnç, doğal olarak oluşan bileşiklerin etkili antibakteriyel ajanlar olarak kullanılması bilim insanlarının ilgisini çekmiştir. Doğal olarak oluşan bileşiklerden Hesperidin'in farklı patojen bakterilere karşı da etkili olabileceği, bakteriyel büyümeyi doğrudan engelleyebileceği belirtilmiştir. Bu çalışmada, Hesperidin'in bazı ATCC suşlarına (*Escherichia coli* ATCC 25922, *Escherichia coli* O157;H7 ATCC 43895, *Salmonella* enteriditis NCTC, *Klebsiella pneumonie* ATCC 1705, *Aeromonas hydrophila* ATCC 7966) karşı etkinliğinin belirlenmesi amaçlanmıştır. Yapılan minimal inhibisyon konstrasyon (MİK) testi sonuçlarına göre Hesperidin için *E. coli*, *E. coli* O157;H7, *S. enteriditis* ve *K. pneumonie* bakterilerine ait MİK değerleri 128 µg/mL iken, *A. hydrophila* bakterisine ait MİK değeri 64 µg/mL olarak belirlenmiştir. Bu sonuçlara ek olarak, pozitif kontrol kolistin için *E. coli, K. pneumonie ve A. hydrophila* bakterilere ait MİK değerleri 0.5 µg/mL olarak belirlenirken, *E. coli* O157;H7 ve *S. enteriditis* bakterilerine ait MİK değerleri sırasıyla 0.5 ve 1 µg/mL olarak belirlenmiştir. Bu çalışma sonuçları Hesperidin'in özellikle su ürünleri yetiştiriciliğinde koruyucu ve alternatif bir antibakteriyel tedavi seçeneği olarak yenilikçi kanıtlar sunabilir.
Anahtar kelimeler: Gram negatif bakteri, Hesperidin, minimal inhibitör konsantrasyon

Determination of Antimicrobial Activity of Hesperidin Microemulsion Against Some Standard (ATCC) Gram Negative Bacteria

Abstract: Increasing antimicrobial resistance to synthetic antibiotics has attracted the interest of scientists in using naturally occurring compounds as effective antibacterial agents. It has been stated that hesperidin can also be effective against different pathogenic bacteria and can directly inhibit bacterial growth. This study aimed to determine the effectiveness of Hesperidin against various ATCC strains (*E. coli* ATCC 25922, *E. coli* O157;H7 ATCC 43895, *S. enteriditis* NCTC, *K. pneumonie* ATCC 1705, *A. hydrophila* ATCC 7966). According to the MIC test results, the minimal ınhibitory concentration (MIC) values of *E. coli*, *E. coli* O157;H7, *S.* enteriditis and *K. pneumonia* bacteria for hesperidin were determined as 128 µg/mL, while the MIC value of *A. hydrophila* bacteria was determined as 64 µg/mL. In addition to these results, the MIC values for colistin for *E. coli*, *K. pneumonia* and *A. hydrophila* bacteria were determined as 0.5 µg/mL, while the MIC values for *E. coli* O157;H7 and *S. enteriditis* bacteria were 0.5 and 1 µg/mL, respectively. was determined as. These study results provide innovative evidence for hesperidin as a preventive and alternative antibacterial treatment, especially in aquaculture.
Keywords: Gram negative bacteria, Hesperidin, minimal inhibitory concentration

Giriş

Hesperidin (C16H14O6) 3',5,7-trihidroksi-4'-metoksi flavanon olarak adlandırılmaktadır. Flavonoidler, bitkilerin farklı kısımlarında geniş çapta dağılan, düşük molekül ağırlıklı polifenolik bitki hücrelerinde bulunan ve tat ve renkten sorumlu olan bir grup bileşiklerdir. Hesperidin, flavonoidlerin flavanon grubuna ait olup, aglikon kısmı ve rutinoz adı verilen bir disakkarit kısmından (glikoz ve ramnoz) oluşmaktadır. Hesperidin, portakal, turunç, limon, greyfurt gibi turunçgillerin farklı kısımlarından, özellikle beyaz kabuklarından önemli miktarlarda doğal olarak elde edilebilmektedir.

Geliş Tarihi/Submission Date : 28.11.2023
Kabul Tarihi/Accepted Date : 13.02.2024

Gram negatif bakteriler arasında 1970'lerde başlayan antibiyotik direncindeki artan artış, kritik bir küresel kriz haline gelmiştir. Son yıllardaki önemli sorun, spesifik patojenleri, özellikle de hastane kaynaklı enfeksiyonlara neden olan, insanlar arasında yayılma potansiyeli olan ve farklı antibiyotiklere karşı direnç geliştirmiş patojenleri tedavi etmek için kullanılabilecek olası alternatiflerin tükenmesidir. Söz konusu bu sebebe yönelik olarak ilaç endüstrisinde yeni antibiyotiklerin keşfinin azalması ile kolistin antibiyotiğine yeniden ilginin artmasına yol açmıştır. Bu bağlamda kolistin Gram negatif patojenlerin neden olduğu enfeksiyonlara karşı kullanılmak üzere yeniden ortaya çıkmıştır (Falagas ve ark., 2005; Bialvaei ve ark., 2015). Son yıllarda sentetik antibiyotiklere karşı artan antimikrobiyal direnç, doğal olarak oluşan bileşiklerin etkili

antibakteriyel ajanlar olarak kullanılmasına neden olmuştur (Guazelli ve ark., 2021). Birçok rapor, Hesperidin'in farklı patojen bakterilere karşı da etkili olabileceğini, bakteriyel büyümeyi doğrudan engelleyebileceğini veya virülens genlerini eksprese ederek dolaylı olarak etki ettiğini göstermiştir (Corciova ve ark., 2015; Farhadi ve ark., 2019; González ve ark., 2021; Suriyaprom ve ark., 2022). Truchado ve ark. (2012), tarafından yapılan bir çalışmada, glikosile flavanonlar (naringin, hesperidin ve neohesperidin) bakımından zengin ekstraktın *Chromobacterium violaceum* ve *Yersinia enterocolitica* üzerindeki inhibitör etkisi test edilmiş ve portakal ekstraktının ve ana flavanon bileşenlerinin *Chromobacterium violaceum* bakterisinde çekirdek algılamayı engellediği rapor edilmiştir (Truchado ve ark., 2012). Ayrıca Hesperidin'in *Yersinia enterocolitica* bakterisinde çevreye salgıladığı laktonların seviyesini azalttığı ve bakteri üremesini inhibe etmeden, biyofilm üretimini inhibe ettiği belirtilmiştir (Bouyahya ve ark., 2022; Chaieb ve ark., 2022). Hesperidin'in farklı merkezi sinir sistemi (MSS) bozukluklarında önemli antioksidan, antiinflamatuar ve nöroprotektif etkiler sağladığı belirtilmiştir (Ikram ve ark., 2019; Muhammad ve ark., 2019). Ayrıca Hesperidin ile yapılan bir çalışmada insanlarda lipopolisakkarid kaynaklı hafıza bozukluğunu in vitro ve in vivo olarak azalttığı rapor edilmiştir (Muhammad ve ark., 2019). Yapılan başka çalışmada streptozotosin verilen diyabetik ve Parkinson hastalığı olan farelerde Hesperidin'in antidepresif etkiye sahip olduğu da belirtilmiştir (Muhammad ve ark., 2019; Antunes ve ark., 2020). Hesperidin aynı zamanda yukarıda belirtilenler dışında UV koruması, yara iyileşmesi ve kutanöz fonksiyonlar ve iyonlaştırıcı radyasyonun neden olduğu hasara karşı radyokoruyucu koruma gibi diğer yararlı sağlık etkileriyle de ilişkilendirilmiştir. Kılcal kırılganlığı azalttığı bu sebeple venöz dolaşım bozukluklarının (bacaklarda şişme, ağrı, gece krampları) ve akut hemoroidal atağa bağlı semptomların tedavisinde bazı araştırmacılar tarafından tavsiye edilmiştir (Hu ve ark., 2018; Fraga ve ark., 2019; Man ve ark., 2019; Musa ve ark., 2019). Bu çalışmada, Hesperidin'in çeşitli bakterilere (*E. coli* ATCC 25922, *E. coli* O157;H7 ATCC 43895, *S. enteriditis* NCTC, *K. pneumonie* ATCC 1705, *A. hydrophila* ATCC 7966) karşı antimikrobiyal etkinliğinin belirlenmesi amaçlanmıştır.

Gereç ve Yöntem

Antibakteriyel etkisinin belirlenmesi amacıyla kullanılan Hesperidin (Sigma-Aldrich Katalog no.520-26-3) ve kolistin (Sigma-Aldrich, Katalog no: C4461) Sigma -Alderich firmasından temin edilmiştir. Stok solüsyonları 100mL/mg dimetil sülfat oksit (DMSO) ile hazırlanmıştır (Karayıldırım, 2017).

Antibakteriyel aktivitenin belirlenmesi

Hesperidin'in antibakteriyel aktivitesi, beş farklı Gram negatif bakterisi (*E. coli* ATCC 25922, *E. coli* O157;H7 ATCC 43895, *S. enteriditis* NCTC, *K. pneumonie* ATCC 1705, *A. hydrophila* ATCC 7966) kullanılarak test edilmiştir. Söz konusu suşlar Blood Agar'a (Merck, Germany Katalog no: 103879) ekilerek 37^{o}C 'de 18-24 saat inkübasyona bırakılmıştır. Analize tabi tutulan ATCC suşlar aracı firmadan (Elips laboratuvar ürünleri San. Ve Tic. Ltd. Şti) temin edilmiştir (Karayıldırım, 2017).

Minimum inhibitör konsantrasyon (MİK) testi

MİK'in belirlenmesi, CLSI (2007) tarafından açıklanan mikrodilüsyon yöntemine göre gerçekleştirilmiştir. Kanlı Agarda (Merck, Germany Katalog no: 103879) 37^{o}C'de 18-24 saat boyunca üreyen bakteriler 1:100 (h/h) oranında ayarlanan Mueller Hinton broth (Merck, Germany, Katalog no: 103872) içerisine inokule edilerek McFarland (0.5 CFU/1.5X10^{8}) standartına göre ayarlanmıştır. Hazırlanmış Hesperidin stok solüsyonları 96 kuyucuklu mikrotitre platelerine (ISOLAB) daha önceden eklenmiş broth üzerine inokule edilmiştir. Kuyucuklardaki Hesperidin konsantrasyonları 256 ile 0.5 µg/mL arasında değişmiştir. Son kuyucukta ise negatif kontrol olarak 100 µL broth ve 100µL bakteri süspansiyonu kullanılmıştır. Tüm platelerin üzeri steril bir plate kapağı ile kapatılarak ve 37 ° C'de 24 saat inkübe edilmiştir. Kuyucuklarda mevcut süspansiyonlar Mueller Hinton agarda (Merck, Germany, Katalog no:103872) ekilerek inkübasyona bırakılmıştır. Hesperidin mikroemülsiyonun etkinliğinin karşılaştırılması amacıyla kolistin antibiyotiği kullanılmıştır. Daha önceden hazırlanan kolistin antibiyotik stok solüsyonları kuyucuklarda 4 ile 0.36 µg/mL arasında olacak şekilde ayarlanmıştır.

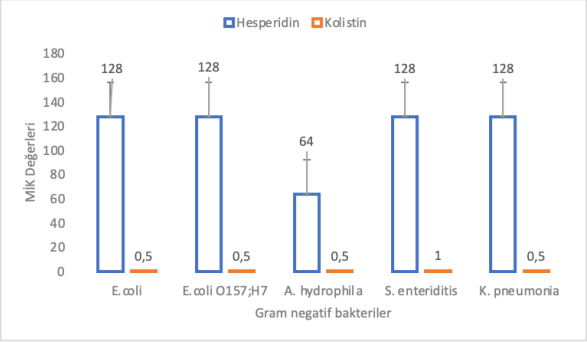
İstatistiksel analiz

Hesperidin'in beş farklı bakteriye karşı MİK değerlerinin sonuçları, Microsoft Office Excel 2019 (Microsoft Corporation, Redmond, WA, ABD) kullanılarak ortalama+standart sapma cinsinden ifade edilmiştir. Ortalamalar arasındaki önemli farklılıklar T-testi (Windows 11 için SPSS; one way ANOVA) ile belirlenmiştir.

Bulgular

Yapılan MİK testi sonuçlarına göre Hesperidin için *E. coli*, *E. coli* O157;H7, S. *enteriditis* ve *K. pneumonie* bakterilerine ait MİK değerleri 128 µg/mL iken, *A. hydrophila* bakterisine ait MİK değeri 64 µg/mL olarak belirlenmiştir. Bu sonuçlara ek olarak, kolistin için *E. coli, K. pneumonie ve A. hydrophila* bakterilerine ait MİK değerleri 0.5 µg/mL olarak belirlenirken, *E. coli* O157;H7 ve S. *enteriditis* bakterilerine ait MİK değerleri sırasıyla 0.5 ve 1 µg/mL olarak belirlenmiştir (Şekil 1). Yapılan tek yönlü varyans analizi (ANOVA) sonucunda MİK yöntemi sonuçlarına göre Gr (-) bak-

teriler arasında MİK değerleri açısından istatistiksel farklılık olmadığı görüldü (P>0,05).



Şekil 1. Çeşitli Gram negatif standart bakterilere göre belirlenen Hesperidin ve Colistin MİK değerleri (µg/mL).

Tartışma ve Sonuç

Hesperidin antioksidan, antiinflamatuar ve antibakteriyel özellikleriyle bilinen bir flavanondur. Ek olarak, önemli oranda tıbbi değere sahip olduğu ve antimikrobiyal, analjezik ve immünomodülatör olduğu farmakolojik olarak kanıtlanmıştır (Yamamoto ve ark., 2000; Gar ve ark.,2001).

Bu çalışmada Hesperidin'in *A. hydrophila* için MİK değeri 64 µg/mL olarak belirlenmiştir. Bu çalışma sonucuna benzer olarak, Abuelsaad ve ark. (2013) tarafından Hesperidin'in *A. hydrophila* bakterisine karşı etkisinin araştırıldığı bir çalışmada MİK değerlerinin 100-12.5 mg/mL arasında değiştiği belirlenmiştir. Mevcut çalışmamızda Hesperidin'in *E. coli*, *E. coli* O157;H7 ve *K. pneumoniae* bakterileri için MİK değerleri 128 µg/mL olarak bulunmuştur. Balakrishnan ve ark. (2021) tarafından yapılan başka bir çalışmada *E. coli, K. pneumoniae* ve *P aeruginosa* bakterilerine karşı Hesperidin'in $MİK_{50}$ değerinin 92.17±3.71µg/mL aralığında olduğu bildirilmiştir. Çalışmamızda Hesperidin'in *E. coli*, *E. coli* O157;H7, S. *enteriditis* bakterileri için MİK değerleri 128µg/mL bulunmuştur. Bu çalışma sonucundan farklı olarak, Yi ve ark. (2008) Hesperidin ile yapılan bir çalışmada *E.coli* ve *S. typhi* bakterilerine ait MİK değerlerinin 800µg/mL olarak bildirilmiştir. Yapılan başka bir çalışmada, Abass ve ark. (2014) *E. coli* ve *S. typhi* bakterilerine ait MİK değerlerini 175-450 µg olarak bulmuştur. Bu çalışmadan farklı olarak Hesperidin'in antimikrobiyel etkisinin araştırıldığı çalışmada *Helicobacter pylori* bakterisine ait MİK değerinin >200 µM olarak bildirilmiştir (Moon ve ark., 2013).

Klinik açıdan önemli Gram-negatif bakteriler arasında çoklu ilaç direncinin ortaya çıkması, kolistin gibi eski antibiyotiklerin yeniden klinik kullanımını artırmıştır. Söz konusu bu artış, *P. aeruginosa*, *E. coli* ve *Klebsiella* spp. gibi Gram-negatif patojen bakterilerde kolistine direncin oraya çıkmasına neden olmuştur. Gram negatif bakterilerde kolistin antibiyotiğe direnç mobil elementlerde bulunmasının mutasyonların yanı sıra kromozomal mutasyonların aracılık ettiği belirtilmiştir (Liu ve ark., 2016; Xavier ve ark., 2016). Bu durum hem hasta bakımı hem de epidemiyolojik sürveyans için klinik mikrobiyoloji laboratuvarları tarafından standartlaştırılmış in vitro duyarlılık testlerine olan acil ihtiyacın önemini artırmıştır. Bununla birlikte, kolistinin katyonik yapısı, plastiğe afinitesi ve agarda zayıf yayılabilirliği gibi doğal özellikleri nedeniyle standartlaştırılmış in vitro duyarlılık testlerini zorlaştırmıştır (Sader ve ark., 2012; Hindler ve Humphries, 2013).

Bu çalışmada kolistin için *E. coli, K. pneumonie ve A. hydrophila* bakterilere ait MİK değerleri 0.5 µg/mL olarak belirlenirken, *E. coli* O157;H7 ve S. enteriditis bakterilerine ait MİK değerleri sırasıyla 0.5 ve 1 µg/mL olarak belirlenmiştir. Bu çalışma sonucuna benzer olarak, yapılan bir çalışmada *E. coli* ve *P. aeruginosa* için MİK değerlerini 0.5–4 µg/ml ve 1–2 µg/ml olarak bildirilmiştir. Başka bir çalışmada *E. coli ve K. pneumonie* için MİK değerleri sırasıyla 8-0.25 µg/ml ve 32-0.5 µg/ml aralığında değiştiği rapor edilmiştir (Matuschek ve ark., 2018). Ancak, bu çalışma sonucundan yüksek olarak, Tan ve Ng, (2006) tarafından yapılan çalışmada ise *E. coli ve K. pneumonie* için MİK değerleri sırasıyla MIC90 ≤2 mg/L ve MIC90 ≤1 mg/L olarak rapor edilmiştir. Buna ek olarak Esposito ve ark. (2018) tarafından yapılan bir çalışmada *K. pneumoniae* suşları için MİK değerlerinin 4-256 mg/L aralığında değiştiği belirtilmiştir. Bir başka çalışmada ise *E. coli* ve *S. enterica* bakterilerine ait MİK değerleri 0.5 µg/mL ve 8 µg/mL olarak bildirilmiştir (Morales ve ark., 2012). Mevcut çalışmamız Hesperidin mikroemülsiyonunun nispeten yüksek bir antibakteriyel tepkiye sahip olduğunu göstermiştir. Buna karşın daha önce yapılan çalışmada Hesperidin'in Gram negatif bakterilere karşı nispeten daha yüksek antibakteriyel etkinliğe sahip olduğu rapor edilmiştir (Basile ve ark., 2000). Ayrıca Hesperidin ile ön tedavinin farelerde enfeksiyonun neden olduğu endotoksik şoku baskılayabildiği ve enfeksiyon sırasında bakteri kolonilerini azaltabildiği gösterilmiştir (Kawaguchi ve ark., 2004).

Sonuç olarak, bu çalışma Hesperidin'in *A. hydrophila* üremesini önemli oranda azalttığını göstermiştir. Buna ilaveten, Hesperidin'in özellikle su ürünleri yetiştiriciliğinde koruyucu ve alternatif bir antibakteriyel tedavi seçeneği olabileceğine ilişkin yenilikçi kanıtlar sunabilir.

Kaynaklar

Abass EH, Zair SK, Mohammud TK, Nagem SA. Recovery of pure Hesperidin from Iraqi Sweet Oranges Peel and study the effect in some bacteria. Baghdad Sci J 2014; 11(2): 455-60.

Abuelsaad AS, Mohamed I, Allam G, Al-Solumani AA. Antimicrobial and immunomodulating activities

of hesperidin and ellagic acid against diarrheic *Aeromonas hydrophila* in a murine model. Life Sci 2013; 93(20); 714-22.

Antunes MS, Cattelan Souza L, Ladd FVL, Ladd AABL, Moreira AL, Bortolotto VC, Araújo SM, Prigol M, Nogueira CW, Boeira SP. Hesperidin ameliorates anxiety-depressive-like behavior in 6-OHDA model of Parkinson's disease by regulating striatal cytokine and neurotrophic factors levels and dopaminergic innervation loss in the striatum of mice. Mol Neurobiol 2020; 57: 3027-41.

Balakrishnan, K, Casimeer SC, Ghidan AY, Al Antary TM, Singaravelu A. Exploration of antioxidant, antibacterial activities of green synthesized hesperidin loaded PLGA nanoparticles. Biointerface Res Appl Chem 2021; 11(6): 14520-28.

Basile A, Sorbo S, Giordano S, Ricciardi L, Ferrara S, Montesano D, Castaldo CR, Vuotto ML, Ferrara L. Antibacterial and allelopathic activity of extract from *Castanea sativa* leaves. Fitoterapia 2000; 1: 110-6.

Bialvaei AZ, Samadi Kafil H. Colistin, mechanisms and prevalence of resistance. Current Med Res Opinion 2015; 31(4): 707-21.

Bouyahya A, Chamkhi I, Balahbib A, Rebezov M, Shariati MA, Wilairatana P, El Omari N. Mechanisms, anti-quorum-sensing actions, and clinical trials of medicinal plant bioactive compounds against bacteria: A comprehensive review. Molecules 2022; 27(5): 1484.

Chaieb K, Kouidhi B, Hosawi SB, Baothman OA, Zamzami MA, Altayeb HN. Computational screening of natural compounds as putative quorum sensing inhibitors targeting drug resistance bacteria: Molecular docking and molecular dynamics simulations. Comput Biol Medi 2022; 145: 105517.

Corciova A, Ciobanu C, Poiata A, Mircea C, Nicolescu A, Drobota M, Marangoci N. Antibacterial and antioxidant properties of hesperidin: β-cyclodextrin complexes obtained by different techniques. J Inclusion Phenom Macrocycl Chem 2015; 81: 71-84.

Esposito EP, Cervoni M, Bernardo M, Crivaro V, Cuccurullo S, Imperi F, Zarrilli R. Molecular epidemiology and virulence profiles of colistin-resistant *Klebsiella pneumoniae* blood isolates from the hospital agency "Ospedale dei Colli," Naples, Italy. Front Microbiol 2018; 9: 1463.

Farhadi F, Khameneh B, Iranshahi M, Iranshahy M. Antibacterial activity of flavonoids and their structure-activity relationship: An update review. Phytother Res 2019; 33(1): 13-40.

Falagas, M. E., Kasiakou, S. K., Saravolatz, L. D. Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. Clinic Infect Dis 2005; 40(9): 1333-41.

Fraga CG, Croft KD, Kennedy DO, Tomás-Barberán FA. The effects of polyphenols and other bioactives on human health. Food Funct 2019; 10(2): 514-28.

Gar A, Garg S, Zaneveld LJD, Singla AK. Chemistry and pharmacology of the citrus bioflavonoid hesperidin. Phytotherapy Res 2001; 15: 655-69.

González A, Casado J, Lanas Á. Fighting the antibiotic crisis: Flavonoids as promising antibacterial drugs against *Helicobacter pylori* infection. Front Cell İnfect Microbi 2021; 11: 709749.

Guazelli CF, Fattori V, Ferraz CR, Borghi SM, Casagrande R, Baracat MM, Verri Jr WA. Antioxidant and anti-inflammatory effects of hesperidin methyl chalcone in experimental ulcerative colitis. Chem Biol Interact 2021; 333: 109315.

Hindler JA, Humphries RM. Colistin MIC variability by method for contemporary clinical isolates of multidrug-resistant Gram-negative bacilli. J Clinic Microbiol 2013; 51(6): 1678-84.

Hu Y, Li Y, Zhang W, Kou G, Zhou Z. Physical stability and antioxidant activity of citrus flavonoids in arabic gum-stabilized microcapsules: Modulation of whey protein concentrate. Food Hydrocoll 2018; 77: 588-97.

Ikram M, Muhammad T, Rehman SU, Khan A, Jo MG, Ali T, Kim MO. Hesperetin confers neuroprotection by regulating Nrf2/TLR4/NF-κB signaling in an Aβ mouse model. Mol Neurobiol 2019; 56: 6293 -309.

Kawaguchi K, Kikuchi S, Hasunuma R, Maruyama H, Yoshikawa T, Kumazawa YA citrus flavonoid hesperidin suppresses infection-induced endotoxin shock in mice, Biol Pharm Bullet 2004; 27: 679-83.

Karayıldırım ÇK. Characterization and in vitro evolution of antibacterial efficacy of novel hesperidin microemulsion. Celal Bayar Univ J Sci 2017; 13(4): 943-7.

Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, 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): 161-8.

Man MQ, Yang B, Elias PM. Benefits of hesperidin for cutaneous functions. J Evid Based Complementary Altern Med 2019: 2676307.

Matuschek E, Åhman J, Webster C, Kahlmeter G.

Antimicrobial susceptibility testing of colistin-evaluation of seven commercial MIC products against standard broth microdilution for *Escherichia coli, Klebsiella pneumoniae*, *Pseudomonas aeruginosa,* and *Acinetobacter* spp. Clinic Microbiol Infect 2018; 24(8): 865-70.

Moon SH, Lee JH, Kim KT, Park YS, Nah SY, Ahn DU, Paik HD. Antimicrobial effect of 7-O-butylnaringenin, a novel flavonoid, and various natural flavonoids against *Helicobacter pylori* strains. Int J Environ Res Public Health 2013; 10(11): 5459-69.

Morales AS, Fragoso de Araújo J, de Moura Gomes VT, Reis Costa AT, Prazeres Rodrigues DD, Porfida Ferreira TS, Rodrigues DP Ferreira TSP, De lima Filsner PHN, Felizardo MR, Micke Moreno A. Colistin resistance in *Escherichia coli* and *Salmonella* Enterica strains isolated from swine in Brazil. Sci World J 2012: 109795.

Muhammad T, Ikram M, Ullah R, Rehman SU, Kim MO. Hesperetin, a citrus flavonoid, attenuates LPS-induced neuroinflammation, apoptosis and memory impairments by modulating TLR4/NF-κB signaling. Nutrients 2019; 11(3): 648.

Musa AE, Omyan G, Esmaely F, Shabeeb D. Radioprotective effect of hesperidin: A systematic review. Medicina 2019; 55(7): 370.

National Committee for Clinical Laboratory Standards: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, Approved Standard-Eighth Edition, Wayne, Pennsylvania: NCCLS Document M7-A6, 2007.

Sader HS, Rhomberg PR, Flamm RK, Jones RN. Use of a surfactant (polysorbate 80) to improve MIC susceptibility testing results for polymyxin B and colistin. Diagn Microbiol Infect Dis 2012; 74(4): 412-4.

Suriyaprom S, Mosoni P, Leroy S, Kaewkod T, Desvaux M, Tragoolpua Y. Antioxidants of fruit extracts as antimicrobial agents against pathogenic bacteria. Antioxidants 2022; 11(3): 602.

Tan TY, Ng SY. The in-vitro activity of colistin in Gram-negative bacteria. Singapore Med J 2006; 47 (7); 621.

Truchado P, Gimenez-Bastida JA, Larrosa M, Castro-Ibáñez I, Espín JC, Tomas-Barberan FA, MT, Allende A. Inhibition of quorum sensing (QS) in *Yersinia enterocolitica* by an orange extract rich in glycosylated flavanones. J Agric Food Chem 2012; 60 (36): 8885-94.

Xavier BB, Lammens C, Ruhal R, Kumar-Singh S, Butaye P, Goossens H, Malhotra-Kumar S. Identification of a novel plasmid-mediated colistin-resistance gene, *mcr-2*, in *Escherichia coli,* Belgium, June 2016. Eurosurveillance 2016; 21(27): 30280.

Yamamoto M, Jokura H, Hashizume K, Oninami H, Shibuya Y, Suzuki A, Hase T, Shimotoyodome A. Hesperidin metabolite hesperetin-7-O-glucuronide, but not hesperetin-3'-O- glucuronide, exerts hypotensive, vasodilatory, and anti- inflammatory activity of extract from *Castanea sativa* leaves, Fitoterapia 2000; 71: 110-6.

Yi Z, Yu Y, Liang Y, Zeng B. In vitro antioxidant and antimicrobial activities of the extract of *Pericarpium Citri Reticulatae* of a new Citrus cultivar and its main flavonoids. LWT 2008; 41(4): 597-603.

ERCİYES ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ
Journal of Faculty of Veterinary Medicine, Erciyes University
Araştırma Makalesi / Research Article
21(3), 148-154, 2024
DOI: 10.32707/ercivet.1587193

Rheum ribes Uygulamasının Diyabetik Ratların Karaciğer ve Böbreklerinde TLR2 ve TLR4'ün İmmunohistokimyasal Lokalizasyonu Üzerine Etkisi*

Ayhan AKGÜN[1,a],Turgay DEPREM[2,b]

[1]Iğdır Üniversitesi, Tuzluca Meslek Yüksekokulu Tıbbi Hizmetler ve Teknikler Bölümü, Iğdır-TÜRKİYE
[2]Kafkas Üniversitesi, Veteriner Fakültesi Histoloji ve Embriyoloji Anabilim Dalı, Kars-TÜRKİYE
ORCID: [a]0000-0001-6585-8655; [b]0000-0002-5523-8150

Sorumlu yazar: Ayhan AKGÜN; E-posta: ayhan.akgun@igdir.edu.tr
Atıf yapmak için: Akgün A, Deprem T. *Rheum ribes* uygulamasının diyabetik ratların karaciğer ve böbreklerinde TLR2 ve TLR4'ün immunohistokimyasal lokalizasyonu üzerine etkisi. Erciyes Univ Vet Fak Derg 2024; 21(3):148-154

Öz: Diyabetes mellitus (DM) çeşitli komplikasyonlara sebep olmaktadır. Bu komplikasyonların en önemlileri hemostazdan sorumlu olan karaciğer ve böbrekte meydana gelen komplikasyonlardır. Işgın (*Rheum ribes, R. ribes*) çeşitli hastalıkların yanı sıra DM'de de tedavi amaçlı kullanılmaktadır. TLR aile üyeleri birçok durumda artmaktadır. Ancak diyabetik hastalarda TLR2 ve TLR4'teki ekspresyon artışı dikkat çekmektedir. Çalışmamızda *R. ribes* ekstraktının diyabetteki etkilerinin yanı sıra diyabetik olgularda belirgin olarak artan TLR2 ve TLR4'ün immünohistokimyasal lokalizasyonunu incelemeyi amaçladık. DM deneklerde Streptozotosin (STZ) 50 mg/kg intraperitoneal uygulanarak oluşturuldu. *R. ribes* ve diyabet + *R. ribes* gruplarına 21 gün boyunca oral gavaj ile 200 mg/kg *R. ribes* ekstraktı verildi. Yapılan incelemede karaciğer dokusunda TLR2 immünreaktivitesi hepatositlerde, V. sentralislerde, sinuzoid endotellerinde; TLR4 immünreaktivitesi V. sentralis, hepatositler ve V. interlobularislerde görüldü. Böbrekte ise TLR2 ve TLR4 immünreaktivite glomerulus hücrelerinde, proksimal tubullerde, distal tubullerde ve medullada gözlendi. TLR2 sitoplazmik immünreaktif olduğu halde TLR4 hem sitoplazmik hem de nükleerdi. Hem TLR2 hem de TLR4 için immünreaktivite diyabet grubu karaciğer ve böbrek dokularında yoğun olarak gözlenirken, *R. ribes* ekstraktı uygulanan diyabet grubu karaciğer ve böbrek dokularında immünreaktivitenin önemli ölçüde azaldığı gözlendi. Sonuç olarak diyabetik ratların karaciğer ve böbrek dokularında *R. ribes* bitkisinin pozitif bir etkiye sahip olduğu belirlenmiştir. Bundan dolayı çeşitli hastalıklarda da kullanılan *R. ribes* bitkisi DM hastalığı için ilaç endüstrisinde yeni tedavi yöntemlerinin geliştirilmesine katkı sağlayacağını düşünmekteyiz.
Anahtar kelimeler: Böbrek, diyabetes mellitus, immunohistokimya, karaciğer, *Rheum ribes*, TLR2, TLR4

Effect of *Rheum ribes* Application on Immunohistochemical Localizations of TLR2 and TLR4 in the Liver and Kidneys of Diabetic Rats

Abstract: Diabetes mellitus (DM) causes various complications. The most important of these complications are those occurring in the liver and kidney, which are responsible for hemostasis. *Rheum ribes* (*R. ribes*) is used for treatment purposes in DM as well as various diseases. TLR family members are increased in many cases. However, the increased expression of TLR2 and TLR4 in diabetic patients is noteworthy. In our study, we aimed to examine the effects of *R. ribes* extract in diabetes as well as the immunohistochemical localization of TLR2 and TLR4, which are significantly increased in diabetic cases. Streptozotocin (STZ) was administered intraperitoneally 50 mg/kg in DM subjects. *R. ribes* and diabetes + *R. ribes* groups were given 200 mg/kg *R. ribes* extract by oral gavage for 21 days. In the examination, TLR2 immunoreactivity in liver tissue was found in hepatocytes, central veins, and sinusoid endothelium; TLR4 immunoreactivity was seen in V. centralis, hepatocytes and V. interlobularis. In the kidney, TLR2 and TLR4 immunoreactivity was observed in glomerulus cells, proximal tubules, distal tubules, and medulla. While TLR2 was cytoplasmic immunoreactive, TLR4 was both cytoplasmic and nuclear. Immunoreactivity for both TLR2 and TLR4 was observed intensely in the liver and kidney tissues of the diabetes group. But it was observed that immunoreactivity was significantly reduced in the liver and kidney tissues of the diabetic group administered *R. ribes* extract. As a result, it was determined that *R. ribes* plant has a positive effect on the liver and kidney tissues of diabetic rats. Therefore, we think that the *R. ribes* plant, which is also used in various diseases, will contribute to the development of new treatment methods in the pharmaceutical industry for DM disease.
Keywords: Diabetes mellitus, immunohistochemistry, kidney, liver, *Rheum ribes*, TLR2, TLR4

Geliş Tarihi/Submission Date : 21.03.2024
Kabul Tarihi/Accepted Date : 12.06.2024

Bu çalışma Kafkas Üniversitesi Bilimsel Araştırma Projeleri Koordinatörlüğü (Proje No: 2021-TS-32) tarafından desteklenmiş olup doktora tezinden türetilmiştir.

Giriş

Diyabetes mellitus (DM), otoimmün bir hastalıktır. DM, vücutta birçok komplikasyona neden olur. Bu komplikasyonlar, vücudun insülin üretememesi veya kullanamamasına bağlı olarak kan şekeri seviyesinin

istem dışı yükselmesiyle oluşmaktadır (Pickup ve Williams, 1991).

Metabolizma ve vücut hemostazının düzenlenmesinden sorumlu olan karaciğer ve böbrek DM'ta önemli rol oynamaktadır (Green ve ark., 2005). Karaciğer, glikoz metabolizmasında önemli bir göreve sahiptir. Bu rol insülin ve glukagon tarafından enerji metabolizmasının düzenlenmesiyle oluşmaktadır. İnsülin karaciğerde glikojen sentezini artırır ve glikoz üretimini engeller. Aksine, glukagon glikoz üretimini indükler ve glikojen sentezini engeller (Kim ve ark., 2011; Kürüm ve ark., 2015). Ayrıca DM, böbrekte de önemli komplikasyonlar oluşturmaktadır. Bu komplikasyonlar böbreklerdeki vasküler dejenerasyonlardan kaynaklanmaktadır. Meydana gelen vasküler dejenerasyonlar vücut hemostazının düzenlenmesinde büyük engel oluşturmaktadır (Kurt ve ark., 2004).

Toll benzeri reseptörler (TLR), deri ve bağırsak mukozası gibi vücut bölümlerinde doğuştan olarak bulunan ve organizmaya giren patojenik yapıları tanıyabilen bir reseptör ailesidir. TLR, patojenik mikroorganizmaları tanır ve onlara karşı bir immun yanıt oluşturabilir (Müştak ve Esendal, 2007). DM ile artan glikoz seviyesi, enzimatik olmayan glikozilasyonuna neden olur. Glikozilasyon sonucu değiştirilmiş proteinler, bağışıklık sistemi tarafından antijen olarak kabul edilir (Aboonabi ve ark., 2014). Doğal savunma reseptörleri olarak bilinen TLR2 ve TLR4, diyabetik hastalarda oluşan proteinlere karşı sentezlenen önemli TLR olarak bilinirler (Devavaraj ve ark., 2011; Kim ve ark., 2011; Sevimli ve Özçelik, 2016).

Işkın olarak bilinen *R. ribes*, Polygonaceae familyasına ait tıbbi bitkilerden biridir. Başta Batı Asya olmak üzere dünyanın ılıman ve subtropikal bölgelerinde (Türkiye, Suriye, Lübnan, Irak, İran, Azerbaycan, Ermenistan, Afganistan, Pakistan) yetişmektedir. Hem gıda maddesi hem de çeşitli hastalıklarda kullanılmaktadır (Akkuş ve Şıktar, 2018). Ayrıca hipoglisemik etkiye sahip olması ve içeriğinde bir çok antioksidanları barındırmasıyla diyabet tedavisine önem kazanmıştır (Konak ve Aktar, 2009; Rafaat ve ark., 2014; Meral, 2017).

Bu çalışmanın amacı, diyabetik ve diyabetik olmayan ratlara *R. ribes* bitki ekstraktı uygulandığında karaciğer ve böbrek dokularında meydana gelen değişiklikleri histopatolojik olarak ortaya koymak ve doğal immün reseptörler olarak bilinen TLR2 ve TLR4'ün bu dokulardaki lokalizasyonlarını ve ekspresyon durumlarını belirlemektir.

Gereç ve Yöntem

Deney prosedürü

Bu çalışma, Kafkas Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu'ndan (KAÜ-HADYEK/2020-128) alınan onay doğrultusunda yapılmıştır. Ratlar, Kafkas Üniversitesi Deney Hayvanları Uygulama ve Araştırma Merkezi'nde; 12 saat aydınlık 12 saat karanlık ritminde ışıklandırılan, 22±2^{o}C'ye ayarlı oda ısısında, standart pelet yem ile beslendi. Ayrıca ratlar, yem ve su alımı serbest olacak şekilde standart plastik kafeslerde barındırıldı. Çalışmamızda deney hayvanı olarak 40 adet erkek Spraque Dawley rat kullanıldı. Deney hayvanları Kontrol (n=8), Sham (n=8), R. ribes (n=8), *R. Ribes* + Diyabet (n=8) ve Diyabet (n=8) olmak üzere beş gruba ayrıldı. Streptozotosin (STZ) (Sigma S0130-100 MG) 0.1 M sitrat tamponunda pH 4.5'da eritilerek 50 mg/kg dozunda diyabetik gruplara intraperitoneal olarak uygulandı. STZ uygulanan gruplarda 72 saat sonra ratlar sekiz saat aç bırakıldı ve kan şekerleri glukometre ile ölçüldü. Kan şekeri düzeyi 250 mg/dl'nin üzerinde olanlar diyabetik olarak kabul edildi (Kanitkar ve Bhonde, 2004). *R. ribes* ve diyabet + *R.ribes* gruplarına 21 gün boyunca 200 mg/kg/gün *R. ribes* ekstraktı distile su içinde eritilerek (Asgharian ve ark., 2018), Sham grubuna ise sadece aynı miktarda distile su oral gavaj yoluyla verildi. Kontrol grubuna sadece ad libitum beslenme yapıldı.

Ekstraktın hazırlanması: Ekstrakt için Van'dan ticari olarak *R. ribes* bitkisi temin edildi. Bitki 10 gün boyunca kurutuldu ve ardından mikserlendi. Toz haline getirilmiş *R. ribes* bitkisi, üç gün süreyle cam bir kapta metil alkol içinde işleme tabi tutuldu. Tortu, karışımdan filtre kağıdı ile ayrıldı. Metil alkolü ekstrakt karışımından uzaklaştırmak için bir evoporatör cihazı kullanıldı. Elde edilen ekstrakt deney başlangıcına kadar oda sıcaklığında saklandı (Gholamhoseinian ve ark., 2009).

Histolojik analiz

Deney sonunda ratların karaciğer ve böbreklerinden doku örnekleri alındı. Dokular %10'luk formaldehit solüsyonunda en az 48 saat tespit edildi. Daha sonra dokular doku takibi işlemlerinden geçirilerek doku blokları hazırlandı. Kesit için hazır hale gelen karaciğer ve böbrek dokuları mikrotomda 5 µm kalınlığında kesildi. Doku kesitleri histolojik inceleme için triple yöntemi (Crossmann'ın üçlü boyaması) ile boyandı.

İmmunohistokimyasal analiz

İmmunohistokimyasal yöntem için avidin-biotin-peroksidaz kompleksi (ABC) tekniği uygulandı (Hsu ve ark., 1981). TLR2 ve TLR4 immünreaktivitesini incelemek amacıyla endojen peroksidaz aktivitesini bloke etmek için %3 H2O2 uygulandı. Antijenleri serbest bırakmak için sitrat tamponunda (pH 6.0) mikrodalga uygulaması yapıldı. Dokular, anti-TLR2 antikoru (ab213676, Poliklonal) (karaciğer dokuları için 1/200, böbrek dokuları için 1/50) ve anti-TLR4 antikoru (sc293072, Monoklonal) (karaciğer dokuları için 1/100, böbrek dokuları için 1/100) ile inkübasyona oda sıcaklığında bir saat bırakıldı. Ardından PBS ile yıkama sonrası kesitlere sekonder antikor

(Biontinylated Goat Anti-Rabbit, Lab Vision 510.991.2800) ilave edilerek 30 dk oda ısısında bekletildi. Kesitlere kromojen uygulaması için DAB (Thermo Scientific) kullanıldı. Son olarak hematoksilen ile karşıt boyama yapıldı. İmmünreaktivite reaksiyon yoğunluğuna göre yarı kantitatif olarak değerlendirildi (Reaksiyon yok: -, Zayıf: +, Orta: ++, Güçlü: +++) (Okihiro ve Hinton, 2000). TLR2 ve TLR4 immünoreaktivitelerinin spesifik olup olmadığını belirlemek için negatif kontrol uygulaması da yapıldı.

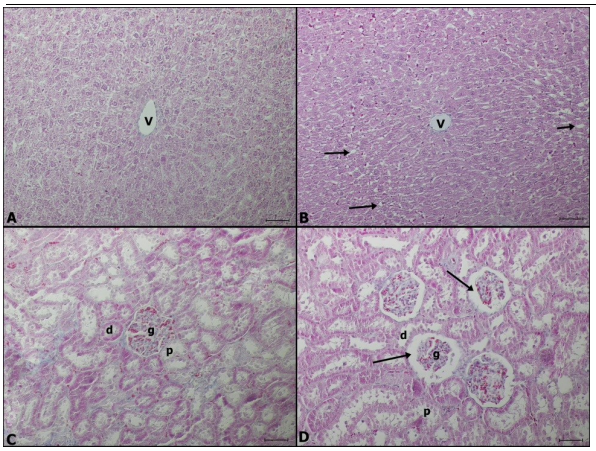
Bulgular

Çalışmamızda kontrol grubuna ait karaciğer dokusunun normal histolojik yapıya sahip olduğu görüldü (Şekil 1-A). Sham ve R. ribes grupları incelendiğinde kontrol grubu ile histolojik olarak anlamlı fark yoktu. Diyabet grubunun karaciğer dokusunda karaciğer loblarının bazı bölgelerinde yaygın olmayan fakat belirgin lipidoz izlendi (Şekil 1-B). Diyabet + *R. ribes* grubunun bulguları, diyabet grubunun bulgularına benzerdi. Kontrol grubu böbrek dokularında ise histolojik görünüm normaldi (Şekil 1-C). Sham ve *R. ribes* grupları için histolojik bulgular kontrol grubundan farklı değildi. Diyabet grubunun böbrek dokularında nefron yapısını oluşturan Bowman aralığında genişlemeler ve glomerüllerde atrofi mevcuttu (Şekil 1-D). Diyabet + *R. ribes* grubu böbrek dokularındaki histolojik bulgular, Diyabet grubu dokularının bulguları ile benzerlik göstermekteydi.

Kontrol, Sham, *R. ribes*, diyabet ve Diyabet + *R. ribes* gruplarına ait karaciğer ve böbrek dokuları TLR2 immünohistokimyasal bulguları açısından incelendi (Tablo 1).

Tablo 1. Gruplara göre TLR2 immünoreaktivite skor tablosu

KARACİĞER	Kontrol Grubu	Sham Grubu	*R. ribes* Grubu	Diyabet Grubu	Diyabet + *R. ribes* Grubu
V. sentralis	+	+	+	+++	++
Hepatositler	-	-	-	+++	++
Sinuzoid endotelleri	+	+	+	+++	++
V. inter lobularis	+	+	+	++	+
A. hepatika	-	-	-	++	+
Duktus biliferus	+	+	+	++	+
BÖBREK					
Glomerulus hücreleri	++	++	++	+++	++
Bowman kapsülü	++	++	++	+++	++
Proksimal tubul	+	+	+	+++	++
Distal tubul	+	+	+	+++	++
İnen henle	++	++	++	+++	++
Çıkan henle	++	++	++	+++	++
Tubulus kollektivus	++	++	++	+++	++



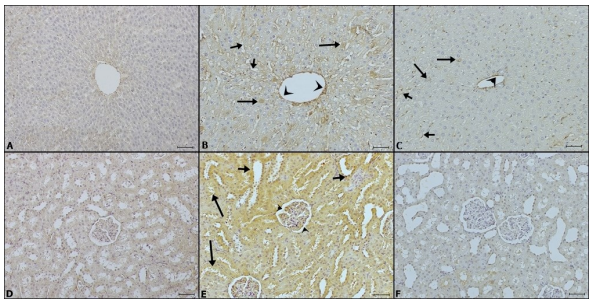
Şekil 1. Kontrol grubu karaciğer dokusu (A). Diyabet grubu karaciğer dokusu (B), Kontrol grubu böbrek dokusu (C), Diyabet grubu böbrek dokusu (D). V. sentralis (V), lipidoz (kısa oklar), glomerulus (g), proksimal tubuller (p), distal tubuller (d), Bowman aralığı (uzun oklar). A,B,C,D Triple Boyama, Bar: 50 µm.

TLR2'nin karaciğerdeki lokalizasyonu V. sentralisler, hepatositler, sinuzoid endotelleri, V. interlobularis, A. hepatikalar ve duktus biliferuslarda gözlendi. Karaciğer dokularında TLR2 immünreaktivitesinin sitoplazmik olduğu gözlendi. Kontrol, Sham ve *R. ribes* grubu karaciğer dokularında TLR2 immünreaktivitesi düşüktü (Şekil 2-A). Diyabetik karaciğer dokularında kontrol grubuna kıyasla yüksek immünreaktivite tespit edildi (Şekil 2-B). Diyabet + *R. ribes* grubunda ise diyabetik karaciğer dokularına göre TLR2 ekspresyonu düşüktü (Şekil 2-C).

TLR2'nin böbrekteki lokalizasyonu glomerulus hücreleri, bowman kapsülleri, proksimal tubuller, distal tubuller, inen henle, çıkan henle ve tubulus kollektivuslarda gözlendi. Böbrek dokularında sitoplazmik TLR2 immünreaktivitesine rastlandı.

Kontrol, Sham ve *R. ribes* grubu böbrek dokularında TLR2 immünreaktivitesi düşüktü (Şekil 2-D). Diyabetik böbrek dokularında kontrol grubuna kıyasla yüksek immünreaktivite gözlendi (Şekil 2-E). Diyabet +

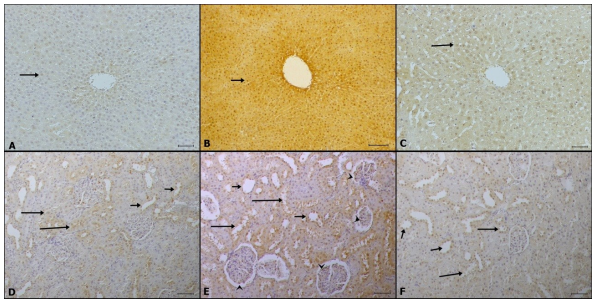
R. ribes grubunda ise diyabetik böbrek dokularına göre ekspresyon düşüktü (Şekil 2-F).



Şekil 2. Kontrol grubunun karaciğer dokusunun TLR2 immünoreaktivitesi (A), Diyabet grubu karaciğer dokusunun TLR2 immünoreaktivitesi (B), R. ribes + Diyabet grubu karaciğer dokusunun TLR2 immünoreaktivitesi (C), Kontrol grubunun böbrek dokusu korteks bölgesinin TLR2 immünoreaktivitesi (D), Diyabet grubunun böbrek dokusu korteks bölgesinin TLR2 immünoreaktivitesi (E), R. ribes + Diyabet grubunun böbrek dokusu korteks bölgesinin TLR2 immünoreaktivitesi (F). V. sentralis endoteli (ok başları), sinüzoid endoteli (kısa oklar), hepatositler (uzun oklar), proksimal tubul (uzun oklar), distal tubul (kısa oklar), glomerüler ağ (ok başları). A-F Bar: 50 µm.

Kontrol, Sham, *R. ribes*, diyabet + *R. ribes* ve diyabet gruplarına ait karaciğer ve böbrek dokularında TLR4 immünohistokimyasal bulguları incelendi (Tablo 2).

Kontrol, Sham ve *R. ribes* grubu karaciğer dokularında TLR4 immünreaktivitesi düşüktü (Şekil 3-A). Diyabetik karaciğer dokularında Kontrol grubuna kıyasla yüksek immünreaktivite gözlendi (Şekil 3-B). Diyabet + *R. ribes* grubunda ise diyabetik karaciğer dokularına göre ekspresyon düşüktü (Şekil 3-C).



Şekil 3. Kontrol grubu karaciğer dokusunun TLR4 immünoreaktivitesi (A), Diyabet grubu karaciğer dokusunun TLR4 immünoreaktivitesi (B), R. ribes + Diyabet grubu karaciğer dokusunun TLR4 immünoreaktivitesi (C), Kontrol grubu böbrek dokusunun TLR4 immünoreaktivitesi (D), Diyabetik böbrek dokusunun TLR4 immünoreaktivitesi (E), R. ribes + Diyabet grubu böbrek dokusunun TLR4 immünoreaktivitesi (F). Hepatositler (ok), proksimal tubul (uzun oklar), distal tubul (kısa oklar), glomerüler ağ (ok başları). (A-F Bar: 50µm).

Tablo 2. Gruplara göre TLR4 immünoreaktivite skor tablosu

KARACİĞER	Kontrol Grubu	Sham Grubu	*R. ribes* Grubu	Diyabet Grubu	Diyabet + *R.ribes* Grubu
V. sentralis	+	+	+	+++	++
Hepatositler	+	+	+	+++	++
Sinuzoid endotelleri	-	-	-	-	-
V. inter lobularis	+	+	+	+++	++
A. hepatika	-	-	-	+++	++
Duktus biliferus	+	+	+	+++	++
BÖBREK					
Glomerulus hücreleri	+	+	+	+++	++
Bowman kapsülü	+	+	+	++	+
Proksimal tubul	++	++	++	+++	++
Distal tubul	++	++	++	+++	++
İnen henle	++	++	++	+++	++
Çıkan henle	++	++	++	+++	++
Tubulus kollektivus	++	++	++	+++	++

TLR4'ün karaciğerdeki lokalizasyonu V. sentralisler, hepatositler, V. inter lobularisler, A. hepatikalar ve duktus biliferuslarda gözlendi. Karaciğer dokularında TLR4 immünreaktivite hem sitoplazmik hem de nükleerdi.

TLR4'ün böbrekteki lokalizasyonu glomerulus hücreleri, bowman kapsülleri, proksimal tubuller, distal tubuller, inen henle, çıkan henle ve tubulus kollektivuslarda gözlendi. Böbrek dokuları için TLR4 immünreaktivite hem sitoplazmik hemde nükleerdi.

Kontrol, Sham ve *R. ribes* grubu böbrek dokularında TLR4 immünreaktivitesi düşüktü (Şekil 3-D). Diyabetik böbrek dokularında Kontrol grubuna kıyasla yüksek immünreaktivite gözlendi (Şekil 3-E). Diyabet + *R. ribes* grubunda ise diyabetik böbrek dokularına göre ekspresyon düşüktü (Şekil 3-F).

Tartışma ve Sonuç

DM'de hiperglisemi lipid, karbonhidrat ve protein metabolizmasının düzensiz çalışmasına neden olur. DM'tan kaynaklı kandaki sürekli yüksek seyreden glikoz seviyesi, enzimatik olmayan glikozilasyona sebep olmasıyla glikozun yabancı proteinlere dönüşümüne sebep olur. Değiştirilmiş proteinler, bağışıklık sistemi tarafından yabancı yapılar olarak algılanır (Aboonabi ve ark., 2014). Doğal savunma reseptörleri içinde yer alan TLR2 veTLR4 bu yabancı proteinler ile etkileşime girerek ekspresyona uğrarlar (Devaraj ve ark., 2008; Kim ve ark., 2011; Sevimli ve Özçelik, 2016)

TLR2 ile DM arasında ilişkiyi ortaya koyan birçok çalışma (Dasu ve ark., 2010; Ehses ve ark., 2010; Karaali ve ark., 2019) olmasına rağmen, yapılan literatür taramalarında TLR2'nin karaciğerde immünlokalizasyonunu araştıran herhangi bir çalışmaya rastlanılmadı. Karaali ve ark. (2019) yaptıkları çalışmada DM hastalarında TLR2 ekspresyonunun kontrol bireylere göre daha yüksek olduğunu bildirmişlerdir. Dasu ve ark. (2010) yaptıkları çalışmada diyabetik bireylerde TLR2'nin kontrol grubuna göre arttığını bildirmişlerdir. Çalışmamızda karaciğer dokularının TLR2 ekspresyonuna bakıldığında diyabet gruplarının kontrol gruplarına kıyasla daha yüksek immünreaktif olduğu gözlendi. Bu bulguya dayalı olarak, çalışmamızda diyabetik olgularda TLR2'nin ekspresyonunda artış gözlemlenmesiyle adı geçen çalışmalarla (Dasu ve ark., 2010; Karaali ve ark., 2019) bizim araştırmamız arasında paralellik olduğu görülmektedir. Yaptığımız çalışmada V. sentralis endotelleri, sinüzoidal endotel hücreleri, hepatositler, V. interlobularis ve A. hepatika epitellerinde TLR2 immünreaktivite gözlendi. Ayrıca ekstrakt uygulanan diyabetik ratların karaciğerlerinde TLR2 immünreaktivitesinde azalma vardı.

Sawa ve ark. (2014) TLR2'nin diyabetik nefropati için önemli olduğunu öne sürmüşlerdir. TLR2'nin glomerüler endotel ve proksimal tubul epitelleri üzerinde lokalize olduğunu bildirdiler. Ayrıca diyabetik farelerde TLR2 ekspresyonunun diyabetik olmayan farelere göre daha yüksek olduğunu gözlemlediler. Devaraj ve ark. (2011) yaptıkları çalışmada TLR2 lokalizasyonunun glomerulusta olduğunu saptamışlardır. Ayrıca diyabetik farelerin böbreklerinde TLR2 ekspresyonunun, kontrol grubundaki TLR2 reaktivitesine kıyasla daha yüksek olduğunu bildirdiler. Mudaliar ve ark. (2013) yaptıkları çalışmada TLR2 ekspresyonunun hem kontrol hem de diyabetik böbrek dokularında görüldüğünü bildirmişlerdir. Ancak diyabetik farelerin böbrek dokularında TLR2 ekspresyonunun, özellikle hasarlı tubullerde daha yoğun immünreaktivite gösterdiğini belirtmişlerdir. Çalışmamızda diyabetik böbrek dokusunda glomerulus, Bowman kapsülü, proksimal tubuller, distal tubuller, inen ve çıkan henle ve tubulus kollektivusu oluşturan bazı hücrelerde daha yoğun immünreaktivite gözlendi. Ayrıca diyabet + *R. ribes* grubunda TLR2 ekspresyonunun diyabetik dokulara göre azaldığını gözlemledik.

Han ve ark. (2016) selastrolün diyabetik ratlarda karaciğer hasarı üzerindeki koruyucu etkilerini ve olası mekanizmalarını araştırmayı amaçlamışlardır. Kontrol grubunda birkaç hepatositte TLR4 reseptör ekspresyonu reaktif iken, diyabetik karaciğer dokularında hepatositlerde ve Kupffer yıldız hücrelerinde daha yüksek TLR4 ekspresyonu gözlemlemişlerdir. Tedavi grubunda bu immünreaktivitenin azaldığını belirtmişlerdir. Buna göre selastrolün diyabetik sıçanların hepatik dokularında organ hasarına karşı koruyucu bir etki sağladığını bildirmişlerdir. Zhao ve ark. (2021), karvakrolün diyabetik farelerde karaciğer hasarı üzerindeki koruyucu etkisini araştırmayı ve potansiyel moleküler mekanizmasını değerlendirmeyi amaçladıklarını bildirmişlerdir. İmmünohistokimyasal olarak, diyabetik farelerin karaciğer dokularında TLR4 ekspresyon seviyesinin kontrol grubuna göre daha yüksek olduğunu ve bu proteinin ekspresyon seviyelerinin karvakrol ile tedavi edilen grupta önemli ölçüde azaldığını bildirmişlerdir. Yaptıkları çalışmalar sonucunda diyabetli farelerde karvakrolün hepatik organ hasarına karşı koruyucu etki sağladığını gözlemlemişlerdir. Yaptığımız çalışmada diyabetik ratlara oral gavaj ile *R. ribes* ekstraktı vererek TLR4 immünolokasyon ve ekspresyon durumunu değerlendirmeyi amaçladık. İncelemelerimize göre diyabet grubundan hepatositler, V. sentralis, V. interlobularis endoteli, duktus biliferus epiteli ve A. hepatikada TLR4 ekspresyonu daha yüksek bulundu. Ekstrakt uygulanan diyabetik ratların karaciğerlerinde immünreaktivitede azalma gözlemledik.

Yuan ve ark. (2018) çalışmasında diyabetik grubun böbrek dokularında TLR4 ekspresyonunun kontrol grubuna göre daha yüksek olduğu bildirmektedirler. TLR4 immünreaktivite böbrek tubullerinde olduğunu gözlemlediler. Lin ve ark. (2012) çalışmalarında diyabetik böbreklerde ağırlıklı olarak proksimal tubuller, distal tubuller ve peritubuler kapillerlerde TLR4 için önemli immünreaktivite gözlendiğini bulmuşlardır. Bununla birlikte, diyabetik nefropatisi olmayan kişilerde çok az reaksiyon gözlemlendiğini bildirmişlerdir. Bizim yaptığımız çalışmada diyabetik böbrek dokularında glomerulus, proksimal tubuller, distal tubuller, inen ve çıkan henle, tubulus kollektivus epitelinin bazı hücrelerinde ve Bowman kapsülünde immünreaktivite tespit edildi. Ekstrakt ile tedavi edilen diyabetik ratların böbreklerinde TLR4 ekspresyonunun önemli ölçüde azaldığı gözlendi. Çalışmamız bahsedilen çalışmalarla diyabetik olgularda TLR4 ekspresyonunun

artışı hakkında paralellik göstermektedir (Lin ve ark., 2012; Yuan ve ark., 2018).

Sonuç olarak, DM'un birçok komplikasyona neden olduğu bilinmektedir. Komplikasyonlar sonucunda dokularda hücresel değişiklikler meydana gelir. Yapılan incelemeler ışığında hücresel değişikliklerin ortaya konması DM'nin mekanizmasının anlaşılmasını sağlayacaktır. Buna göre, çalışmamızda *R.ribes* bitkisinin ekstraktını kullanarak diyabetteki etkisini inceledik. Deneysel olarak oluşturulan DM'lu ratların karaciğer ve böbrek dokuları incelendi. Dokularda TLR2 ve TLR4 ekspresyon farkı belirlenerek çalışmanın önemi ortaya konuldu. DM'ta TLR2 ve TLR4'ün yüksek ekspresyon düzeylerine ulaştığı tespit edilirken, *R.ribes* ekstraktı kullanılan diyabetli deneklerde bu ekspresyonların önemli ölçüde azaldığı belirlendi. Deneysel olarak oluşturduğumuz diyabetik dokularda doğal bağışıklıkta görevli alan TLR2 ve TLR4'ün ekspresyonları, DM'nin mekanizmasının ve komplikasyonlarının daha iyi anlaşılmasında önemli bir role sahip olabileceği düşüncesini oluşturmuştur.

Teşekkür: 2021-TS-32 no'lu proje ile çalışmasının yapılmasındaki katkılarından dolayı KAÜ Bilimsel Araştırma Projeleri Koodinatörlüğü Birimi'ne teşekkür ederiz.

Kaynaklar

Aboonabi A, Rahmat A, Orthman F. Effect of pomegranate on histopathology of liver and kidney on generated oxidative stress diabetic ınduced rats. J Cytol Histol 2014; 6(1): 1-5.

Akkuş Y, Şıktar E. *Rheum ribes* (ışkın otu)'in metanol ekstresinin düzenli aerobik yüzme egzersizi uygulanan rat dokularında antioksidan ve histopatolojik etkileri, Doktora tezi, Atatürk Üniver Kış Sporları ve Spor Bil Enst, Erzurum 2018.

Asgharian S, Hoseinkhan, H, Bijad E, Lorigooini Z, Rafieian-Kopaei, M. Protective effect of hydroalcoholic *Rheum ribes L*. extract in male rat model of lead acetate-induced nephrotoxicity. J Nephropathol 2018; 7(2): 83-7.

Dasu MR, Devaraj S, Park S, Jialal I. Increased toll-like receptor (TLR) activation and TLR ligands in recently diagnosed type 2 diabetic subjects. Diabetes Care 2010; 33(4): 861-8.

Devaraj S, Dasu MR, Rockwood J, Winter W, Griffen SC, Jialal I. Increased toll-like receptor (TLR) 2 and TLR4 expression in monocytes from patients with type 1 diabetes: further evidence of a proinflammatory state. J Clin Endocrinol Metab 2008; 93(2): 578 -83.

Devaraj S, Tobias P, Kasinath BS, Ramsamooj R, Afify A, Jialal I. Knockout of toll-like receptor-2 attenuates both the proinflammatory state of diabetes and incipient diabetic nephropathy. Arterioscler Thromb Vasc Biol 2011; 31(8): 1796-804.

Ehses JA, Meier DT, Wueest S, Rytka J, Boller S, Wielinga PY, Donath MY. Toll-like receptor 2-deficient mice are protected from insulin resistance and beta cell dysfunction induced by a high-fat diet. Diabetologia 2010; 53(8):1795-806.

Gholamhoseinian A, Moradi MN, Sharifi-far F. Screening the methanol extracts of some Iranian plants for acetylcholinesterase inhibitory activity. Res Pharm Sci 2009; 4(2): 105-12.

Green TJ, Venn BJ, Skeaff CM, Williams SM. Serum vitamin B-12 concentrations and atrophic gastritis in older New Zealanders. Eur J Clin Nutr 2005; 59 (2): 205-10.

Han LP, Li CJ, Sun B, Xie Y, Guan Y, Ma, ZJ, Chen LM. Protective effects of celastrol on diabetic liver injury via TLR4/MyD88/NF-κB signaling pathway in type 2 diabetic rats. J Diabetes Res 2016; 2641248.

Hsu SM, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 1981; 29(4): 577-80.

Kanitkar M, Bhonde R. Existence of islet regenerating factors within the pancreas. Rev Diabet Stud 2004; 1(4): 185-92.

Karaali ZE, Candan G, Aktuğlu MB, Velet M, Ergen A. Toll-like receptor 2 (TLR-2) gene polymorphisms in type 2 diabetes mellitus. Cell J 2019; 20(4): 559-63.

Kim DH, Lee JC, Kim S, Oh SH, Lee MK, Kim KW, Lee MS. Inhibition of autoimmune diabetes by TLR2 tolerance. J Immunol 2011; 187(10): 5211-20.

Konak A, Aktar O. Medikal antropoloji çerçevesinde Tunceli/Ovacık'ta geleneksel sağaltma yöntemleri. CÜ Sosyal Bilimler Dergisi 2009; 35(2): 156-87.

Kurt M, Atmaca A, Gürlek A. Diyabetik nefropati. Hacettepe Med J 2004; 35: 12-7.

Kürüm A, Kocamış H, Deprem T, ÇINAR M. Determining gene expression profile of GPX 1 in the liver of diabetic rats treated with capsaicin by real-time PCR. Kafkas Univ Vet Fak Derg 2015; 21(2): 259-63.

Lin M, Yiu WH, Wu HJ, Chan LY, Leung JC, Au WS, Tang SC. Toll-like receptor 4 promotes tubular inflammation in diabetic nephropathy. J Am Soc

Nephrol 2012; 23(1):86-102.

Meral N. Farklı sıcaklık derecelerinin uşkun bitkisinin antioksidan aktivitesi ve fenolik profili üzerine etkisi. YYÜ Tar Bil Derg 2017; 27(1): 88-94.

Mudaliar H, Pollock C, Komala MG, Chadban S, Wu H, Panchapakesan U. The role of toll-like receptor proteins (TLR) 2 and 4 in mediatinginflammation in proximal tubules. Am J Physiol Renal Physiol 2013; 305(2): 143-54.

Müştak HK, Esendal ÖM. Toll benzeri reseptörler. Etlik Veteriner Mikrobiyoloji Dergisi 2007; 18: 33-8.

Okihiro MS, Hinton DE. Partial hepatectomy and bile duct ligation in rainbow trout (*Oncorhynchus mykiss*): Histologic, immunohistochemical and enzyme histochemical characterization of hepatic regeneration and biliary hyperplasia. Toxicol Pathol 2000; 28 (2): 342-56.

Pickup JC, Williams G. Textbook of Diabetes. Second edition. Wiley-Blackwell 1997; pp. 151-5.

Rafaat K, Aboul-Ela M, El-Lakany A. Alloxan-induced diabetic thermal hyperalgesia, prophylaxis and phytotherapeutic effects of *Rheum ribes L.* in mouse model. Arch Pharm Res 2014; 44(8): 1-10.

Sawa Y, Takata S, Hatakeyama Y, Ishikawa H, Tsuruga E. Expression of toll-like receptor 2 in glomerular endothelial cells and promotion of diabetic nephropathy by Porphyromonas gingivalis lipopolysaccharide. PloS one 2014; 9(5): e97165.

Sevimli T, Özçelik N. Stz (streptozotosin) ile diyabet oluşturulmuş ratların böbrek dokusunda silibinin'in HIF-1α (hypoxıa-inducible factor-1 alpha) ve TLR-2 (toll like receptor 2) genlerinin mRNA düzeylerine etkisi, Doktora Tezi, SDÜ Sağ Bil Enst, Isparta 2016.

Yuan S, Liu X, Zhu X, Qu Z, Gong Z, Li J, Liu F. The role of TLR4 on PGC-1α-mediated oxidative stress in tubular cell in diabetic kidney disease. Oxid Med Cell Longev 2018; 2018: 6296802.

Zhao W, Chen L, Zhou H, Deng C, Han Q, Chen Y, Li S. Protective effect of carvacrol on liver injury in type 2 diabetic db/db mice. Mol Med Rep 2021; 24 (5): 741.

ERCİYES ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ
Journal of Faculty of Veterinary Medicine, Erciyes University

Research Article / Araştırma Makalesi
21(3), 155-161, 2024
DOI: 10.32707/ercivet.1587311

Antimicrobial Effect of Partially Purified Bacteriocins on *Pseudomonas aeruginosa**

Eda HACIOMEROGLU[1,a], Gizem CUFAOGLU[1,b]

[1]Kırıkkale University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Kırıkkale-TÜRKİYE
[2]Kırıkkale University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Kırıkkale-TÜRKİYE
ORCID: [a]0000-0003-1859-4399; [b]0000-0001-8639-532X

Corresponding author: Gizem ÇUFAOĞLU; E-mail: gizemcufaoglu@kku.edu.tr
How to cite: Hacıömeroğlu E, Çufaoğlu G. Antimicrobial effect of partially purified bacteriocins on *Pseudomonas aeruginosa*. Erciyes Univ Vet Fak Derg 2024; 21(3):155-161

Abstract: Bacteriocins are commonly used in foods to inhibit the growth of spoilage and pathogenic bacteria, thus extending the shelf life of food products. Interest in bacteriocins is increasing because of the increasing tendency of consumers to use healthy, natural and additive-free products in foods. In this study, it was aimed to investigate the control of *P. aeruginosa* in milk by using partially purified bacteriocins produced from lactic acid bacteria (LAB) strains. Among the 13 reference LAB strains, four strains that showed the highest antimicrobial activity by the agar spot test were selected for bacteriocin production. The bacteriocins were partially purified with 40% ammonium sulfate. The antibacterial activity of bacteriocins on *P. aeruginosa* strains was determined in arbitrary unit by the well diffusion method. Then, UHT milk samples inoculated with *P. aeruginosa* and bacteriocin cocktail were stored at +4°C for a week and bacterial counts were performed on the 1st, 3rd, 5th, and 7th days. The LAB strains that displayed the widest clear zones on *P. aeruginosa* were determined as *L. plantarum*, *L. paraplantarum*, *L. fermentum* and *L. pentosus* and the antimicrobial activities of the partially purified bacteriocins of these strains were 640, 640, 160 and 80 AU/ml, respectively. Among tested *P. aeruginosa* strains, the highest antibacterial effect was observed against *P. aeruginosa* ATCC 15442 (>18mm). In the milk model, the bacteriocin cocktail caused a decrease of approximately 2 log cfu/ml in the number of bacteria for up to three days and the number remained constant until the end of the seventh day. However, the decrease in the number of bacteria was not statistically significant (P>0.05). As a result, bacteriocins obtained from *Lactobacillus* strains showed antibacterial effect on *P. aeruginosa* on agar medium but could not achieve a significant decrease on the milk. However, bacteriocins, which have generally been proven to efficient on Gram-positive bacteria, have been determined to be effective on *P. aeruginosa*, a Gram-negative bacterium. Moreover, this study emphasizes that in addition to *in-vitro* experiments, products to be used for biocontrol purposes in foods are also needed to complement with food models.
Keywords: Bacteriocin cocktail, lactic acid bacteria, milk, *P. aeruginosa*, storage

Kısmi Saflaştırılmış Bakteriyosinlerin *Pseudomonas aeruginosa* Üzerindeki Antimikrobiyal Etkisi

Öz: Bakteriyosinler, patojen ve/veya gıdalarda bozulmaya neden olan bakterilerin büyümesini engellemek ve gıdaların raf ömrünü uzatmak amacıyla yaygın olarak kullanılmaktadır. Tüketicilerin gıdalarda sağlıklı, doğal ve katkı maddesi içermeyen ürünleri kullanma eğiliminin artması nedeniyle bakteriyosinlere olan ilgi de artmaktadır. Bu çalışmada laktik asit bakterisi (LAB) suşlarından üretilen kısmi saflaştırılmış bakteriyosinler kullanılarak sütte *P. aeruginosa*'nın kontrolünün araştırılması amaçlanmıştır. On üç adet referans LAB suşu arasında agar spot testiyle en yüksek antimikrobiyal aktiviteyi gösteren dört suş, amonyum sülfatla kısmi olarak saflaştırılmıştır. Bakteriyosinlerin *P. aeruginosa* suşları üzerindeki antibakteriyel aktivitesi kuyu difüzyon yöntemiyle arbitrary unit (AU/ml) olarak belirlenmiştir. Ek olarak, *P. aeruginosa* ile kontamine edilen UHT süt örneklerine bakteriyosin kokteyli eklenmiş bir hafta boyunca +4°C'de inkübe edilerek 1., 3., 5. ve 7. günlerde bakteri sayımları yapılmıştır. *P. aeruginosa* üzerinde en geniş zon sergileyen LAB suşlarının *L. plantarum*, *L. paraplantarum, L. fermentum* ve *L. pentosus* olduğu ve bu suşlardan elde edilen kısmi saflaştırılmış bakteriyosinlerinin antimikrobiyal aktivitelerinin sırasıyla 640, 640, 160 ve 80 AU/ml olduğu belirlenmiştir. Test edilen *P. aeruginosa* suşları arasında en yüksek antibakteriyel etki *P. aeruginosa* ATCC 15442'ye (>18mm) karşı gözlenmiştir. Süt modelinde ise, bakteriyosin kokteyli bakteri sayısında üç güne kadar yaklaşık 2 log kob/ml azalmaya neden olmuş ve sayı yedinci günün sonuna kadar sabit kalmıştır. Bununla birlikte, bakteri sayısındaki azalmanın istatistiksel olarak anlamlı olmadığı tespit edilmiştir (P>0.05). Sonuç olarak çalışmada elde edilen bakteriyosinlerin *P. aeruginosa*'ya karşı *in-vitro* ortamda antibakteriyel etki gösterdiği tespit edilmesine karşın gıda modelinde anlamlı bir sonuç elde edilememiştir. Bu çalışmanın verileri gıdalarda biyokontrol amaçlı kullanılacak maddelerin *in-vitro* analizlerin yanı sıra gıda modelleri ile tamamlanmasının önemi vurgulamıştır.
Anahtar kelimeler: Bakteriyosin kokteyli, laktik asit bakterileri, muhafaza, *P. aeruginosa*, süt

Geliş Tarihi/Submission Date : 29.02.2024
Kabul Tarihi/Accepted Date : 13.06.2024

* This study was presented as an oral presentation and full text at the Erzurum II. International Health Sciences and Multidisciplinary Approaches Congress.

Introduction

Foodborne illnesses have been a global concern for years. Despite the use of modern food storage techniques, the rate of illness and death due to foodborne pathogens is still increasing, especially in countries without appropriate food safety monitoring systems. On the other hand, consumers' renewed interest in ready-to-eat foods that are minimally processed and contain additives is a challenge for the food industry. The search for alternative natural compounds to increase food safety and shelf life has become inevitable (Sharma et al., 2017). In this context, bacteriocins are an option that cannot be ignored among the alternatives. Bacteriocins are peptides or proteins synthesized in the ribosomes of Gram-positive and Gram-negative bacteria and released into the extracellular environment (Cotter et al., 2013). Bacteriocins are secreted by some types of bacteria to kill other bacteria. They generally vary in length between 30-60 amino acids and have been associated with many bacterial species such as *Lactobacillus, Pediococcus, Leuconostoc, Lactococcus*, *E. coli, Staphylococcus* and *Enterococcus* (Chen et al., 2003; Mills et al., 2011). Bacteriocins are potential alternatives for use in the food and pharmaceutical industries to prevent food spoilage and the growth of pathogenic bacteria (Verma et al., 2017). It is recognized by the US Food and Drug Administration (FDA) with the "Generally Recognized as Safe (GRAS)" status (Johnson et al., 2018).

Food spoilage is defined as loss of quality in terms of color, odor, texture and, in general, loss of sensory properties and is associated with a microbiological, chemical or physical source (Petruzzi et al., 2017). Psychotropic *Pseudomonas* species are among the most common bacteria that cause spoilage, especially in refrigerated foods (Tirloni et al., 2021). *Pseudomonas* species produce heat-stable lipolytic and proteolytic enzymes that play a very important role in reducing the quality and shelf life of raw and processed milk (Dogan and Boor, 2003). When raw milk is contaminated with *Pseudomonas*, lipolytic enzymes cause hydrolyzation of milk fat, leading to the development of off-flavors, rancidity, and texture changes. Proteolytic enzymes cause degradation of casein and the formation of bitter peptides, which further contribute to spoilage (Erkmen and Bozoglu, 2016). Because of these enzymes are heat stable, they do not vanish after pasteurization. On the other hand, *P. aeruginosa* attracts attention as a foodborne pathogen in various food groups such as water, milk, meat, fruits and vegetables (Chatterjee et al., 2016). On the light of these information, we aimed to investigate the antibacterial effect of partially purified LAB bacteriocins on *P. aeruginosa in-vitro* conditions and in milk model.

Materials and Methods

Strains

Thirteen reference LAB strains were included in the study following: *L. pentosus* ATCC 16366*, L. gasseri* ATCC 33323*, L. paraplantarum* ATCC 10641*, L. plantarum* ATCC 10241*, L. fermentum* ATCC 14931*, L. paracasei* ATCC 25302*, L. casei* ATCC 334, *L. brevis* ATCC 8287*, L. diolivorans* ATCC 4356*, L. acidophilus* ATCC 19435*, L. curvatus* ATCC 25601*, L. buncheri* ATCC 4005*, L. rhamnosus* ATTC 53103. Additionally, both multidrug-resistant strains with various virulence properties and commonly used laboratory reference strains with different virulence properties were used; *P. aeruginosa* ATCC 15442, *P. aeruginosa* ATCC 27853, *P. aeruginosa* PAO1, Vim-2, and Imp-13.

Determination of LAB strains effective against P. aeruginosa

Among 13 LAB strains, first it was intended to determine the ones which produce bacteriocins effective against *P. aeruginosa*. For this purpose, serial dilutions of LAB strains were prepared, and appropriate 0.1 ml was inoculated to MRS agar to obtain approximately 30 colonies on the petri dish. Also, *P. aeruginosa* strains were incubated individually at 37°C overnight. After incubation, 200 µl of each strain (10^9 cfu/ml) were inoculated in LB soft agar and overlaid on the MRS agar plates which had LAB colonies on it. Petri dishes were incubated at 37°C overnight. LAB strains that formed transparent zones around the colonies were determined as bacteriocin producers effective against *P. aeruginosa* and selected for further analyses (Kaya and Simsek, 2019).

Agar spot assay

LAB strains that formed clear zones on the *P. aeruginosa* mixed culture were checked once again. For this purpose, 200 µl of the active P*. aeruginosa* mix culture enriched the night before was inoculated into LB soft agar and poured onto LB agar and MRS agar as a second layer. Petri dishes were incubated at 37° C overnight in an aerobic environment to ensure that *P. aeruginosa* strains showed turbidity. The next day, each petri dish was divided into four and 20 µl of active LAB strains were added and incubated overnight at 30°C in an anaerobic environment. At the end of the incubation, four strains that formed the largest zone around the areas where LAB was dropped were selected to obtain bacteriocin (Elyass et al., 2015).

Partial purification of bacteriocins with ammonium sulfate

Selected LAB strains were enriched in 250 mL MRS broth medium and incubated at 30ºC for 24 hours. At the end of the incubation, the developing cultures

were centrifuged at 10,000 rpm for 30 minutes at 4° C. Then, the supernatant filtered through 0.22 µm microporous membrane filters (ISOLAB Laborgerate GmbH, Eschau, Germany). To eliminate the antimicrobial effect that may arise from organic acids or the pH of the MRS broth, the pH of the supernatants was adjusted to 6.5-7.0 with a pH meter (Ohaus, 3100, USA) using 5 N NaOH or 5 N HCl. Then, ammonium sulfate was slowly added to the final concentration of 40% and stirred until dissolved and incubated at +4ºC overnight. On the next day, the samples were centrifuged (Hermle Z326K, Germany) at 13,000 rpm for 45 minutes at +4°C. The upper phase was discarded, and the remaining precipitate was dissolved in 4 mL of sterile 0.05 M potassium phosphate buffer (pH 7.0). The suspended sediment mixtures were filtered through 0.22 µm filters again and stored at -20°C as crude extracts of bacteriocins (Zhao et al., 2020).

Antimicrobial activity of partially purified bacteriocins

Antimicrobial activity of partially purified bacteriocins was determined by well diffusion method. Double-layer LB agars were prepared with five *P. aeruginosa* strains, both separately and mixed. Wells were opened on the surface of the double-layered medium using a glass pasteur pipette. The bottom of the wells was covered by placing 10 µl of MRS agar at the bottom of the wells. After 100 µl of crude bacteriocins were added to the wells, they were incubated at 37°C for 24 hours. At the end of the incubation, bacteriocins that formed a zone around the well were evaluated as positive and the resulting zone diameters were measured and recorded (Lei et al., 2020; Parlindungan et al., 2021).

To determine the quantitative bacteriocin activity, crude bacteriocins were diluted twofold (1/2, 1/4, 1/8, 1/16, 1/32, 1/64) with phosphate buffer (20 mM, pH 7.0). One hundred µl of each dilution added to the wells opened in double-layer LB agar containing *P. aeruginosa* ATCC 15442, which gave the widest zone as a result of the well diffusion test. Petri dishes were left at room temperature for one hour to allow diffusion and then incubated at 37ºC for 24 hours. Inhibition zones around the wells were measured (mm) and recorded. Antimicrobial activity of bacteriocins were expressed in Arbitrary Unit (AU/ml) using the formula as follows: $AU/ml = 2^n \times (1000/x)$ [n: the last dilution showing any inhibition zone; x: the volume of the bacterium added to each hole (Zhao et al., 2020; Parlindungan et al., 2021).

Determination of antibacterial effect in milk model

Bacteriocin cocktail consisted of four bacteriocins which formed the widest zones were selected to examine the antibacterial effect on *P. aeruginosa* in milk. The reason for using a bacteriocin cocktail is that cocktails have a greater potential to lead to synergistic antimicrobial effects, so the overall activity of the cocktail would be bigger than the sum of the individual bacteriocins. The bacteriocin cocktail was prepared by mixing equal amounts of each bacteriocin. Then, UHT milk was added to the bacteriocin cocktail at a final concentration of 190 AU/ml and the test *P. aeruginosa* strain to a final concentration of 10^4 cfu/ml. For the control group, the samples were prepared with milk and bacterial culture only. The milk samples were stored at +4°C for 7 days. In addition to the initial day counts, *P. aeruginosa* counts were performed by planting on LB agar on the 1st, 3rd, 5th, and 7th days of storage. The experiment was carried out in three parallel repetitions (Kaya and Sımsek, 2019). The data was analyzed using the student t-test, considering the P value less than 0.05 as statistically significant.

Results

LAB strains with antimicrobial effect against P. aeruginosa

Among the thirteen ATCC strains, only transparent zones around *L. plantarum* and *L. fermentum* colonies were observed with the spread method, which was described in “Determination of LAB strains effective against *P. aeruginosa*” section. In addition to these strains, with the agar spot test *L. pentosus* and *L. paraplantarum* also displayed wide clear zones on *P. aeruginosa* mixed culture. Therefore *L. plantarum, L. fermentum, L. pentosus* and *L. paraplantarum* were selected to obtain bacteriocins.

Antimicrobial activity of partially purified bacteriocins

Based on the agar spot test results, *L. pentosus*, *L. plantarum, L. paraplantarum* and *L. fermentum* were selected for further bacteriocin production. The inhibition zone diameters formed by the partially purified bacteriocins by the well diffusion method against each of the *P. aeruginosa* strains and their mixtures are given in Table 1. Four bacteriocins formed the largest zones for *P. aeruginosa* ATCC 15442 strain. The antimicrobial activities of these four strains on *P. aeruginosa* ATCC 15442 were determined as 640 AU/ml for Bc-Pla and Bc-Para (from *L. plantarum* and *L. paraplantarum*), followed by Bc-Fer (from *L. fermentum*) with 160 AU/ml and Bc-Pen (from *L. pentosus*) with 80 AU/ml.

Table 1. The inhibition zone diameters of partially purified bacteriocins on *P. aeruginosa* ATCC 15442

Strain	Bacteriocin	Zone
***P. aeruginosa* ATCC 15442**	Bc-Pen	18 mm
	Bc-Fer	20 mm
	Bc-Para	20 mm
	Bc-Pla	21 mm
***P. aeruginosa* ATCC 27853**	Bc-Pen	17 mm
	Bc-Fer	19 mm
	Bc-Para	20 mm
	Bc-Pla	20 mm
***P. aeruginosa* PAO1**	Bc-Pen	14 mm
	Bc-Fer	16 mm
	Bc-Para	14 mm
	Bc-Pla	16 mm
***P. aeruginosa* VIM-2**	Bc-Pen	14 mm
	Bc-Fer	15 mm
	Bc-Para	16 mm
	Bc-Pla	16 mm
***P. aeruginosa* Imp-13**	Bc-Pen	14 mm
	Bc-Fer	16 mm
	Bc-Para	17 mm
	Bc-Pla	17 mm
***P. aeruginosa* mix**	Bc-Pen	14 mm
	Bc-Fer	13 mm
	Bc-Para	14 mm
	Bc-Pla	14 mm

Bc-Pen: bacteriocin of L. pentosus, Bc-Pla: bacteriocin of L. plantarum,
Bc-Para: bacteriocin of L. paraplantarum, Bc-Fer: bacteriocin of L. fermentum.

Antimicrobial effect of the bacteriocins in milk model

Milk samples with bacteriocin cocktail and *P. aeruginosa* were stored at +4°C for a week and bacterial counts were made on the 1st, 3rd, 5th and 7th days. Accordingly, while a decrease of 1.71 log cfu/ml was observed in the control group on the 1st day, 0.72 log cfu/ml reduction was recorded in the experimental group. However, on the 3rd day, while no logarithmic decrease was observed in the control group, there was a 1.01 log cfu/ml decrease in the experimental group. By the fifth and seventh days, the bacterial count remained at approximately 3 log cfu/ml in both groups. As a result, it was determined that the bacteriocin cocktail used in this study provided a 1.73 log cfu/ml reduction at refrigerator temperature (4°C) for up to three days, and the number of bacteria remained constant after three days. However, the decrease in the experimental group was not statistically significant (P>0.05). The average results of three parallel repeats are shown in Figure 1.

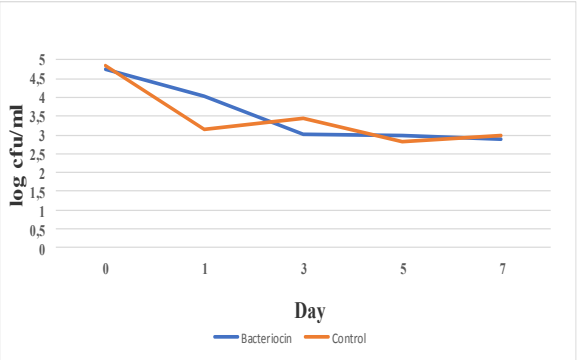


Figure 1. Antibacterial effect of bacteriocin cocktail in UHT milk against *P. aeruginosa* ATCC 15442.

Discussion and Conclusion

In this study, the antimicrobial effect of bacteriocins obtained from LAB strains on *P. aeruginosa* was investigated *in-vitro* and in milk model. Firstly, LAB strains with antibacterial effects on *P. aeruginosa* mix culture were identified. Afterwards, LAB strains that formed the largest clear zones against *P. aeruginosa* strains were distinguished. In this context, *L. pentosus, L. fermentum*, *L. paraplantarum* and *L. plantarum* strains were selected to obtain bacteriocins and further used in milk model.

The inhibitory effect of various LAB strains against Gram-positive and Gram-negative pathogens has been reported by many researchers. Shokri et al. (2017) isolated a total of 57 *Lactobacillus* strains from local yogurt and milk samples and examined their antibacterial effects against *P. aeruginosa* strains. Among the *Lactobacillus* strains, cell-free supernatants of two *Lactobacillus* strains (L1 and L2) were reported to show inhibition ranging from 12-20 mm in diameter against all 80 *P. aeruginosa* strains in the well diffusion method. Elyass et al. (2015) examined the antibacterial activity of *Lb. curvatus* M3 and *P. pentosaceus* N2 bacteriocins using the agar well test. While *Lb. curvatus* M3 showed a zone of 13-19 mm against *S. aureus*, *B. subtilis*, *E. coli* and 6-12 mm against *E. faecalis*, *P. pentosaceus* N2 showed a zone of over 20 mm against *S. aureus* and 13 -19 mm against *B. sublitis*, 6-12 mm zone against *E. coli* and *E. faecalis*. As a result of screening the inhibitor activity spectrum, it was reported that 7 out of 10 test bacteria were inhibited by both bacteriocins but could not inhibit *P. aeruginosa* and *Salmonella Typhi* isolates. In another study, the antibacterial activity of *Pediococcus acidilactici* BAMA 15 was investigated against *E. coli* and *S. aureus* and the zones were measured as 6.44 mm and 7.53 m, respectively. Researchers reported that of Gram-negative bacteria was more resistant than Gram-positive bacteria to the bacteriocin (Nasution et al., 2023). In the research conducted to isolate probiotic lactic acid bacteria from kiwi fruit pulp, a total of eight isolates were found and two of them were stated to be probiotic LAB strains. The antibacterial effect of isolates A2 and A5 against pathogenic bacteria such as *Staphylococcus*, *Pseudomonas* and *E. coli* was examined. The isolates exhibited inhibition against Gram-positive bacteria but not against Gram-negative bacteria such as *E. coli*. It was concluded that lactic acid bacteria have inhibitory properties primarily against Gram-positive bacteria. Overall, it has been stated that lactic acid bacterial strains are generally ineffective against Gram-negative bacteria due to the resistance provided by the outer membrane (Kamaliya et al., 2023).

In this study, it was observed that the inhibition activity of the bacteriocins differed on *P. aeruginosa* ATCC 15442. The highest bacteriocin inhibition activities were detected in the bacteriocins of *L. plantarum* and *L. paraplantarum* strains, with 640 AU/ml. In a study by Elhag et al. (2014), lactic acid bacteria were isolated from fresh sausages, intestines of different animals, saliva, cheese and cucumber. *Salmonella* spp., *S. Typhi, S. aureus*, *B. subtilis*, *B. cereus*, *B. stearothermophilus*, *B. pantotheticus*, *E. coli* and *Pediococcus* BFE 2306 strains were included in the study. Pellets of lactic acid bacteria (including *E. faecalis* (3 isolates), *E. avium*, *P. pentosaceus* (3 isolates), *P. domanosus*, *Lb. murinus* (2 isolates), *L. gasseri* (2 isolates), *Lb. acidophilus*, *L. plantarum*, *Lb. alimentarius* and *L. rhamnosus*, *E. faecalis*, *P. pentosaceus* and *Lb. murinus* were examined and it has been reported that the majority of partially isolated bacteriocins show either weak or no antimicrobial activity against the mentioned microorganisms (0.00-640 AU/ml).

It is possible to use more than one bacteriocin as a cocktail to increase the effect in the control of foodborne pathogens. In this study, the effect of the bacteriocin cocktail against *P. aeruginosa* in milk was investigated at refrigerator temperature. Although a decrease of approximately 2 log cfu/ml was observed in the number of bacteria for up to three days and no increase or decrease in the number of bacteria was observed until the seventh day, the results were not statistically significant when compared to the control group ($P>0.05$). The fact that the bacteriocin cocktail did not show a significant antibacterial activity may be associated with the fact that the pathogenic bacteria used were Gram-negative. As mentioned above, studies report that Gram-positive bacteria are generally more sensitive to bacteriocin (Nasution et al. 2023, Kamaliya et al. 2023, Elyass et al., 2015). The cell wall structure of Gram-positive bacteria has a lower lipopolysaccharide, lipoprotein and phospholipid composition than Gram-negative bacteria (Cao-Hoang et al., 2010). It is reported that the simpler cell wall structure of Gram-positive bacteria plays an important role on bacteriocin activity (Kusharyati et al., 2021). Additionally, although there are many characterized bacteriocins with potential for use in foods, biocontrol effectiveness in foods depends on various factors such as pH, temperature, food composition, and target pathogen/strain. Therefore, it is necessary to establish standardized conditions for the use of each bacteriocin in each food matrix (Prudencio et al., 2015). On the other hand, taking into account the pathogenic bacterial load in foods in a real-life scenario, each log decrease in the number of bacteria can be considered promising in terms of food safety.

There are studies on the application of bacteriocins in milk. However, up to our knowledge this is the first study focused on antimicrobial effect of bacteriocins on *P. aeruginosa* on this food model. In a study, a new broad-spectrum bacteriocin from fermented foods, Garviecin LG34, was obtained. While the numbers of *S. aureus* and *L. monocytogenes* in the control group increased over time during the 12-day incubation period at 4°C, significant differences were observed in the number of bacteria in the milk samples containing bacteriocin. It was reported that the number of *S. aureus* bacteria in whole milk, low-fat milk and skim milk in the experimental group decreased by 1.0, 2.9 and 4.1 log cfu/ml, respectively. In the same experimental setup, at the end of 12 days of incubation, a decrease of 2.0, 4.1 and 4.9 log cfu/ml was recorded in *L. monocytogenes* numbers, respectively. Additionally, the antimicrobial effect of

Garviecin LG34 bacteriocin against these two pathogens has been reported to be strongest in skim milk and weakest in whole milk. It has been stated that whole milk contains a large amount of fat globules, and its surface can be absorbed by garviecin LG34, which may cause a decrease in inhibition (Gao et al., 2023). In a study conducted by Verma et al. (2017) to increase the shelf life of raw buffalo milk, pediocin PA -1 was added to raw buffalo milk at 1%, 5% and 10% (v/v) concentrations contaminated with 10^5 cfu/ml *S. aureus*. It was stated that the number of *S. aureus* in milk was counted as 5, 4 and 3 log cfu/ml, respectively, depending on the percentage of Pediocin PA-1 addition, while it increased to 9 log cfu/ml in the control group. In another study, it was reported that 10,000 IU/mL nisin managed to reduce the number of *S. aureus* in milk by 4.68 log cfu/ml after 4 hours of incubation at 37°C, but the surviving cells again caused an increase in the number of bacteria at the end of 24 hours. It has been stated that this situation indicates the presence of nisin-resistant bacteria (Arques et al., 2011).

As a result, in this study, it was determined that bacteriocins obtained from *Lactobacillus* strains showed an antibacterial effect on *P. aeruginosa* on agar medium but could not achieve a significant decrease on the milk. However, bacteriocins, which have generally been proven to efficient on Gram-positive bacteria, have been determined to be effective on *P. aeruginosa*, a Gram-negative bacterium. Moreover, this study shows that in addition to in-vitro experiments, products to be used for biocontrol purposes in foods are also complemented with food models. In this regard, it is thought that detailed studies to understand how bacteria can survive and adapt in complex environments such as food matrix will be effective in increasing the success in biocontrol with bacteriocins. It is believed that as when the need for food safety increases day by day, characterizing target-specific bacteriocins in detail, standardizing their use and commercializing them with new generation strategies become important.

Acknowledgements

This article contains the findings of Eda Hacıömeroğlu's Master thesis of Kırıkkale University Institute of Health Sciences, Department of Food Hygiene and Technology.

Conflict of Interest

The authors state that there is no conflict of interest.

References

Arqués JL, Rodríguez E, Nuñez M, Medina M. Combined effect of reuterin and lactic acid bacteria bacteriocins on the inactivation of foodborne pathogens in milk. Food Contr 2011; 22: 457-61.

Cao-Hoang L, Chaine A, Gregoire L, Wache Y. Potential of nisin-incorporated sodium caseinate films to control *Listeria* in artificially contaminated cheese. Food Microbiol 2010; 27: 940-4.

Chatterjee M, Anju C, Biswas L, Kumar VA, Mohan CG, Biswas R. Antibiotic resistance in *Pseudomonas aeruginosa* and alternative therapeutic options. Int J Med Microbiol 2016; 306: 48-58.

Chen H, Hoover DG. Bacteriocins and their food applications. Compr Rev Food Sci Food Saf 2003; 2 (3): 82-100.

Cotter PD, Ros RP, Hill C. Bacteriocins-a viable alternative to antibiotics? Nat Rev Microbiol 2013; 11: 95-105.

Dogan B, Boor KJ. Genetic diversity and spoilage potentials among *Pseudomonas* spp. isolated from fluid milk products and dairy processing plants. Appl Environ Microbiol 2003; 69: 130-8.

Elhag NB, Babiker ERB, Mahdi AA. Effect of bacteriocins-producing lactic acid bacteria on target microorganisms. J Agri-Food Appl Sci 2014; 2 (5):155-62.

Elyass ME, Altayar MA, Mahdi AA, Abdelrawaf SS, Shigidi MT, Attitalla IH. Characterization and evaluation of antimicrobial activity of bacteriocins from *Lactobacillus curvatus* and *Pediococcus pentosaceus*. J Infect Non Infect Dis 2015; 1: 001.

Erkmen O, Bozoglu TF. Food microbiology: Principles into practice. Volume 1, New York: Wiley, 2016 p.311.

Gao Y. Antibacterial mechanism of garviecin LG34 against *S. aureus* and *L. monocytogenes* and its application in dairy products preservation. Research Square 2023; 2-18.

Johnson EM, Jung Y, YuJin Y, Jayabalan R, Yang SH, Suh JW. Bacteriocins as food preservatives: Challenges and emerging horizons. Crit Rev Food Sci Nutr 2018; 58(16): 2743-67.

Kamaliya DB, Javia BM, Gadhvi MS, Dudhagara DR. Isolation and characterization of probiotic bacteria from fruit pulp, screening of probiotic properties and production of bacteriocin and probiotic curd. Open J Biol Sci 2023; 8(1): 33-42.

Kaya HI, Simsek O. Characterization of pathogen-specific bacteriocins from lactic acid bacteria and their application within cocktail against pathogens in milk. LWT-Food Sci Technol 2019; 115: 108464.

Kusharyati DF, Satwika TD, Mariana A, Rovik A. Potential screening of bacteriocinogenic-lactic acid bacteria rom mangrove sediment of Logending

Beach for fisheries product preservation. J Trop Biodivers Biotechnol 2021; 6(1): 61927.

Lei S, Zhao R, Sun J, Ra J, Ruan X, Zhu Y. Partial purification and characterization of a broad-spectrum bacteriocin produced by a *Lactobacillus plantarum* zrx03 isolated from infant's feces. Food Sci Nutr 2020; 8(5): 2214-22.

Mills S, Serrano L, Griffin C, O'connor, PM, Schaad G, Bruining, C. Inhibitory activity of *Lactobacillus plantarum* LMG P-26358 against *Listeria innocua* when used as an adjunct starter in the manufacture of cheese. Microb Cell Fact 2011; 10: S7.

Nasution SA, Fachrial E, Ginting CN, Liena Piska F. Bacteriocin-producing *Pediococcus acidilactici* BAMA 15 isolated from "naniura" traditional foods in North Sumatra, Indonesia. Biodiversitas 2023; 24: 2830-5.

Parlindungan E, Dekiwadia C, Jones OA. Factors that influence growth and bacteriocin production in *Lactiplantibacillus plantarum* B21. Process Biochem 2021; 107: 18-26.

Petruzzi L, Corbo MR, Sinigaglia M, Bevilacqua A. Chapter 1-Microbial spoilage of foods: Fundamentals. In The MicroBiological Quality of Food-Foodborne Spoilers; Bevilacqua A, Corbo MR, Sinigaglia M, eds. Sawston, Cambridge, UK; Woodhead Publishing, 2017; pp. 1-21.

Prudêncio CV, dos Santos MT, Vanetti MCD. Strategies for the use of bacteriocins in Gram-negative bacteria: relevance in food microbiology. J Food Sci Technol 2015; 52(9): 5408-17.

Sharma C, Singh BP, Thakur N, Gulati S, Gupta S, Mishra SK, Panwar H. Antibacterial effects of *Lactobacillus* isolates of curd and human milk origin against food-borne and human pathogens. Biotech 2017; 7: 31.

Shokri D, Khorasgani, MR, Mohkam M, Fatemi SM, Ghasemi Y, TaheriKafrani A. The inhibition effect of *Lactobacilli* against growth and biofilm formation of *Pseudomonas aeruginosa*. Probiotics Antimicrob Proteins 2017; 10(1): 34-42.

Tirloni E, Bernardi C, Stella S. *Pseudomonas* spp. are food grade organic acids efficient against these spoilage microorganisms in fresh cheeses? Foods 2021; 10: 891.

Verma SK, Sood SK, Saini RK and Saini N. Pediocin PA-1 containing fermented cheese whey reduces total viable count of raw buffalo (*Bubalis bubalus*) milk. LWT-Food Sci Technol 2017; 83: 193-200.

Zhao R, Lu Y, Ran J, Li G, Lei S, Zhu Y and Xu B. Purification and characterization of bacteriocin produced by *Lactobacillus rhamnosus* zrx01. Food Biosci 2020; 38: 100754.

ERCİYES ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ
Journal of Faculty of Veterinary Medicine, Erciyes University
Research Article / Araştırma Makalesi
21(3), 162-167, 2024
DOI: 10.32707/ercivet.1587330

Effects of Potassium Dichromate and Boron on Oxidative Stress and DNA Damage in Rats*,**

Meryem EREN[1,a], Meryem ŞENTÜRK[1,b]

[1]University of Erciyes, Faculty of Veterinary Medicine, Department of Biochemistry, Kayseri-TÜRKİYE
ORCID: [a]000-0003-1339-0493; [b]0000-0002-3763-8310

Corresponding author: Meryem EREN; E-mail: erenmeryem@hotmail.com, meren@erciyes.edu.tr
How to cite: Eren M, Şentürk M. Effects of potassium dichromate and boron on oxidative stress and DNA damage in rats. Erciyes Üniv Vet Fak Derg 2024; 21(3):162-167

Abstract: In this study, the effects of potassium dichromate ($K_2Cr_2O_7$) and boron (B, as boric acid) on oxidative stress and DNA damage in rat serum and liver were investigated. Sixty female Sprague-Dawley rats were divided into six groups of 10 animals each. The first group was kept as the control group. The second and third groups received 5 and 10 mg/kg B, respectively, the forth group received 10 mg/kg $K_2Cr_2O_7$, and the fifth and sixth groups received 5 and 10 mg/kg B plus $K_2Cr_2O_7$, respectively, orally for two weeks. Compared with the control groups, serum MDA levels increased ($P<0.01$) and TAC levels decreased ($P<0.001$) in the $K_2Cr_2O_7$ group. Serum MDA levels decreased in the $K_2Cr_2O_7$+5 and 10 mg/kg B groups, but a significant decrease was found in the $K_2Cr_2O_7$+10 mg/kg B group ($P<0.01$). Serum TAC levels showed a numerical increase in $K_2Cr_2O_7$+B groups. The liver MDA level was significantly decreased in the $K_2Cr_2O_7$+10 mg/kg B group compared to the other groups ($P<0.01$). There was no difference in plasma 8-OHdG levels between the groups. A positive correlation was observed between liver B and Cr levels ($P<0.05$). In this study, serum MDA and TAC levels were negatively affected in rats administered 10 mg/kg $K_2Cr_2O_7$. In contrast, administration of 10 mg/kg B to the $K_2Cr_2O_7$ group had positively effected on serum and liver lipid peroxidation indicators.
Keywords: Boron, DNA damage, oxidative stress, potassium dichromate, rat

Ratlarda Potasyum Dikromat ile Bor'un Oksidatif Stres ve DNA Hasarı Üzerine Etkileri

Öz: Bu çalışmada, sıçanlarda potasyum dikromat ($K_2Cr_2O_7$) ve borun (B, borik asit olarak) serum ve karaciğer oksidatif stres ve DNA hasarı üzerine etkileri araştırıldı. Altmış dişi Sprague-Dawley sıçan, her birinde 10 hayvan bulunan altı gruba ayrıldı. Birinci grup kontrol grubu olarak tutuldu. İkinci ve üçüncü gruplara sırasıyla 5 ve 10 mg/kg B; dördüncü gruba 10 mg/kg $K_2Cr_2O_7$; beş ve altıncı gruplara ise 5 ve 10 mg/kg B ile birlikte $K_2Cr_2O_7$ 2 hafta boyunca oral olarak verildi. Kontrol grupları ile karşılaştırıldığında $K_2Cr_2O_7$ grubunda serum MDA düzeyleri artarken ($P<0.01$), TAC düzeyleri azaldı ($P<0.001$). Serum MDA düzeyleri $K_2Cr_2O_7$+5 ve 10 mg/kg B gruplarında azaldı; ancak, anlamlı azalma $K_2Cr_2O_7$+10 mg/kg B grubunda ($P<0.01$) tespit edildi. Serum TAC düzeyleri $K_2Cr_2O_7$+B gruplarında sayısal artış gösterdi. Karaciğer MDA düzeyi, $K_2Cr_2O_7$+10 mg/kg B grubunda diğer gruplara göre anlamlı düzeyde azaldı ($P<0.01$). Gruplar arasında plazma 8-OHdG düzeyleri açısından fark belirlenemedi. Karaciğer B ve Cr düzeyleri arasında pozitif korelasyon saptandı ($P<0.05$). Bu çalışmada 10 mg/kg $K_2Cr_2O_7$ uygulanan ratlarda serum MDA ve TAC düzeyleri olumsuz etkilenirken, $K_2Cr_2O_7$ grubuna 10 mg/kg B verilmesi, serum ve karaciğer lipid peroksidasyon göstergeleri üzerine olumlu etki yaptığı söylenebilir.
Anahtar kelimeler: Bor, DNA hasarı, oksidatif stres, potasyum dikromat, rat

Introduction

Chromium (Cr) occurs at 0, 2^+, 3^+, and 6^+ oxidation states at the level of a metallic element and the 3^+ and 6^+ valance compounds are biologically most important (Mertz, 1969; Mc Dowell, 1992; Cohen et al., 1993; Barceloux, 1999; Zayed and Terry, 2003). All chemical forms of Cr, except chromates, can be removed quickly from the blood. The plasma Cr level is not a good indicator of the level of Cr in tissues since there is no balance between the levels of Cr in the tissues and circulation (Mc Dowell, 1992). The reticuloendothelial system shows a great affinity for Cr (Mertz, 1969); it has been reported that Cr is found to be less in blood, muscle, heart, lung, and brain compared to kidney, liver, pancreas, and spleen (Anderson and Polansky, 1995). Potassium dichromate ($K_2Cr_2O_7$) is an inorganic chemical substance generally used as an oxidizing agent in various laboratory and industrial areas (Danadevi et al., 2004; Patel et al., 2016). The Cr^{+6} is the most stable and strong oxidizing agent after Cr^{+3}, especially in the acidic environment. Hexavalent chromium binds oxygen as a chromate (CrO_7^{2-}) or dichromate ($Cr_2O_7^{2-}$) with a strong oxidative capacity (Mc Dowell, 1992; Nickens et al., 2010; Fedala et al., 2021; Orhan et al.,

Geliş Tarihi/Submission Date : 01.04.2024
Kabul Tarihi/Accepted Date : 10.07.2024

* This study was presented as a poster at the 8th National Veterinary Biochemistry and Clinical Biochemistry Congress (Bursa, TÜRKİYE, 22 - 24 Eylül 2016. ss.120-121).
** This research was supported by Erciyes University Research Fund. Project no: TSA-12-4017.

2022). Chromate ions (Cr^{+6}) can cross the cell membrane very quickly. In addition, the fact that chromates are very strong oxidants and irritants causes them to be more toxic than Cr^{+3}. Potassium dichromate is the most toxic form of Cr and is considered a carcinogen in humans and animals. It has been reported that the carcinogenic effect of CrO_7^{2-} ions causes DNA lesions and mutations (Mertz, 1969; Patlolla et al., 2009; Nickens et al., 2010; Patel et al., 2016). Excessive production of reactive oxygen species (ROS), which are produced during the reduction of Cr^{+5} and Cr^{+6}, reveals the toxic effects of Cr (Patlolla et al., 2009; Nickens et al., 2010; Patel et al., 2016; Bashandy et al., 2021; Fedala et al., 2021). Reactive oxygen species are also important for their toxic effects in that they damage macromolecules such as lipids, proteins, carbohydrates, and nucleic acids, disrupting the structure and function of cell membranes and causing cell death by inactivating enzymes (Nordberg and Arner, 2001; Klaunig et al., 2004).

Boron (B) is a dark brown nonmetal and is found in nature in the forms of borax (BX), colemanite, boronatrocalcite, and boric acid (BA) (Underwood and Suttle, 1999) and is widely used in industry (Butterwick et al., 1989). Although B was accepted to be essential for plants in 1923, it has recently been found that it may be an essential element for animals and humans (Mc Dowell, 1992; Nielsen, 1988; 1994). Although there is literature reporting that the amount of B that should be taken daily can vary between 0.5-1.0 mg in humans (Nielsen, 1991; WHO, 1996), there is no definite recommendation for humans and animals (NRC, 1994). Although it is thought that B can react with bio substances containing cis-hydroxyl groups (for example, glycolipids and phosphoinositides) and may be effective in the continuity of cell membrane functions or stability, hormone receptors, and transmembrane signals, its biochemical function in human and animal tissues is little known (Nielsen, 1990; 1991; Mc Dowell, 1992; Nielsen, 1994). Various studies have shown that B element is effective on bone, mineral, energy metabolism, immune and endocrine function and lipid peroxidation, antioxidant system and DNA damage (Türkez et al., 2007; İnce et al., 2010, 2012, 2014; Küçükkurt et al., 2015a,b, 2017; Acaröz et al., 2018, 2019; Çakır et al., 2018; İnce et al., 2019; Sarıca et al., 2019). Adequate literature has not been found regarding the effect of B element against oxidative stress caused by $K_2Cr_2O_7$ (Iztleuov et al., 2019, Sarıca et al., 2019). In this study, the effects of $K_2Cr_2O_7$, the most toxic form of Cr, and B element (as boric acid) on malondialdehyde (MDA), total antioxidant capacity (TAC), and DNA damage in serum/liver were determined in rats.

Material and Methods

Experimental design

Ethical approval for the study was obtained from the Erciyes University Animal Experiments Ethics Committee in Turkey (decision dated 11.04.2012 and numbered 12/56).

In the study, 60 female Sprague-Dawley rats were divided into six groups of ten. Control group (Group 1) rats were given 2 ml of distilled water; Groups 2 and 3 received 5 and 10 mg/kg (live weight)/day B, respectively; group 4 received 10 mg/kg $K_2Cr_2O_7$; 5th group 10 mg/kg $K_2Cr_2O_7$ + 5 mg/kg (live weight)/day B; group 6 also received $K_2Cr_2O_7$ + 10 mg/kg (live weight)/day B. Animals were given distilled water, $K_2Cr_2O_7$ and B (in the form of boric acid) by gavage for two weeks. The $K_2Cr_2O_7$ (Mohammed and Saber, 2011) and B (Price et al.1997) doses applied in this study were determined according to the results of previous studies. Researchers (Mohammed and Saber, 2011) stated that 10 mg/kg $K_2Cr_2O_7$ administration caused oxidative stress in their study in rats. The researchers (Price et al. 1997) determined that the addition of B up to 10 mg/kg (live weight) did not cause any adverse effects in rats, and negative effects began to be seen at doses above 10 mg, and the levels of NOAEL (No Observed Adverse Effect Level) that did not show any negative effects according to blood B levels were determined as 10 mg B/kg (bw). They reported that the low levels (LOAEL) at which signs of toxicity may develop were 13 mg B/kg, bw/day. Water and feed were given to the animals *ad libitum* throughout the experiment.

Collection of samples and biochemical analysis

At the end of the experiment, 2-3 ml of blood was taken from the animals as intra-cardiac (i.c.) into tubes with anticoagulant (Li-heparin) to determine 8-OHdG levels. The blood samples were centrifuged at 1300 x g for 10 minutes and their plasma was separated. To determine serum MDA, TAC, Cr, and B levels, 3-5 ml blood samples taken into tubes without anticoagulant were centrifuged at 1300 x g for 10 minutes and their serum was separated. In addition, liver tissue samples were taken to determine the tissue MDA level.

Serum/tissue MDA (Cayman, 10009055, USA), serum TAC (Rel Assay Diagnostics, RL 024, Turkey), and plasma 8-OHdG (NWK 8-OHdG 02, Northwest Life Science Specialist and LLC) analyses were measured in µQuant Bio-Tek ELISA reader using ELISA kit. Tissue B and Cr analyses were performed at Erciyes University Technology Research and Application Center on an ICP/MS (Agilent 7500a series) device.

Statistical analysis

Statistical analysis of the data was performed using the SPSS 20.0 package program for Microsoft. One-way analysis of variance (ANOVA) and Kruskal Wallis test were applied to determine the difference between groups. Data were analyzed with the Levene test for assumptions of homogeneity of variance and the Shapiro-Wilk test for assumptions of normal distribution (P>0.05). Duncan's Multiple Range Test and Bonferroni test were used to determine which group was responsible for the differences between the groups. Data were given as the mean and standard error of the means. The statistical significance level was determined as P<0.05. Spearman's Rho correlation coefficient was used for correlation analysis.

Results

In this study, compared to the control group, only the $K_2Cr_2O_7$ group had an increase in serum MDA level (P<0.01) and a decrease in the TAC level (P<0.001). Administration of 5 and 10 mg/kg B to the groups with $K_2Cr_2O_7$ decreased serum MDA levels, but a significant decrease was detected in the group given $K_2Cr_2O_7$ +10 mg/kg B (P<0.01). It was observed that the serum TAC levels increased numerically with the addition of B to the groups with $K_2Cr_2O_7$ (Table 1).

Table 1. Serum MDA, TAC, and plasma 8-OHdG levels in control, $K_2Cr_2O_7$, and B administered rats

Parameters	N	Control	5B	10B	$K_2Cr_2O_7$	$K_2Cr_2O_7$ +5B	$K_2Cr_2O_7$ +10B	P
MDA (nmol/mL)	8	5.18 ± 0.38^{bc}	5.32 ± 0.42^{bc}	5.55 ± 0.49^{bc}	7.49 ± 0.83^{a}	6.86 ± 0.48^{ab}	4.76 ± 0.57^{c}	**
TAC (mmol/L)	8	4.43 ± 0.29^{a}	4.25 ± 0.34^{abc}	4.33 ± 0.36^{ab}	2.64 ± 0.19^{d}	3.39 ± 0.18^{cd}	3.46 ± 0.34^{bcd}	***
8-OHdG (ng/mL)	8	0.277 ± 0.003	0.263 ± 0.007	0.272 ± 0.003	0.285 ± 0.008	0.270 ± 0.005	0.267 ± 0.004	-

$^{a-d}$: The difference between values with different letters on the same line is important.
5B: 5 mg/kg Boron, 10B: 5 mg/kg Boron
*-: Not significant, p>0.05; ** P<0.01; ***: P<0.001*

Liver MDA level showed a statistically significant decrease only in the $K_2Cr_2O_7$+10 mg/kg B group (P<0.01) compared to the other groups. The correlation coefficient between liver B and Cr levels was 0.556 and a positive correlation was found (P<0.05; Table 2).

Table 2. Liver MDA, B, and Cr levels in control, $K_2Cr_2O_7$, and B administered rats

Parameters	N	Control	5B	10B	$K_2Cr_2O_7$	$K_2Cr_2O_7$ +5B	$K_2Cr_2O_7$ +10B	P
MDA (nmol/mL)	8	9.50 ± 0.26^{a}	10.18 ± 0.19^{a}	9.80 ± 0.20^{a}	10.37 ± 0.56^{a}	8.45 ± 0.58^{ab}	7.38 ± 1.20^{b}	**
B (ppb)	7	0.13 ± 0.04^{d}	0.44 ± 0.02^{b}	0.51 ± 0.07^{b}	0.29 ± 0.02^{c}	1.96 ± 0.16^{a}	2.41 ± 0.28^{a}	***
Cr (ppb)	7	0.071 ± 0.003^{c}	0.59 ± 0.004^{c}	0.062 ± 0.005^{c}	0.919 ± 0.051^{b}	1.252 ± 0.025^{a}	1.239 ± 0.063^{a}	***

$^{a-c}$: The difference between values with different letters on the same line is important.
5B: 5 mg/kg Boron, 10B: 5 mg/kg Boron
*** P<0.01; ***: P<0.001*

Discussion and Conclusion

Hexavalent chromium (Cr^{+6}) is known to be a potential hepatotoxic and nephrotoxic agent associated with oxidative stress and inflammation (Patlolla et al., 2009; Patel et al., 2016; Navya et al., 2018; Orhan et al., 2022). In the study in which 2.5, 5, 7.5 and 10 mg/kg $K_2Cr_2O_7$ were applied to the diet in rats, it was determined that the MDA levels in both liver and kidneys increased significantly in the groups given 7.5 and 10 mg/kg $K_2Cr_2O_7$ compared to the control group (Patlolla et al., 2009). Likewise, in another study (Orhan et al., 2022), it was observed that administration of $K_2Cr_2O_7$ (15 mg/kg, i.p.) increased MDA levels, which is an indicator of lipid peroxidation, and decreased antioxidant enzymes in rat liver and kidney tissues. Bashandy et al. (2021) administered 10 mg/kg $K_2Cr_2O_7$ orally to rats for 8 weeks and found that glutathione, SOD, and CAT activities in testes decreased, while MDA and NO levels increased. These investigators suggested that the elevated MDA and NO levels in the testicles of rats exposed to $K_2Cr_2O_7$ were probably due to oxidative damage caused by the inability of antioxidant enzymes to scavenge oxidants produced in testicular tissue. Fedala et al. (2021) also administered the same dose of $K_2Cr_2O_7$ subcutaneously to pregnant Wistar albino rats and found that this substance supports hypothyroidism, oxidative stress, genotoxicity, and histological changes in the thyroid gland. Similarly, Patel et al. (2016) demonstrated the presence of genotoxicity and oxidative stress caused by $K_2Cr_2O_7$ (0.625, 1.25, and 2.5 mg/kg) toxicity was given orally by gavage for 28 days in Wistar rats. In another study (Navya et al., 2018), it was determined that 30 mg/kg $K_2Cr_2O_7$ given by gavage for 28 days in Wistar albino rats increased liver enzymes and TBARS levels in serum, decreased antioxidant enzymes in liver tissue, and it stated that increased toxic overload by reactive oxygen species may result in irregular expression in genes. An increase in the activity of lipid peroxidation was observed, possibly due to the formation of the hydroxyl radical catalyzed by chromium as indicated by Patel et al. (2016).

Ince et al. (2010), in their study on rats, found that

adding boric acid and borax to the diet (100 mg B/kg-live weight) significantly reduced the blood MDA level; they stated that there was a numerical decrease in MDA levels in the liver, kidney, and heart, which was not statistically significant, and that it also had positive effects on DNA damage. It has been reported that B can strengthen the antioxidant defense system of tissues by causing changes in oxidative metabolism through an undefined mechanism (Pawa and Ali, 2006; Çoban et al., 2015). In a study that produced hepatotoxicity with CCl_4 in mice; it has been stated that 50, 100 and 200 mg/kg (live weight) boric acid significantly reduces liver MDA levels and increases liver GSH, SOD and CAT activities. It has been stated that the hepatoprotective effect of boric acid may lead to both increased antioxidant defense system activity and inhibition of lipid peroxidation (İnce et al., 2012). Additionally, Türkez et al. (2007) found that 15 mg/L B component increased both SOD and CAT activities in erythrocytes, whereas 500 mg/L B decreased both SOD and CAT activities. On the other hand, Çakır et al. (2018) stated that 5 and 10 mg/kg B (i.p) reduced serum MDA levels in diabetic rats, but did not affect TAC levels. Acaroz et al. (2018) in their study on rats; showed that orally administered element B (5, 10, and 20 mg/kg) had a healing effect on oxidative stress and inflammation caused by acrylamide and alleviated tissue damage by preventing the decrease of antioxidant enzymes and suppressing lipid peroxidation. Likewise, the antioxidant, anti-inflammatory, and regulating effects of B (5, 10, and 20 mg/kg) in a dose-dependent manner on oxidative stress, inflammatory gene expression, metabolic and histopathological changes caused by Bisphenol A in rats have been revealed (Acaröz et al., 2019). Pawa and Ali (2006) 4 mg/kg of borax; Çoban et al. (2015) and Küçükkurt et al. (2017) also found that different doses of B (5, 10 and 20 mg/kg, respectively) may have positive effects on maintaining the oxidant/antioxidant balance in case of oxidative stress in rats in a dose-dependent manner.

Adequate literature has not been found regarding the effect of B element against oxidative stress caused by $K_2Cr_2O_7$ (Iztleuov et al., 2019, Sarıca et al., 2019). In a study (Iztleuov et al., 2019), the cardioprotective effects of orally administered 22.5 and 225 mg/kg sodium tetraborate on chromium intoxication ($K_2Cr_2O_7$), lipid profile correction, and oxidative stress were investigated. In this study, sodium tetraborate administered to rats at a level of 700 mg/L in their drinking water for 21 days increased both MDA levels and antioxidant system activity in the heart tissue, and it was revealed that lower doses had a positive effect on lipid peroxidation and antioxidant system. In a previous study we conducted on this subject, it was observed that lipid peroxidation in the brain tissue of rats treated with $K_2Cr_2O_7$ (10 mg/kg, i.p) decreased significantly after the application of 5 and 10 mg/kg B, but there was no significant change in antioxidant enzyme (SOD, CAT, and GSH-Px) levels (Sarıca et al., 2019).

In the presented study, it was observed that there was no statistical difference between the groups in terms of plasma 8-OHdG levels, but the plasma 8-OHdG level showed a numerical increase only in the $K_2Cr_2O_7$ group compared to the control. However, it was observed that the application of 5 and 10 mg/kg B to the $K_2Cr_2O_7$ groups caused a non-statistical numerical decrease in plasma 8-OHdG levels. Additionally, it was found that 5 and 10 mg/kg B given to groups with $K_2Cr_2O_7$ reduced serum MDA levels; and decreased liver MDA levels with statistical significance only in the group administered $K_2Cr_2O_7$ + 10 mg/kg B. It was also observed that serum TAC levels increased with the application of 5 and 10 mg/kg B to the $K_2Cr_2O_7$ groups, although not statistically. As a result, it was seen that B may have positive effects on oxidative stress and DNA damage caused by $K_2Cr_2O_7$ in rats. It was concluded that new research is needed in which different doses and compounds can be used to determine the exact effects of the boron element on metabolism.

Acknowledgment

We sincerely acknowledge Erciyes University Research Fund for its financial support [Project Number: TSA-12-4017].

References

Acaröz U, İnce S, Arslan Acaröz D, Gürler Z, Demirel HH, Küçükkurt İ, Eryavuz A, Kara R, Varol N, Zhu K. Bisphenol-A induced oxidative stress, inflammatory gene expression, and metabolic and histopathological changes in male Wistar albino rats: protective role of boron. Toxicol Res 2019; 7(3): 503-12.

Acaröz U, İnce S, Arslan Acaröz D, Gürler Z, Küçükkurt İ, Demirel HH, Arslan HO, Varol N, Zhu K. The ameliorative effects of boron against acrylamide-induced oxidative stress, inflammatory response, and metabolic changes in rats. Food Chem Toxicol 2018; 118: 745-52.

Anderson RA, Polansky MM. Dietary and metabolite effects on trivalent chromium retention and distribution in rats. Biol Trace Elem Res 1995; 50(2): 97-108.

Barceloux DG. Chromium. Clin Toxicol 1999; 37(2): 173-94.

Bashandy SAE, Ebaid H, Al-Tamimi J, Ahmed-Farid OAH, Omara EA, Alhazza IM. Melatonin alleviated potassium dichromate-induced oxidative stress and reprotoxicity in male rats. Hindawi BioMed Res Int

2021, Article ID 3565360, 1-12.

Butterwick L, De Oude N, Raymond K. Safety assessment of boron in aquatic and terrestrial environments. Ecotox Environ Safe 1989; 17: 339-71.

Coban FK, Ince S, Kucukkurt I, Demirel HH, Hazman Ö. Boron attenuates malathion-induced oxidative stress and acetylcholinesterase inhibition in rats. Drug Chem Toxicol 2015; 38(4): 391-9.

Cohen MD, Kargacin B, Klein CB, Costa M. Mechanisms of chromium carcinogenicity and toxicity. Crit Rev Toxicol 1993; 23: 255-81.

Çakır S, Eren M, Şentürk M, Soyer Sarıca Z. The effect of boron on some biochemical parameters in experimental diabetic rats. Biol Trace Elem Res 2018; 184(1): 165-72.

Danadevi K, Rozati R, Saleha BB, Grover P. Genotoxic evaluation of welders occupationally exposed to chromium and nickel using the Comet and micronucleus assays. Mutagenesis 2004; 19: 35-41.

Fedala A, Adjroud O, Abid-Essefi S, Timoumi R. Protective effects of selenium and zinc against potassium dichromate-induced thyroid disruption, oxidative stress, and DNA damage in pregnant Wistar rats. Environ Sci Pollut Res Int 2021; 28 (18): 22563-76.

Ince S, Keles H, Erdoğan M, Hazman Ö, Küçükkurt İ. Protective effect of boric acid against carbon tetrachloride-induced hepatotoxicity in mice. Drug Chem Toxicol 2012; 35: 285-92.

Ince S, Kucukkurt I, Cigerci İH, Fidan AF, Eryavuz A. The effects of boric acid and borax supplementation on lipid peroxidation, antioxidant activity, and DNA damage in rats. J Trace Elem Med Biol 2010; 24: 161-4.

Ince S, Kucukkurt I, Demirel HH, Acaröz U, Arslan Acaröz D, Varol N. Boron ameliorates arsenic-induced DNA damage, proinflammatory cytokine gene expressions, oxidant/antioxidant status, and biochemical parameters in rats. J Biochem Mol Toxicol 2019; 33:e22252.

Ince S, Kucukkurt I, Demirel HH, Arslan Acaröz D, Abal E, Cigerci İH. Protective effects of boron on cyclophosphamide induced lipid peroxidation and genotoxicity in rats. Chemosphere 2014;108:197-204.

Iztleuov M, Temirova G, Bashbayeva M, Komyekbay Z, Iztleuov Y, Madikhan Z, Yemzharova G. Effect of sodium tetraborate on oxidative damages in heart tissue in chromium intoxication. Biomed & Pharmacol J 2019; 12(2): 609-18.

Klaunig JE, Kamendulis LM. The role of oxidative stress in carcinogenesis. Annu Rev Pharmacol Toxicol 2004; 44: 239-67.

Küçükkurt İ, Demirel HH, Arslan Acaröz D, İnce S, Eryavuz A. Potential protective effect of boron against gentamicin-induced oxidative stress on rat tissues. Kocatepe Vet Derg 2017; 10(4): 300-7.

Küçükkurt İ, İnce S, Akbel E,Karabağ Çoban F. The effects of dietary boron compounds in supplemented diet on hormonal activity and some biochemical parameters in rats. Toxicol Indust Health 2015; 31 (3): 255-60.

Küçükkurt İ, İnce S, Demirel HH, Türkmen R, Akbel E, Çelik Y. The effects of boron on arsenic induced lipid peroxidation and antioxidant status in male and female rats. J Biochem Mol Toxicol 2015; 29 (12): 564-71.

McDowell LR. Minerals in Animal and Human Nutrition. Academic Press 1992; pp. 265-370.

Mertz W. Chromium Occurrence and Function in Biological Systems. Physicological Reviews. Washington: Walter Reed Army Medical Center, 1969; p. 163-239.

Mohammed NE, Saber RA. Effect of aqueous extract of damsissa (*Ambrosia maritima*) on the biochemical changes induced by potassium dichromate in rats. J Am Sci 2011; 7(3): 234-42.

National Research Council. Nutrient Requirements of Poultry. Ninth Edition, Washington DC: National Academy of Sciences Press, 1994; p. 15.

Navya K, Phani Kuamr G, Chandrasekhar Y, Anilakumar KR. Evaluation of potassium dichromate ($K_2Cr_2O_7$)-induced liver oxidative stress and ameliorative effect of *Picrorhiza kurroa* extract in Wistar albino rat. Biol Trace Elem Res 2018; 184: 154-64.

Nickens KP, Patierno SR, Ceryak S. Chromium genotoxicity: a double-edged sword. Chem Biol Interact 2010; 188: 276-88.

Nielsen FH. Biochemical and physiological consequences of boron deprivation in humans. Environ Health Perspect 1994; 102(suppl 7): 59-63.

Nielsen FH. Boron-An overlooked element of potential nutritional importance. Nutr Today 1988; 4-7.

Nielsen FH. New essential trace elements for the life sciences. Biol Trace Elem Res 1990; 26-27: 599-611.

Nielsen FH. Nutritional requirements for boron, silicon, vanadium, nickel, and arsenic: Current knowledge and speculation. FASEB J 1991; 5:

2661-7.

Nordberg J, Arner ES. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. Free Radical Biol Med 2001; 31: 1287-312.

Orhan S, Ruhi Turkmen R, Demirel HH, Akosman M, Fırat F. An investigation of the effect of chlorogenic acid on potassium dichromate- induced oxidative stress in rats. Research Square (preprint) 2022; 1-27.

Patel G, Joshi DV, Patel BJ, Raval SH, Lambade PS. Potassium Dichromate induced genotoxicity and oxidative stress in Wistar rats (*Rattus norvegicus*). Indian J Vet Pathol 2016; 40(1): 42-6.

Patlolla AK, Barnes C, Yedjou C, Velma VR, Tchounwou PB. Oxidative Stress, DNA Damage, and antioxidant enzyme activity ınduced by hexavalent chromium in Sprague-Dawley rats. Environ Toxicol 2009; 24(1): 66-73.

Pawa S, Ali S. Boron ameliorates fulminant hepatic failure by counteracting the changes associated with the oxidative stress. Chem-Biol Inter 2006; 160: 89-98.

Price CJ, Strong PL, Murray FJ, Goldberg MM. Blood boron concentrations in pregnant rats fed boric acid throughout gestation. Reprod Toxicol 1997; 11(6): 833-42.

Sarıca Soyer Z, Eren M, Şentürk M. Effect of boron on the potassium dichromate induced oxidative damage in brain tissue of Sprague Dawley rats. Pak J Zoology 2019; 51(5): 1905-10.

Türkez H, Geyikoğlu F, Tatar A, Keleş S, Özkan A. Effects of some boron compounds on peripheral human blood. Z Naturforsch 2007; 62: 889-96.

Underwood EJ, Suttle NF. The Mineral Nutrition of Livestock. Third Edition. New York: CABI Publishing, 1999; pp. 513-42.

WHO. Trace Elements in Human Nutrition and Health: Boron. Geneva, 1996; pp. 175-82.

Zayed AM, Terry N. Chromium in the environment: factors affecting biological remediation. Plant Soil 2003; 249: 139-56.

ERCİYES ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ
Journal of Faculty of Veterinary Medicine, Erciyes University
Research Article / Araştırma Makalesi
21(3), 168-175, 2024
DOI: 10.32707/ercivet.1587417

Retrospective Analysis of Feline Ocular Diseases: Insights into Prevalence, Breed Predispositions, and Anatomical Localizations in a Veterinary Hospital in the Northeastern Anatolian Region of Turkey: 310 Cases (2016-2023)

Ayşe GÖLGELİ BEDİR[1a], Latif Emrah YANMAZ[2b], Sıtkıcan OKUR[1c], Mümin Gökhan ŞENOCAK[1d], Ferda TURGUT[1e], Yakup KOCAMAN[3f], Ömer Tarık ORHUN[1g], Uğur ERSÖZ[1h], Elif DOĞAN[4ı], Zafer OKUMUŞ[1i], Büşra BAYKAL[1j]

[1]Atatürk Üniversitesi Veteriner Fakültesi Cerrahi Anabilim Dalı, Erzurum-TÜRKİYE
[2]Burdur Mehmet Akif Ersoy Üniversitesi Veteriner Fakültesi Cerrahi Anabilim Dalı, Burdur-TÜRKİYE
[3]Yozgat Bozok Üniversitesi Veteriner Fakültesi Cerrahi Anabilim Dalı, Yozgat-TÜRKİYE
[4]Kastamonu Üniversitesi Veteriner Fakültesi Cerrahi Anabilim Dalı, Kastamonu-TÜRKİYE
ORCID: [a]0000-0002-9798-8638; [b]0000-0001-5890-8271; [c]0000-0003-2620-897X; [d]0000-0002-8855-8847 ; [e]0000-0003-2956-7548; [f]0000-0002-3580-7782; [g]0000-0003-4184-8879; [h]0000-0002-1687-2327, [ı]0000-0002-3321-8116; [i]0000-0001-5880-1415; [j]0009-0005-2787-1249

Corresponding author: Ayşe GÖLGELİ BEDİR; E-mail: aysegolgeli@atauni.edu.tr
How to cite: Bedir AG, Yanmaz LE, Okur S, Şenocak MG, Turgur F, Kocaman Y, Orhun ÖT, Ersöz U, Doğan E, Okumuş Z, Baykal B. Retrospective analysis of feline ocular diseases: insights into prevalence, breed predispositions, and anatomical localizations in a veterinary hospital in the Northeastern Anatolian Region of Turkey: 310 cases (2016-2023). Erciyes Univ Vet Fak Derg 2024; 21(3):168-175

Abstract: In this retrospective investigation, the medical records of feline patients exhibiting ocular symptoms were comprehensively collected from Atatürk University, Veterinary Faculty, Animal Hospital over the period ranging from 2016 to 2023. A complete investigation was carried out a total of 427 cases with ocular disease observed in 310 cats. A statistical analysis was made to identify patterns associated with breed, age, gender, and anatomical localization. No evidence of gender predisposition to ocular diseases was found, while a higher incidence was noted in kittens aged 0-6 months, resulting in 57.4% of cases. In relation to breed predisposition, it was observed that ocular diseases demonstrated a higher prevalence among Mix breed and British shorthair cat breeds. The predominant conditions observed within patients were adnexa diseases, which represented 50.2% of cases, followed by nasolacrimal system disorders, which represented 27.9% of cases. In summary, it was shown that brachycephalic breeds presented a higher prevalence of nasolacrimal disorders, whereas mix breed breeds indicated a higher incidence of ocular diseases.
Keywords: Adnexa, British shorthair, cat, eye, nasolacrimal

Kedi Oküler Hastalıklarının Retrospektif Analizi: Türkiye'nin Kuzeydoğu Anadolu Bölgesindeki Bir Veteriner Hastanesinde Prevalans, Irk Yatkınlıkları ve Anatomik Lokalizasyonlara İlişkin Görüşler: 310 Vaka (2016-2023)

Öz: Bu retrospektif araştırmada, Atatürk Üniversitesi Veteriner Fakültesi Hayvan Hastanesi'nden 2016-2023 yılları arasında oküler semptomlar gösteren kedi hastalarının tıbbi kayıtları kapsamlı bir şekilde toplandı. Toplam 310 hastada gözlenen 427 oküler hastalık vakası üzerinde tam bir inceleme yapıldı. Irk, yaş, cinsiyet ve anatomik lokalizasyon ile ilişkili kalıpları belirlemek için istatistiksel bir analiz yapıldı. Oküler hastalıklara cinsiyet yatkınlığına dair bir kanıt bulunmazken, 0-6 aylık yavru kedilerde vakaların %57.4'ünü oluşturan daha yüksek bir insidansda olduğu belirlendi. Irk yatkınlığı ile ilgili olarak, oküler hastalıkların Mix breed ve British shorthair kedi ırkları arasında daha yüksek bir prevalans gösterdiği gözlemlendi. Hastalarda gözlemlenen baskın hastalıklar, vakaların %50.2'sini temsil eden adneksa hastalıkları olup, bunu vakaların %27.9'unu temsil eden nazolakrimal sistem bozuklukları izledi. Özetle, brakisefalik ırklarda nazolakrimal bozuklukların daha yüksek prevalans gösterdiği, melez ırklarda ise oküler hastalıkların daha yüksek insidans gösterdiği belirlendi.
Anahtar kelimeler: British shorthair, göz, göz kapakları, kedi, nasolakrimal

Introduction

Ophthalmology plays an important part in the recognition and treatment of disorders affecting animals. The ocular system has a significant the level of complication in both its anatomical make up and physiological mechanisms. The previously identified organ be sensitised and show clinical signs even as a result of slight changes in the physiological balance of localised or systemic diseases (Scountzou, 2003; Akın and Samsar, 2005).

Ocular diseases are frequently observed in feline species. Hence, the assessment of ocular illness prevalence based on factors such as breed, sex, and age retains significant importance in establishing the diagnosis of ocular diseases (Gould and McLellan,

Geliş Tarihi/Submission Date : 26.11.2023
Kabul Tarihi/Accepted Date : 21.08.2024

2014; Akinrinmade and Ogungbenro, 2015; Park et al., 2023). Congenital ocular malformations have been reported in dogs and cats in previous studies (Glaze 2005; Kuehn et al., 2016; Saraiva and Delgado, 2020; Bott and Chahory, 2022). Although there have been many studies on glaucoma, entropion, cherry eye and cataract in cats, there are few studies on the prevalence and epidemiologic analysis of ocular diseases in cats (Chahory et al., 2004; Williams and Kim, 2009; McLellan and Teixeira, 2015; Guyonnet et al., 2019).

Ophthalmologic studies may determine the frequency of ocular diseases and may assist in limiting down potential diagnoses and treatment alternatives (Kanski 2007). While studies investigating the incidence of ocular disorders in dogs and cats have been conducted in France and South Korea (Glaze 2005; Guyonnet et al., 2019; Bott and Chahory, 2022; Sarfaty et al., 2022). The objective of this retrospective study was to investigate the prevalence of ocular disorders in cats, examining the influence of factors such as breed, gender, and age at a Veterinary Hospital in the Northeastern Anatolian Region of Turkey.

Materials and Methods

Medical records review

In this retrospective study, the medical records of feline patients presenting with ocular symptoms at Atatürk University, Veterinary Faculty, Animal Hospital in Erzurum-Türkiye were collected from January 2016 to July 2023. As this study was considered retrospectively and in an observational manner, no institutional or client approval was obtained. Data were gathered for each feline subject, encompassing variables such as sex, age at initial presentation, medical history, length and manifestation of clinical signs, findings from physical examination, diagnosis, treatment administered, and clinical outcomes. An investigation was conducted to determine if both oculars of the patients were impacted. Simultaneously, these structures were categorized based on their localization as adnexa, nasolacrimal canal, cornea, globe/ orbit, üvea, lens.

To perform a statistical comparison of the age ranges across patients, the age scale of cats was utilized as a reference point (Quimby et al., 2021). The patients in the study were categorized into four groups based on their age: patients aged 0-6 months were assigned the Group 1, patients aged 7 months-2 years were assigned the Group 2, patients aged 3-6 years were assigned the Group 3, and patients aged 7-10 years were assigned the Group 4.

Ophthalmic examination

Veterinarians in the Department of Surgery at Atatürk University Veterinary Faculty Animal Hospital, Erzurum, Turkey carried a comprehensive ophthalmic examination on cats referred to the facility. Threat response, palpebral, glare, and pupil light reflexes were evaluated. Subsequently, Schirmer tear test I (AkSchirmer), fluorescein staining (Flu-Glo; Akorn Pharmaceuticals), ultrasonographic imaging (Mindray,Vetus 8;China), ophthalmoscopic (Aesculap AC-635 C, Braun, Tuttlingen, Germany) and intraocular pressure measurements using a rebound tonometer (TonoVet; Icare), were performed in both oculars of all patients. If required, pupillary dilation was performed using a topical 0.5% tropicamide solution (Mydriaticum; Théa). STT-1 was applied to each ocular with the designated portion of the strip placed in the lateral third of the ventral conjunctival fornix. A stopwatch was used to time the tests and the wet portion of the strip was measured in millimeters after one minute.

Statistical analysis

Descriptive statistical analysis was performed to examine many factors including breed, sex, age at initial presentation, anatomical location, diagnosis, and the unilateral or bilateral nature of disorders. This study was conducted using commercial software, specifically Excel 2016 by Microsoft. The prevalence of each ocular disease in the reference population and the 95% confidence interval (CI) of the estimates were calculated. The breed, age, and sex of the cats were expressed as proportions relative to the overall population of cats, whereas the prevalence of diseases was presented as proportions relative to the total number of diagnosed cases. All these variables were subjected to parametric and nonparametric tests. The calculation of odds ratios (ORs) was performed to compare the prevalence of a variable in the study population to that in the reference population. The statistical software package SPSS (Version 26.0; IBM) was utilized for conducting the analysis. A p-value less than or equal to 0.05 was considered to be statistically significant.

Figure 1: Laceretion in 3 eyelids (a), Entropion (b), Proptosis due to trauma (c).

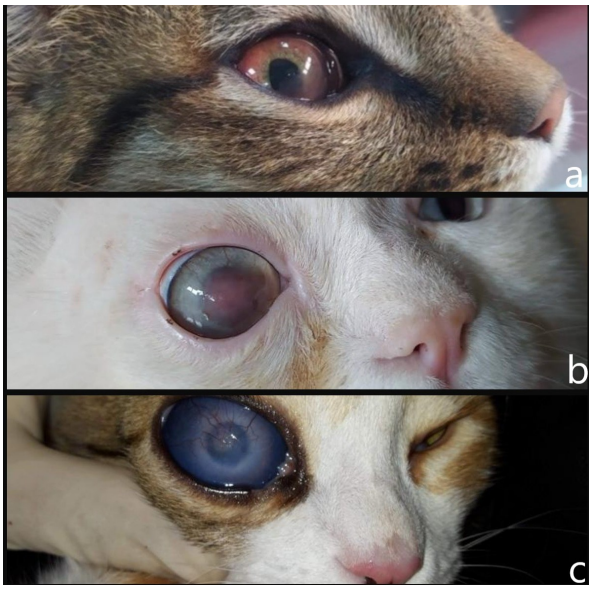


Figure 2: Anterior uveitis (a), Pannus (b), Glaucoma (c).

Results

A total of 427 out of 6000 feline patients who sought medical attention at Atatürk University Animal Hospital from June 2016 to July 2023 exhibited ocular problems, accounting for 7.12% of the observed cases (Figure 1 and Figure 2). In total, a sample size of 425 ocular disease, from 310 cats, were presented and subjected to examination. A total of 6 different breeds were included in the study (Figure 3). Among these breeds, there were 132 cats with brachycephalic features, including Scottish fold, British shorthair and Persian. The most common breeds in the study were 164 Mix breeds, 85 British shorthair, 35 Scottish fold and 12 Persian cats.

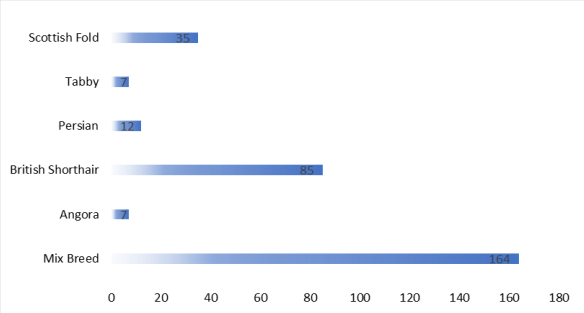


Figure 3: The prevalence rate (%) ocular disease in cats, varies among different breeds.

From the feline subjects examined in the research, a total of 152 (49%) individuals were identified as male, while 149 (48%) individuals were classified as female (Figure 4). The gender of nine feline within the age range of 0-2 months could not be ascertained. Among the participants included in our study, it was seen that 178 (57.4%) individuals fell into the kitten category, 104 (33.5%) individuals were classified as Junior, 26 (8.4%) individuals were categorized as prime, and a mere 2 (0.6%) individuals were identified as adult (Figure 5). The study examined seven ocular findings based on their anatomical positioning, including the adnexa, nasolacrimal system, cornea, globe/orbit, uvea and lens, and gloucoma (Figure 6).

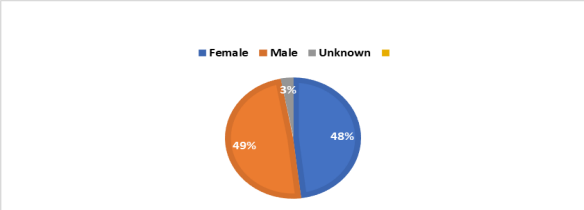


Figure 4: The Prevalence rate (%) of ocular disorders according to gender .

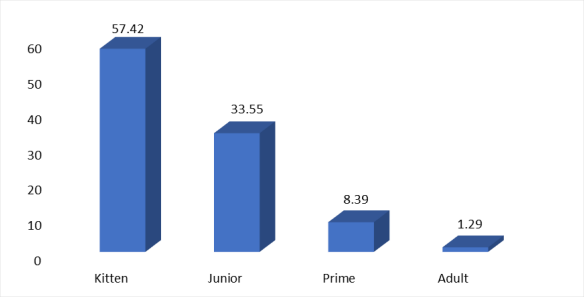


Figure 5: Prevalence rate (%) of ocular disorders according to age scale of cats.

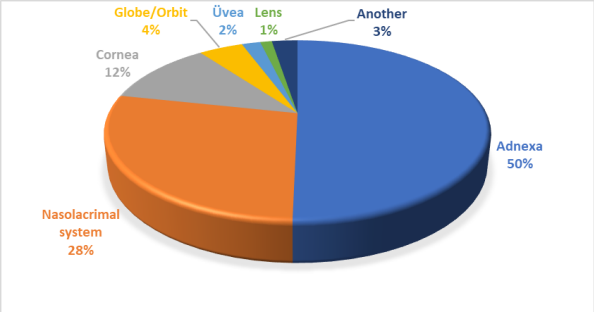


Figure 6: Prevalence rates (%) of ocular disorders according to the anatomical location in cats.

Adnexa

The prevalence rate of adnexa disorders was 50.2% including conjunctivitis (39.3%), entropion (3.51%), cherry ocular (2.11%), chemosis (1.87%), blepharitis and others (Table 1). The incidence of adnexa diseases in mix breed cats was higher than the incidence of adnexa diseases in other breeds (46.6%, ORs: 1.02; 95% CI: 0.67-1.5; P<0.05). This was followed by British shorthair and Scottish fold. When it related to the occurrence of adnexa diseases in cats, there was no statistical difference between male and female cats (ORs: 0.72; 95% CI: 0.47-1.08; P=1.11). When adnexa diseases were analyzed according to age groups, it was found that adnexa disease was more likely to be seen in the 1-year age group (ORs: 0.67; 95% CI: 0.46-0.98; P<0.05, Table 2).

Table 1: Prevalence of ophthalmic disorders according to location in cats in this study

Ocular location	Disorder	Number of disorders	%
Adnexa	Conjunctivitis	168	**39.3**
	Entropion	15	**3.51**
	Cherry eye	9	**2.11**
	Chemozis	8	**1.87**
	Blepharitis	6	**1.40**
	Horner syndrome	3	**0.70**
	Traumatic wound	3	**0.70**
	Trichiasis	1	**0.23**
	Symblepharon	1	**0.23**
	Subtotal	214	**50.2**
Nasolacrimal system	Epifora	70	**16.4**
	Micropuncta	40	**9.4**
	Keratoconjunctivitis sicca	9	**2.1**
	Subtotal	119	**27.9**
Cornea	Corneal edema	25	**5.85**
	Corneal perforation	14	**3.28**
	Keratitis	7	**1.64**
	Corneal ulceration	5	**1.17**
	Subtotal	51	**11.9**
Globe/orbit	Ocular trauma	10	**2.34**
	Foreign body	4	**0.94**
	Buphthalmos	2	**0.47**
	Microphthalmia	2	**0.47**
	Orbital fracture	1	**0.23**
	Subtotal	19	**4.45**
Uvea	Uveitis	8	**1.87**
	Subtotal	8	**1.87**
Lens	Lens luxation	3	**0.7**
	Cataract	2	**0.47**
	Subtotal	5	**1.17**
Another	Secondary glaucoma	11	**2.58**
	Subtotal	11	**2.58**
Total		427	**100**

Table 2: Localization of eye disease prevalence according to breeds in this study

	Breeds	Odds ratio	95% CI	Prevalance (%)	P VALUE
Adnexa	Mix breed	1.023	0.678 to 1.54	46.6	P<0.001
	Angora	0.336	0.074 to 1.53	3.42	
	British shorthair	1.285	0.75 to 2.21	26.8	
	Persian	0.449	0.14 to 1.48	5.87	
	Tabby	0.598	0.131 to 2.72	3.42	
	Scottish fold	0.979	0.461 to 2.08	13.7	
Nasolacrimal system	Brachiocephalic	2.178	1.440 to 3.296	50.1	P=0.04
	Non-brachiocephalic	0.496	0.327 to 0.751	49.9	
Cornea	Mix breed	0.105	0.066 to 0.165	56.6	P<0.001
	Angora	0.336	0.074 to 1.53	5.19	
	British shorthair	0.0531	0.026 to 0.11	22.3	
	Persian	0.0408	0.005 to 0.32	2.81	
	Tabby	0.0748	0.009 to 0.63	2.63	
	Scotish fold	0.0579	0.019 to 0.169	10.5	
Globe/Orbit	Mix breed	1.429	0.697 to 2.94	57.9	P=0.036
	Angora	0.997	0.055 to 18.1	3.55	
	British shorthair	0.756	0.25 to 2.29	24.4	
	Persian	1.392	0.17 to 11.4	6.78	
	Tabby	0.997	0.06 to 18.09	3.55	
	Scotish fold	0.211	0.012 to 3.56	3.73	
Lens	Mix breed	0.753	0.145 to 3.92	30.1	P=0.64
	Angora	3.703	0.187 to 73.2	9.22	
	British shorthair	0.726	0.084 to 6.3	17.6	
	Persian	5.545	0.596 to 51.5	16.5	
	Tabby	3.703	0.187 to 73.2	9.22	
	Scotish fold	1.794	0.204 to 15.8	17.3	
Uvea	Mix breed	0.944	0.28 to 3.182	37	P=0.93
	Angora	2.373	0.125 to 45	6.32	
	British shorthair	0.910	0.19 to 4.365	22.2	
	Persian	1.424	0.078 to 26	6.47	
	Tabby	2.373	0.125 to 45	6.32	
	Scotish fold	2.288	0.47 to 11.3	21.6	
Another (Gloukom)	Mix breed	1.394	0.549 to 3.54	55.5	P=0.11
	Angora	1.736	0.093 to 32.1	5.64	
	British shorthair	0.324	0.041 to 2.54	11.33	
	Persian	1.042	0.06 to 18.7	5.78	
	Tabby	10.873	1.89 to 62.3	15.8	
	Scottish fold	0.367	0.021 to 6.36	5.92	

Nasolacrimal system

The prevalence rate of nasolacrimal system was 27.9% including epifora (16.4%), micropuncta (9.4%), keratoconjuktivitis sicca (2.21%) (Table 1). Nasolacrimal system diseases were more frequently reported in brachiocephalic breeds than in other breeds (50%; ORs: 2.17; 95% CI: 1.44.-3.29; P<0.05). When it related to the occurrence of nasolacrimal system in cats, there was no statistical difference between male and female cats (ORs: 1.02; 95% CI: 0.58-1.82; P=0.93). When nasolacrimal system diseases were analyzed according to age groups, it was found that nasolacrimal system disease was more likely to be seen in the 1-year age ve 2-year group (ORs: 0.97; 95% CI: 0.604-1.55; P<0.05 and ORs: 0.77; 95% CI: 0.42-1.14; P<0.05, Table 2).

Cornea

In our investigation, the incidence of corneal diseases was found to be 11.9%. Corneal edema accounts for 5.85% of the recorded cases, but corneal perforation represents 3.28% of the total incidences. Four patients with ocular edema and perforation were found to exhibit entropion. The mix breed breed had the highest prevalence of corneal disorders, as reported in the study (56.7%; ORs: 0.105; 95% CI: 0.07-0.16;

P<0.05), followed by British shorthair breed (22.3%; ORs: 0.05; 95% CI: 0.03-0.11; P<0.05) and scottich breed (10.4%; ORs: 0.06; 95% CI: 0.02-0.17; P<0.05). When it related to the occurrence of corneal disease in cats, there was no statistical difference between male and female cats (ORs: 0.88; 95% CI: 0.47-1.64; P=0.68). When corneal disease were analyzed according to age groups, it was found that cornea was more likely to be seen in the 1-year age ve 2 -year group (ORs: 1.00; 95% CI: 0.59-1.67; p<0.05 and ORs: 0.94; 95% CI: 0.5 -1.77; P<0.05, Table 2).

Globe/orbit

The incidence of globe and orbital diseases is 4.45% and 2.34%, respectively, causing of ocular trauma. It was found that globe/ orbital diseases were more likely to be seen in mix breed breed cats (48.9%) and British shorthair breed cats (22.6%) compared to other breeds (ORs: 1.47; 95% CI: 0.68-3.07; P<0.05 and ORs: 0.76; 95% CI: 0.25-2.27; P<0.05). There was no statistically significant difference when globe and orbital disease were analyzed in terms of gender compared to other diseases (ORs: 0.67; 95% CI: 0.19 -2.44; P=0.55). When cornea were analyzed according to age groups, it was found that cornea was more likely to be seen in the 1-year age ve 2-year group (ORs: 1.00; 95% CI: 0.59-1.67; P<0.05 and ORs: 0.94; 95% CI: 0.5 -1.77; P<0.05, Table 2).

Other diseases

Secondary gloucoma (2.59%) accounted for all of the gloucoma data in our study. Uveitis was observed in 8 patients in our study, and uveitis constituted 1.88% of our data. Of the lens diseases (1.18%), 0.71% were caused by lens luxation and 0.47% by cataract. No race, age, or gender predisposition was found in these diseases (P>0.05, Table 2).

Discussion and Conclusion

According to the data collected during the study, it was determined that kittens exhibited an increased risk for developing ocular problems associated with conjunctival diseases. Furthermore, our study indicated a statistically significant prevalence of the mix breed breed across all the obtained data. Moreover, the findings of our study indicated the absence of substantial correlation between gender and the incidence of ocular disorders.

The majority of felines included in our research were within the age of one year (57.42%). Previous research has indicated (21.7%) a higher incidence of eye diseases in kittens (Park et al., 2023). Similarly, a previous study carried out in puppies indicated that ocular disease have high prevalence in puppies rather than adult dogs (Akinrinmade and Ogungbenro, 2015). According to the research that was performed, it was observed that the ocular opening of kittens often occurs within a timeframe of 10 to 14 days subsequent to their birth. The occurrence of premature eye opening in kittens has been found to be associated with many ocular diseases, including corneal dryness, keratitis, corneal ulcers, and conjunctivitis. There exists a claim suggesting that kittens not receive enough amount of maternal milk exhibit reduced levels of antibodies, making them more vulnerable to acquiring infectious diseases (Giger and Casal, 1997; Larson and Schultz, 2021).

There were no notable gender disparities detected in the development of our analysis. Previous research conducted in the field of veterinary medicine suggests that there is no evidence of discernible difference in the occurrence of ocular-related diseases between genders (Saraiva and Delgado, 2020; Bott and Chahory, 2022). The primary source of our data is derived from felines adhering to the brachycephalic and mix breeds. These findings may potentially suggest recent regional breeding patterns or a potentially increased prevalence of congenital ocular defects in the specified breeds. It is also suggested that the prevalence of ocular diseases has increased due to the circumstance that mix breed felines predominantly lack permanent residences and reside within urban areas.

The study's findings indicate that eye problems mostly affect the adnexa of felines. The incidence of conjunctival abnormalities (38.8%) was observed to be the highest among a range of adnexal conditions. The conjunctiva, a crucial part in protecting the cornea from dryness and facilitating the movement of the eyelids and globe, is susceptible to inflammation caused by infections on frequently. Nevertheless, conjunctivitis may result from structural problems, trauma, or hypersensitivity reactions, either alone or in conjunction with these underlying conditions (Hartmann et al., 2010). Previous studies have indicated that conjunctival diseases were found to be the most commonly seen condition in prior investigations conducted on canines and felines (Akinrinmade and Ogungbenro, 2015; Turan, 2022). It is postulated that the conjunctiva is influenced by a multitude of congenital or viral pathologies (Stanley, 1988; Narfstrom, 1999).

During the course of our analysis, it was determined that the nasolacrimal system (28%) had the second highest frequency of occurrence. The current investigation involved an intake of 90 individuals who had been diagnosed with epiphora. Among this group, 60 individuals exhibited micropuncta and were classified as belonging to the barachiocephalic racial group. According to a study, there is a notable prevalence of epiphora in individuals of brachiocephalic racial backgrounds. This phenomenon can be ascribed to the variation of the nasolacrimal duct from its normal anatomical location (Glaze, 2005; Anagrius et al.,

2021).

A further investigation on the prevalence of feline ocular disorders revealed that the cornea exhibited the highest occurrence rate. This observation can be attributed to the chronic and recurring character of corneal disease (Park et al., 2023). In accordance with an alternative investigation, it was found that corneal sequestration emerged as the most commonly observed disease, with a significant proportion of felines exhibiting brachiocephalic facial features. (Sarfaty et al., 2022). The prevalence of corneal disorders was found to be 10.9% in our study. After conducting this retrospective study, we found that ocular diseases were more prevalent among cats aged 0-6 months. Additionally, no gender predisposition was observed among the participants in the study. The majority of the patients belonged to mix breed and brachiocephalic breeds, with the most common affected areas being the conjunctiva, nasolacrimal system, and cornea, respectively.

Reference

Anagrius KL, Dimopoulou M, Moe AN, Petterson A, Ljungvall I. Facial conformation characteristics in Persian and Exotic Shorthair cats. JFMS 2021; 23: 1089-97.

Akın F, Samsar E. Göz Hastalıkları. Retina Hastalıkları. Ankara: Medipres Yayıncılık, 2005; ss. 99-122.

Akinrinmade JF, Ogungbenro OI. Incidence, diagnosis and management of ocular affections in dogs. Sokoto J Vet Sci 2015; 13(3): 9-13.

Bott MMP, Chahory S. Epidemiology and clinical prensentation of feline presumed hereditary or breed-related ocular diseases in France: retrospective study of 129 cats. JFMS 2022; 24: 1274-82.

Chahory S, Crasta M, Trio S, Clerc B. Three cases of prolapse of the nictitans gland in cats. Vet Ophthalmol 2004; 7(6): 417-9.

Giger U, Casal Glaze ML. Feline colostrum-friend or foe: Maternal antibodies in queens and kittens. J Reprod Fertil Suppl 1997; 51: 313-6.

Glaze MB. Congenital and hereditary ocular abnormalities in cats. Clin Tech Small Anim Pract 2005; 20(2): 74-82.

Gould D, McLellan GJ. BSAVA Manual of Canine and Feline Ophthalmology. Third Edition: British Small Animal Veterinary Association. Quedgeley, 2014.

Guyonnet A, Donzel E, Bourguet A, Chahory S. Epidemiology and clinical presentation of feline cataracts in france: A retrospective study of 268 cases. Vet Ophthalmol 2019; 22(2): 116-24.

Hartmann A, Hawley J, Werckenthin C, Lappin M, Hartmann K. Detection of bacterial and viral organisms from the conjunctiva of cats with conjunctivitis and upper respiratory tract disease. JFMS 2010; 12: 775-82.

Kanski JJ. Clinical Ophthalmology: A Systematic Approach; Butterworth Heinemann: Boston, MA, 2007; Volume 629.

Kuehn MH, Lipsett KA, Menotti-Raymond M, Whitmore SS, David VA, O'Brien SJ, Zhao Z, Jens JK, Snella EM, Ellinwood NM, McLellan GJ. A mutation in LTBP2 causes congenital glaucoma in domestic cats (*Felis catus*). PLoS One 2016;11 (5): e0154412.

Larson LJ, Schultz RD. Canine and feline vaccinations and immunology. Miller L, Janeczko S, Hurley KF. eds. In: Infectious Disease Management in Animal Shelters. Second Edition. NJ: Wiley Blackwell, 2021; pp.191-20.

McLellan GJ, Teixeira LB. Feline glaucoma. Vet Clin North Am Small Anim Pract 2015; 45(6):1307-33.

Narfstrom K. Hereditary and congenital ocular disease in the cat. JFMS 1999; 1: 135-41.

Park J, Kang S, Lee E, Seo K. Prevalence of feline ophthalmic disorders in South Korea: A retrospective study (2009–2021). JFMS 2023: 25(2); 1098612X231151478.

Saraiva IQ, Delgado E. Congenital ocular malformations in dogs and cats: 123 cases. Vet Ophthalmol 2020; 23: 964-78.

Sarfaty H, Ezra-Elia R, Sebbag L. Prevalence and characteristics of ocular diseases in Sphynx cats: A retrospective assessment (2012-2021) and comparison with non-Sphynx cats. Vet Ophthalmol 2022; 25(5): 367-75.

Scountzou E. Ophthalmic examination in dogs and cats. J Hell Vet Med Soc 2003; 54(4): 329-34

Stanley RG. Superficial stromal keratitis in the dog. Aust Vet J 1988; 65(10): 321-3.

Turan K. Kedi ve köpeklerde göz hastalıkları prevalansı, Master's thesis, Afyon Kocatepe Üniv Sağ Bil Enst, Afyon 2022.

Williams DL, Kim JY. Feline entropion: A case series of 50 affected animals (2003-2008). Vet Ophthalmol 2009; 12(4): 221-6.

Quimby J, Gowland S, Carney HC, DePorter T, Plummer P, Westropp J. 2021 AAHA/AAFP feline life stage guidelines. JFMS 2021; 23(3): 211-33.

ERCİYES ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ
Journal of Faculty of Veterinary Medicine, Erciyes University
Araştırma Makalesi
21(3), 176-183, 2024
DOI: 10.32707/ercivet.1587434

Parvoviral Enteristisli Köpeklerde Endotel Hücre Spesifik Molekül-1 (Endocan) Düzeyleri*

Mehmet Mustafa OFLAZ[1,a], Vehbi GÜNEŞ[2,b]

[1]Tarım ve Orman Bakanlığı, Kahramanmaraş İl Tarım ve Orman Müdürlüğü, Andırın İlçe Tarım ve Orman Müdürlüğü, Kahramanmaraş-TÜRKİYE
[2]Erciyes Üniversitesi, Veteriner Fakültesi İç Hastalıkları Anabilim Dalı, Kayseri-TÜRKİYE
ORCID: [a]0000-0003-3235-6151; [b]0000-0002-4047-4409

Sorumlu yazar: Vehbi GÜNEŞ; E-posta: vgunes@erciyes.edu.tr
Atıf yapmak için: Oflaz MM, Güneş V. Parvoviral enteristisli köpeklerde endotel hücre spesifik molekül-1 (endocan) düzeyleri. Erciyes Üniv. Vet. Fak. Derg. 2024; 21(3):176-183

Öz: Endocan veya Endotelyal Hücre Spesifik Molekül-1 (ESM-1), hücre adezyonu, inflamasyon ve tümör gelişimi gibi süreçlerin düzenlenmesinde anahtar bir rol oynadığı kanıtlanmıştır. Bu çalışmada proinflamatuar ve proanjiyojenik moleküllerin varlığında Endocan düzeylerindeki artış nedeniyle Parvoviral Enteritis'li (PVE) köpeklerde potansiyel yangısal biyobelirteç rolünün araştırılması amaçlandı. Çalışmaya 6-20 haftalık, farklı ırklarda, 40 adet (24 erkek, 16 dişi) ishal (kanlı/kansız), halsizlik şikâyeti bulunan ve köpek parvovirüs (CPV) antijen pozitif köpek dâhil edildi. Kontrol grubunu; 20 adet sağlıklı köpek (12 erkek, 8 dişi) oluşturdu. Tüm köpeklerin serum örneklerinde; Endocan, IL-6 ve CRP düzeyleri sandviç ELISA metodu ile analiz edildi. Ortalama Endocan düzeylerinin PVE'li köpeklerde (68.07 ng/L; 17.30-115.55) sağlıklı gruba göre (11.92 ng/L; 10.32-13.58) istatistiksel açıdan (P<0.001) anlamlı düzeyde yüksek olduğu saptandı. Hasta grubundaki hem ortalama CRP düzeyi (20.87±6.34 mg/L), hem de ortalama IL-6 düzeyleri (2.32±0.84 pg/ml), sağlıklılardan (2.24±0.66 mg/L ve 1.07±0.61 pg/ml) istatistiksel açıdan önemli oranda (P<0.01) yüksekti. Ayrıca Endocan ile CRP düzeyleri ve IL-6 değerleri arasındaki pozitif korelasyonlar nedeni ile Endocan'ın yangısal sürecin sistemik bir bileşeni olduğu değerlendirildi. Sonuçta PVE'li köpeklerde Endocan düzeylerinin hastalarda önemli bir biyobelirteç olduğunu destekleyen bulgular elde edildi.
Anahtar kelimeler: Biyobelirteç, endocan, köpek, parvoviral enteritis

Endothelial Cell Specific Molecule-1 (Endocan) Levels in Dogs with Parvoviral Enteritis

Abstract: Endocan or Endothelial Cell Specific Molecule-1 (ESM-1) has been proven to play a key role in the regulation of processes such as cell adhesion, inflammation and tumor development. In this study, we aimed to investigate the role of Endocan as a potential inflammatory biomarker in dogs with Parvoviral Enteritis (PVE) due to increased Endocan levels in the presence of proinflammatory and proangiogenic molecules. The study included 40 dogs (24 males, 16 females), 6-20 weeks old, of different breeds, with diarrhea (bloody/bloodless), weakness and canine parvovirus (CPV) antigen positive dogs. The control group consisted of 20 healthy dogs (12 males, 8 females). Endocan, IL-6 and CRP levels in serum samples of all dogs were analyzed by sandwich ELISA method. Mean Endocan levels were significantly higher in dogs with PVE (68.07 ng/L; 17.30-115.55) compared to the healthy group (11.92 ng/L; 10.32-13.58) (P<0.001). Both mean CRP level (20.87±6.34 mg/L) and mean IL-6 levels (2.32±0.84 pg/ml) in the patient group were statistically significantly (P<0.01) higher than the healthy group (2.24±0.66 mg/L and 1.07±0.61 pg/ml). In addition, due to the positive correlations between Endocan and CRP levels and IL-6 values, Endocan was considered to be a systemic component of the inflammatory process. In conclusion, findings supporting that Endocan levels in dogs with parvoviral enteritis is an important biomarker in patients were obtained.
Keywords: Biomarker, dog, endocan, parvoviral enteritis

Giriş

Köpek/Canine parvovirüs (CPV) enfeksiyonu akut, bulaşıcı ve ölümcül viral bir hastalık olup, nadiren yetişkin köpeklerde görülmekle birlikte; çoğunlukla 6-20 haftalık yaş grubundaki genç aşılanmamış veya eksik aşılanmış köpeklerde yaygındır. Hastalık akut, fibrinli, nekrotik ve hemorajik enteritis bazen de myokarditis ile seyreder. Etken zarfsız, tek sarmallı bir DNA virüsü olup, günümüzde CPV-2a ve CPV-2b serotiplerinin dünya genelinde salğınlara, klinik hastalığa ve ölümlere neden olduğu bildirilmektedir (Charoenkul ve ark., 2019). İlk kez 2000 yılında tespit edilen daha virulent olan CPV-2c adı verilen üçüncü bir suş da dünya genelinde giderek yayılmaktadır (Polat ve ark., 2019). Canine parvovirüs, viral replikasyon amacıyla; bağırsak kript hücrelerini, myokardı ve lenfoid organları hedefler. Parvoviral enteritisin en yaygın klinik belirtileri nonspesifik olup anoreksi, depresyon, ateş, letarji, iştahsızlık, kusma ve ilk klinik belirtilerden sonraki 24-48 saat içinde gelişen ince bağırsak ishalidir. İshalin görünümü yumuşak mukoid bir sıvı veya çoğunlukla kanamalıdır (Prittie, 2004;

Geliş Tarihi/Submission Date : 07.03.2024
Kabul Tarihi/Accepted Date : 26.08.2024

*Bu çalışma Erciyes Üniversitesi Bilimsel Araştırma Proje Birimi tarafından desteklenen TYL-2021-9233 kodlu projeden özetlenmiştir.

Lamm ve ark., 2008). Gastrointestinal sıvı kayıpları ile hipovolemik şoka ilerleyen intersitisyel dehidratasyon hızla meydana gelebilir. Klinik belirtilerin şiddeti yaşa, koruyucu antikor titresine ve hastalık süresine göre değişebilir. Klinik belirtilere ek olarak, fiziki muayenede mukozal solgunluk, gecikmiş kapiller dolum zamanı, ateş veya hipotermi ve abdominal ağrı görülebilir. Bazı olgularda ince bağırsak invaginasyonu ve buna bağlı karın palpasyonunda ağrılı, sert, tübüler yumuşak doku kitlesi belirlenebilir (Rallis ve ark., 2000; Mazzaferro, 2020). Etkilenen hayvanlarda bağışıklık sisteminin yetersizliği, bağırsak bakterilerinin translokasyonundan kaynaklanan bakteriyemi ile birleştiğinde, septik şok, sistemik inflamatuar yanıt sendromu, multiorgan yetmezliği ve tedavi edilmezlerse ölüm riski ortaya çıkar (Goddard ve Leisewitz, 2010; Ford ve ark., 2017). Bu nedenle parvoviral enteritis olgularında patogenez ve prognozda yangısal süreç ve sepsis gelişimi önemli rol oynar.

Endotel hücreleri sepsis patogenezinde önemli bir role sahiptir. Endotelden salınan Endocan'ın (endotel hücre spesifik molekül-1, ESM-1), insanlardaki sepsis çalışmasında düzeylerinin anlamlı olarak yükseldiği, ayrıca endotel disfonksiyonunu, multiorgan yetmezliğini ve sepsisteki sağ kalımı göstermede iyi bir biyobelirteç olduğu kanıtlanmıştır (Büyüktiryaki ve ark., 2017). Endocan'ın, başlıca akciğer damar sisteminden eksprese edildiği de gösterilmiştir. Yapısal olarak, Endocan/ESM-1, ICAM-1 ve LFA-1 integrinleri ile etkileşime girebilen ve dolayısıyla yangısal olayları önleyen 50 kDa'lık bir proteoglikandır. Vazküler endotelin; iltihaplanma, pıhtılaşma, anjiyogenez ve tümör invazyonunda, öncelikle reseptör/ligand etkileşimlerinin ayarlanması ile ve farklı mediyatörlerin sekresyonunda önemli bir rol oynadığı saptanmıştır (Hsu ve ark., 2019). Naseri ve ark. (2020) tarafından CPV'li köpeklerde Endocan düzeylerinin, sağlıklı köpeklere kıyasla CPV'li köpeklerde daha yüksek olduğu gösterilmiştir. Ayrıca ölen köpeklerde yüksek Endocan düzeylerinin saptanması nedeniyle CPV'li köpeklerde mortaliteyi tahmin etmek için prognostik bir biyobelirteç olabileceğini belirtmişlerdir (Naseri ve ark., 2020).

Parvoviral enteritis'li (PVE) hastalarda endotelyal hücreler üzerine endotoksin veya sitokin aracılı prokoagulant etkinin oluşabileceği bildirilmektedir. Hasarlı bağırsak duvarı ve villus atrofisine bağlı malabsorbsiyona; sıvı ve protein kaybına, bakteriyel sepsis ve endotoksemiye neden olur. Bu tablo da hızlı bir şekilde şok ve ölüme yol açabilir. Bu nedenle Endocan gibi damar endotelinden köken alan bir biyobelirtecin PVE'li köpeklerde koagulopati ve sepsis gibi nedenlerle kandaki düzeylerinin artması beklenebilir. Bu açıdan parvoviral enteritis gibi sistemik hastalığa neden olan köpeklerde teşhis ve prognozda önemli olduğu düşünülen Endocan'ın etkinliğinin ortaya konulması ilişkili sitokin ve akut faz yanıtın birlikte değerlendirilmesi önemlidir. Yaygın olarak CPV ile enfekte olmuş köpeklerde çeşitli patolojik biyobelirteçler araştırılmaya devam etmektedir. Genellikle testlerin morbidite veya mortaliteyi tahmin etmede tek başına yararlı olmayacağı belirtilmektedir (Kocatürk ve ark., 2010; McClure ve ark., 2013). Bu nedenle PVE'in patogenezinde rol oynayan yeni ve güvenilir biyobelirteçlerin araştırılması ilgi çekici olmaya devam etmektedir.

Endotel hücreleri tarafından eksprese edilen Endocan, yangısal süreçlerde endotel disfonksiyonu için yeni bir biyobelirteç olabileceği ifade edilmesi nedeniyle, bu çalışmada doğal parvovirüs enfeksiyonu (CPE) geçirmekte olan hasta köpeklerin plazma örneklerinde yangısal bir biyobelirteç olarak Endocan düzeyleri ile ilişkili sitokin (IL-6) ve akut faz proteini (CRP) düzeylerinin araştırılması amaçlanmıştır.

Gereç ve Yöntem

Bu çalışmada, Erciyes Üniversitesi, Veteriner Fakültesi, İç Hastalıklar Anabilim Dalı, Küçük Hayvan Hastanesine tedavi amacıyla getirilen, sahipleri tarafından çalışmaya katılma onayı alındıktan sonra PVE tanısı konulan ve sağlık kontrollerinde sağlıklı olduğu belirlenen köpeklerden elde edilen kan numuneleri ve hayvanlara ait veriler kullanıldı. Çalışma öncesi Erciyes Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu'na (EÜHADYEK) başvuruldu. Hayvan deneyleri yerel etik kurulu tarafından verilen 18/008 sayılı kararı ile çalışma gerçekleştirildi.

Çalışma grupları ve örnek alımları

Çalışmaya, 6-20 haftalık yaşlarda, farklı ırklarda, 24 erkek ve 16 dişi olmak üzere toplam 40 adet CPV antijen pozitif köpek dâhil edildi. Kontrol grubunu ise; aynı yaş aralığında olan 12 erkek ve 8 dişi toplam 20 adet sağlıklı köpek oluşturdu. Köpeklerde altı haftalık yaştan sonra parvovirusun neden olduğu hastalığın genel klinik belirtilerinden olan; anoreksi, kusma, ishal (sıkça hemorajik) ve letarji belirtileri gösteren; dehidrasyon, ateş ve solgun mükoz membranları bulunan köpekler ile lökopeni, anemi, panhipoproteinemi ve hipoglisemi gibi laboratuvar bulguları olan hasta köpekler ile CPV antijen pozitif köpekler bu araştırmaya dâhil edildiler. Dört ila altı haftadan önceki yaşlar da enteritisden daha çok solunum güçlüğü (dispne) ve myokarditis nedeniyle ani ölüm geliştiği için bu köpekler araştırma dışı bırakıldılar. Ayrıca Campylobacteriosis, clostridiasis, giardiasis ve isospora enfeksiyonları gibi parvovirus dışında gastroenteritis ve kusma semptomlarına yol açan bakteriyel ve paraziter etkenleri barındıran köpekler de çalışma dışı bırakıldı.

Hasta grubunda ortalama 6-9 ay arasında, farklı ırklarda (Golden Retriever, Cocker Spaniel, Rottweiler, Melez, Kangal melezi, Terrier, Beagle, Cavalier King Charles, Border Collie) ve cinsiyette (Erkek, n=24; Dişi, n=16) akut PVE'li köpekler yer aldı. Sağlıklı kontrol grubunu ise sağlık kontrolü amacıyla kliniğe

getirilen ortalama 5-8 aylık, farklı ırklarda (Rottweiler, French Bulldog, Melez, Kangal, Pomerian, Alman Çoban Köpeği, Belçika Malinois) ve cinsiyette (Erkek, n=12; Dişi, n=8) tamamen sağlıklı köpekler oluşturdu.

Hasta ve Kontrol grubu köpeklerin ilk olarak klinik muayeneleri yapılarak beden ısısı, kalp ve solunum sayıları ölçüldü. Klinik olarak iştahsızlık, depresyon, durgunluk, kusma ve kanlı ishal semptomları gösteren hayvanlardan kliniğe getirildikleri gün ve tek seferde olmak üzere antikoagülanlı (EDTA) ve boş tüplere sefalik venden kan örnekleri alındı. Köpeklerden rektal swap ile dışkı örnekleri alındı ve örnekler parvovirus antijeni yönünden hızlı tanı testine (Asan Pharm Parvo, CPV Ag, Güney Kore) tabi tutuldu. Test sonucu parvovirus antijeni yönünden pozitif olan hastalar tez çalışmasının çalışma grubuna dâhil edildi.

Serum biyokimya ve ELISA analizleri

Hematolojik analizler Veteriner Fakültesi Hayvan Hastanesi Klinik Hematoloji ve Biyokimya Laboratuvarında bulunan tam kan sayım cihazında (Mindray BC2800), biyokimyasal analizler ise aynı birimdeki biyokimya analizöründe (Randox Monaco) gerçekleştirildi. Kan CRP, IL-6, Endocan ve biyokimyasal analizleri için sefalik ven'den vakumlu, jelli kan toplama tüplerine (Hemolab BD) 5'er ml kan örneği alındı. Alınan kan örneği 15 dakika oda ısısında bekletildikten sonra santrifüj (Hettich, Zentrifugen) edilerek (3000xrpm, 15 dakika) serum örnekleri çıkartıldı. Kan CRP, IL-6 ve Endocan ELISA testlerinin ölçümünde kullanılacak plazma ve serumlar her bir örnek için iki ependorf olmak üzere ve her bir ependorfda 500 µl plazma ve serum örneği olacak biçimde analiz işlemlerine kadar -20ºC de depolandı. ELISA analizleri ilk örnek alımından sonraki sekizinci ayda tamamlandı. Serum örneklerinde BUN, Total Protein, GGT, Glukoz, Kreatinin, AST, ALP ve Albumin değerleri analiz edildi.

Çalışma ve kontrol köpeklerden alınan serum örneklerinden CRP (201-15-0161/SunRedBio), IL-6 (201-15-0128/SunRedBio), Endocan (SRB-T-87983/ SunRedBio) analizleri belirtilen ticari test kitleri kullanılarak yapıldı. Kantitatif ELISA yöntemiyle test prosedürüne göre analiz edildi ve sonuçlar Biotek ELx800 ELISA cihazında 450 nm'de okundu.

İstatistiksel analizler

Verilerin istatistiksel analizi Statistical Package for the Social Sciences (SPSS for Windows version 25.0) paket programı ile yapıldı. Verilerin normal dağılıma uygunluğu Shapiro-Wilk testi, Q-Q ve histogram grafikleri ile belirlendi. Normal dağılım gösteren değişkenler ortalama±standart sapma olarak ifade edildi. Normal dağılım göstermeyen değişkenler ise ortanca (25^{th}-75^{th} yüzdelik) olarak ifade edildi. Ayrıca varyansların homojenliği Levene testi ile kontrol edildi. CRP, IL-6 ve Endocan değişkenleri açısından hasta ve sağlıklı gruplar bağımsız iki örneklem t testi (student t testi) ile analiz edildi. Normal dağılım göstermeyen veriler ise Mann-Whitney U testi testi ile analiz edildi. Parvoviruslu ve sağlıklı köpeklerde Endocan düzeylerinin duyarlılık ve özgüllükleri hesaplandı. Bu köpeklerde Endocan eşik değerinin belirlenmesi için ROC eğrisi analizi yapıldı. Endocan'ın Parvoviral enfeksiyonların patogenezinde rol alan bir biyobelirteç olup olmadığı ve köpeklerde infeksiyöz durumların takibi için kullanılan CRP düzeylerine göre muhtemel avantaj ve dezavantajları araştırmak amacıyla pearson korelasyonu uygulandı. İstatistiksel farklılığın $P<0.05$ olması anlamlı kabul edildi.

Bulgular

Klinik bulgular

Çalışma grubunu oluşturan köpeklerin tümünde durgunluk, depresyon, hipertermi (10 olgu), letarji, kusma, diyare ve hemorajik diyare gözlendi. Ayrıca tedavinin ikinci gününden itibaren kanlı ishal görülmeyen vakalarda kanlı ishal de görüldü. Tedavinin ilk günü uygulanan tedaviden sonra 25 olguda kusma sıklığında azalma gözlendi. Nispeten ishalde azalma ve çevreye olan ilgilerinde artış tespit edildi. Hastalara üçüncü günden itibaren yüksek enerjili bir diyet ile besleme yapıldı. Bazı hastalar tedaviye 72. saatin sonunda cevap vermeye başladılar ve iştah geri geldi. Bu hastalara yüksek enerjili diyete ancak o zaman geçilebildi. Çalışma grubundaki 11 köpekte tedaviye rağmen düzelme gözlenmedi ve hastaneye getirilmelerinden sonraki 1. ve 2. günlerde öldüler. Parvovirüs enfeksiyonu olan çalışma grubu köpeklerinde ortalama vücut sıcaklığı, ortalama solunum sayısı ve ortalama nabız sayısı düzeyleri sağlıklı köpeklerden istatistiksel açıdan anlamlı düzeyde daha yüksek bulundu ($P<0.001$).

Hematoloji ve serum biyokimya analizi bulguları

Ortalama WBC, lenfosit, granülosit, RBC, hemoglobin (Hgb) ve trombosit (Plt) değerleri açısından çalışma grubunda elde edilen düzeylerin kontrol grubuna göre düşük seyrettiği belirlendi. Özellikle WBC, monosit ve granülosit değerlerinin ortalamasının CPV'li köpeklerde istatistiki açıdan anlamlı oranda düşük ($P<0.001$) ve PLT değerlerinin ortalamasının istatistiksel açıdan önemli oranda yüksek olduğu belirlendi ($P<0.001$).

Çalışma grubuna ait biyokimyasal analiz bulgularında birtakım sapmalar gözlendi. Özellikle total protein, albümin, glukoz, kreatinin ve ALP düzeyleri kontrol grubuna göre farklıydı. Belirlenen biyokimyasal değişiklikler; hipoproteinemi, düşük kreatinin, hiperglisemi, ALP aktivitesinde artış ve albümin düzeylerinde düşme olarak kaydedildi.

Serum Endocan, CRP ve IL-6 bulguları

Ortanca Endocan konsantrasyonunun PVE'li köpeklerde 68.07 ng/L (17.30-115.55) ve sağlıklılarda ise 11.92 ng/L (10.32-13.58) olduğu saptandı. Hasta grubunun ortanca Endocan seviyesi sağlıklı grubun ortanca değerinden istatistiksel açıdan anlamlı düzeyde yüksekti (P<0.001). Ortalama CRP düzeyleri ise PVE'li köpeklerde 20.87±6.34 mg/L, sağlıklılarda 2.24±0.66 mg/L idi. Ortalama IL-6 düzeyi hasta köpeklerde 2.32±0.84 pg/ml, sağlıklılarda ise 1.07±0.61 pg/ml idi. Hasta grubunun ortalama CRP ve IL-6 düzeyleri, sağlıklılardan istatistiksel açıdan önemli oranda (P<0.01) farklıydı (Tablo 1).

Tablo 1. Gruplar arası ortalama/ortanca Endocan, IL-6 ve CRP konsantrasyonlarının karşılaştırılması

Parametreler	Kontrol Grubu (n=20)	Çalışma Grubu (n=40)	P değeri
Endocan (ng/L)	11.92 (10.32-13.58)	68.07 (17.30-115.55)	<0.001
CRP (mg/L)	2.24±0.66	20.87±6.34	<0.001
IL-6 (pg/ml)	1.07±0.61	2.32±0.84	0.010

***CRP:** C-reaktif protein, **IL-6:** İnterlökin 6*

Endocan, CRP ve IL-6 parametrelerinin korelasyon analizi sonuçları Tablo 2 de verildi. Endocan ile CRP arasında pozitif yönde, orta düzeyde ve istatistiksel olarak anlamlı bir korelasyon görüldü (P= 0.002, r= 0.595). Endocan ile IL-6 arasında pozitif yönde, orta düzeyde ve istatistiksel olarak anlamlı bir korelasyon görüldü (P<0.001, r= 0.597). IL-6 ile CRP arasında pozitif yönde, orta düzeyde ve istatistiksel olarak anlamlı bir korelasyon görüldü (P= 0.004, r= 0.507) (Tablo 2).

Tablo 2. Endocan, CRP ve IL-6 parametrelerinin Korelasyon analizi sonuçları

	Endocan (ng/L)	CRP (mg/L)	IL-6 (pg/ml)
Endocan (ng/L)	r=1.000 -		
CRP (mg/L)	r=0.595 P=0.002	r=1.000 -	
IL-6 (pg/ml)	r=0.597 P<0.001	r=0.597 P=0.004	r=1.000 -

***CRP:** C-reaktif protein, **IL-6:** İnterlökin 6*

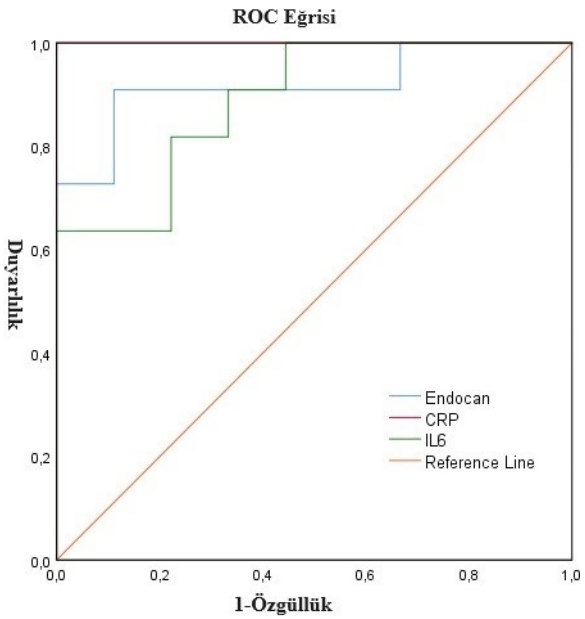
Bu çalışmada Endocan için kesme noktası 15.85 alındığında duyarlılık, özgüllük, ROC AUC ve LR değerleri hesaplandı (sırasıyla; %91, %11, 0.919, 8.18). CRP ve IL-6 değişkenlerine ait duyarlılık, özgüllük, LR ve AUC değerleri Tablo 3'de ifade edildi.

ROC analiz grafiği (Şekil 1) ve gruplar arası Endocan sonuçlarını gösteren grafik (Şekil 2) aşağıda verilmiştir. Tanı performansları açısından CRP'nin en iyi ayırt edici belirteç olduğu görüldü. Endocan'ın ise gerçek pozitiflik oranının ya da duyarlılığının IL-6'ya göre daha iyi olduğu, fakat CRP'ye göre daha düşük olduğu saptandı.

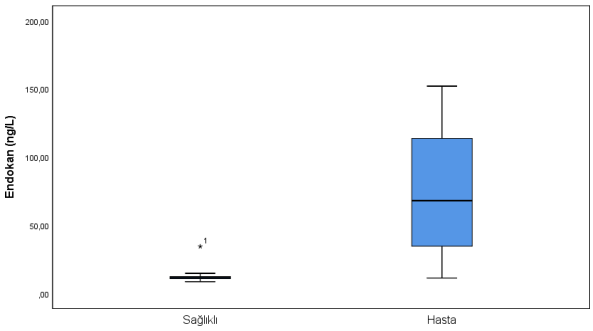
Tablo 3. Endocan, CRP ve IL-6 kesme noktası için duyarlılık, özgüllük, olasılık oranı ve işlem karakteristiği eğrisi (ROC) altındaki kalan alan (AUC) değerleri

Değişkenler	Kriter	Duyarlılık	Özgüllük	AUC	LR
Endocan (ng/L)	>15.85	0.909	0.111	0.919	8.18
CRP (mg/L)	>2.94	1.000	0.111	1.000	9.00
IL-6 (pg/ml)	>1.90	0.636	0.111	0.889	5.73

***CRP:** C-reaktif protein, **IL-6:** İnterlökin 6, **AUC:** Çalışma özellikleri altındaki alan, **LR:** Olasılık oranı*



Şekil 1. ROC analizi sonuçları.



Şekil 2. Gruplar arası Endocan konsantrasyonlarının karşılaştırılması.

Tartışma ve Sonuç

Akut faz proteinleri (AFP), enfeksiyöz hastalıklarda (Ok ve ark., 2015), immün aracılı hastalıklarda (Bathen-Noethen ve ark., 2008; Mitchell ve ark., 2009), neoplazilerde (Nielsen ve ark., 2007; Planellas ve ark., 2009; Shida, 2011) klasik inflamasyon belirteçlerinden daha hızlı ve daha kısa bir yarı ömür ile tepki verirler. Köpeklerde CRP düzeyleri çeşitli yangısal durumlarda en çok araştırılan AFP'dir. Kan CRP seviyeleri, köpeklerde inflamatuar bir uyarıdan sonraki ilk 8-24 saat içinde artar ve 48 saat sonra pik noktaya ulaşan değerler 1-2 hafta içinde normal seviyelere döner (Christensen ve ark., 2014; Hindenberg ve ark., 2018). Bu araştırmada kontrol grubundaki köpeklerdeki ortalama CRP düzeylerinin 2.24±0.66 mg/L olduğu, PVE'li köpeklerde ise ortalama 20.87±6.34 mg/L olduğu saptanmıştır. Parvoviral enfeksiyonlu köpeklerde sağlıklı deneklere göre; serum CRP seviyeleri 10 kat daha yüksek olabileceği bildirilmiştir (McClure ve ark., 2013). Kocatürk ve ark. (2010) ölüm oranının %91 oranında görüldüğü parvoviral enteritli köpeklerde 92.4 mg/L'nin üzerindeki CRP seviyelerini rapor etmişlerdir. Sağlıklı köpeklerin CRP konsantrasyonları genellikle <10mg/L'dir. Ancak klinik açıdan sağlıklı bazı köpekler biraz daha yüksek değerlere (25 mg/L'ye kadar) sahip olabilir. Bazı araştırmalarda CRP aralığının ~10-20 mg/l'nin altında olduğu da gösterildi (Hillström ve ark., 2014; Ok ve ark., 2015; Hindenberg ve ark., 2018). Başbuğ ve ark. (2020) ölen ve yaşayan parvoviral enteritisli köpeklerin CRP düzeyini kontrol grubuna göre önemli düzeyde yüksek ve CRP eşik değerinin ise 120.50 mg/L olduğunu belirlemiştir. Doku hasarı ve enfeksiyonlarda yüksek CRP seviyesinin zayıf prognozu gösterdiği belirtilse de, kısa yarılanma zamanı ve akut dönemde ortaya çıkması nedeniyle daha çok diagnostik açıdan önemli bir biyobelirteçtir (Kocatürk ve ark., 2010). Çalışmada elde edilen ortalama CRP düzeylerinin diğer çalışmalardan farklı biçimde düşük bulunmasının çalışmalardaki köpeklerin enfeksiyonun farklı (erken veya geç) dönemlerinde olduğunu göstermektedir. Araştırmada önceki çalışmalarla uyumlu olarak hasta grubunda sağlıklılara göre yaklaşık 10 katı CRP konsantrasyonlarının anlamlı olarak daha yüksek olduğu görülmüştür (Kocatürk ve ark., 2010, Başbuğ ve ark., 2020). Ayrıca PVE için CRP analizlerinin özgüllük ve duyarlılığının da oldukça yüksek olduğu saptanmıştır. Bu araştırmada; yangının ilk aşamasında oluşan ve önemli proinflamatuar sitokinlerden olan IL-6 düzeylerinin de sağlıklılara oranla önemli oranda yüksek olduğu belirlenmiştir. Savunma sisteminin cevabı olarak yangısal hücrelerden salgılanan bu tür mediatörler karaciğerden akut faz proteinlerinin üretimine yol açarlar. İnsan çalışmalarında daha çok araştırılan bir belirteç olan IL-6 (Declue ve ark., 2012; Miao ve ark., 2013), IL-1 ve TNF-α'ya ek olarak, erken akut faz reaksiyonunda yer alan üç ana sitokinden biridir. Esas olarak monositler ve makrofajlar tarafından üretilirken, kronik inflamatuar hastalıkta IL-6'nın ana kaynağı T lenfositlerdir. IL-6 salınımı lipopolisakkaritler, viral enfeksiyonlar veya nekrotik hücreler tarafından salınan ürünler tarafından indüklenir (Naugler ve Karin, 2008). IL-6 kendi başına serum amiloid A (SAA) ve CRP gibi AFP'nin üretimini indükler (Castell ve ark., 1988). Bu araştırmada da IL-6 düzeyleri sağlıklılara oranla hastalarda önemli oranda yüksek bulunmuştur. Ayrıca CRP ve IL-6 arasındaki orta derecede belirlenen pozitif korelasyon yukarıdaki araştırmalarda belirtildiği gibi; IL-6 nın CRP üretimi ile paralel olduğu ve bu sitokinin CRP üretimini indüklediğini desteklemektedir. Parvoviral hastalığın enterik formunda kript epitellerinde nekroz oluşmaktadır. Villus ve lamina propria, kript epitelinin kaybı ve dökülen villöz epitel hücrelerinin yerine konamaması sonucu önemli oranda hasara uğrar. Sindirimin bozulması ve malabsorbsiyon sonucunda oluşan diyare, dehidrasyon, elektrolit dengesizliği, endotoksik şok veya sekonder septisemiye yol açabilir (Nandi ve Kumar, 2010). İnsanlarda sistemik inflamatuar yanıt

sendromu ve sepsis veya septik şok gibi yangısal durumlarda endotelial glikokalix (eGCX) yapının bozulacağı bildirilmiştir (Nelson ve ark., 2008). Endotelyal hücreye özgü molekül-1 (ESM-1) veya Endocan, eGCX tabakanın hasarına bağlı olarak kan serumunda düzeylerinin artması nedeniyle sepsis gibi kritik durumlar için umut vadedici bir biyobelirteç olduğu belirtilmektedir (Uchimido ve ark., 2019). Çalışmamızda elde edilen ve kontrol grubuna göre önemli oranda artış gösteren Endocan düzeyleri bu hayvanlarda endotel düzeyinde bir yıkımlanmanın olduğunun göstergesi olarak kabul edilmiştir. Köpeklerde damar endoteli hasarının belirlenmesi için standart bir yöntem olmamakla birlikte parvoviral enfeksiyonlu köpeklerde Endocan düzeylerinin araştırıldığı son bir araştırmada; sağlıklı köpeklere oranla parvoviral enfeksiyonlu köpeklerde daha yüksek ESM-1 (Endocan) seviyelerinin eGCX hasarını gösterebileceğini de belirlemişlerdir (Naseri ve ark., 2020). Ayrıca, parvoviral enfeksiyon nedeniyle hayatta kalamayan köpeklerde serum ESM-1'in yüksek seviyeleri, serum ESM-1'in CPV'li köpeklerde mortaliteyi tahmin etmede faydalı bir biyobelirteç olabileceği de aynı yazarlar tarafından ortaya konulmuştur. Araştırmamızda önceki çalışmalardan farklı olarak hasta grubundaki köpeklerde CRP, IL-6 bulguları ile birlikte Endocan analizleri birlikte değerlendirildi. Sonuçta yangı öncesi sitokin (IL-6) ve ilgili akut faz proteini (CRP) bulgularının ortalama Endocan bulguları ile sağlıklılara göre artış tarzında paralel seyretmesi (Tablo 1) nedeniyle Endocan'ın CPE için de CRP'ye benzer biçimde önemli bir biyobelirteç adayı olduğunu göstermektedir. Yapılan pearson korelasyon analizlerinde de bu üç parametre arasında orta düzeyde pozitif korelasyonların belirlenmesi (Tablo 2) nedeniyle korelasyon sonuçları da yukarıdaki yargıyı desteklemektedir. Bu çalışmada aynı zamanda Parvoviral enteritislerde Endocan düzeylerinin eşik değerleri, spesifite ve sensitiviteleri de ortaya konulmuştur (Tablo 3). Elde edilen sonuçlar bu alanda yapılan son çalışma olan Naseri ve ark., (2020)'nın çalışmasında elde edilen sonuçlardan farklı olarak belirlenmiştir. Önceki araştırmada, 0.821'lik AUC, %100'lük bir duyarlılık ve %67'lik bir özgüllük Endocan için ortaya konulmuştu. Bu çalışmada ise; Endocan için kesme noktası 15.85 ng/L alındığında duyarlılık, özgüllük, ROC AUC ve LR değerleri sırasıyla; %91, %11, 0.919, 8.18 olduğu belirlenmiştir. Bu değerlerin yukarıdaki verilerle karşılaştırıldığında sağlıklı gruba göre hasta köpeklerde Endocan seviyelerinin önemli düzeyde artması nedeniyle belirli bir oranda endotelial hasarını desteklemiştir. Ancak Naseri ve ark.'nın (2020) yaptıkları son çalışmadaki hem sağlıklı hem de çalışma grubundaki köpekler ile kıyaslandığında bu tez çalışmasının bulgularının Endocan aktivitesi yönünden daha düşük düzeyde olduğu görülmüştür. Bu farklılığın temel nedenleri arasında; öncelikle olguların farklılığı, kullanılan ELISA kitlerinin farklılığı, kitlerin farklı duyarlılık ve standart konsantrasyonlarında olması, ve son olarak her iki çalışma da kullanılan hayvanların ve örneklemenin alındığı andaki enfeksiyon düzeylerinin veya günlerinin farklı olması, bu çok hassas analizin çalışmalar arasında farklı düzeylerde seyredebileceğini düşündürmüştür. Fakat her iki çalışmanın kendi kontrol grupları ve spesifik laboratuvar şartları ile değerlendirilmesi her bir çalışmanın özgünlüğünü ortaya koymaktadır.

Sonuçta; parvoviral enfeksiyonda yangısal durumu belirleyen ve küçük hayvan hekimliği alanındaki güncel teşhis parametrelerinden Endocan, IL-6 ve CRP seviyelerindeki pozitif korelasyonlar nedeniyle bu üç parametrenin de parvoviral enfeksiyonlarda yangısal sürecin değerlendirilmesinde etkin faktörler olduğu belirlenmiştir. Yukarıdaki sonuçlara rağmen bu çalışmayı sınırlayan bazı faktörler de söz konusudur. Bunlar; Örneklerin ELISA sürecinde çift olarak çalışılmasına rağmen, kontrol ve çalışma grubundaki her bir köpekten bir kez kan örneğinin alınması, sağ kalan ve ölen köpekler arasında parametreler arasında farklılığın belirlenememesi, çalışma grubu köpeklerinde sepsis, SIRS kriterlerinin kesin biçimde belirlenememesi nedeniyle sepsis için yukarıdaki parametrelerin tam olarak geçerliliğinin kanıtlanamaması, ölen köpeklerde histopatolojik analizlerin yapılmaması gibi eksiklikler sayılabilir. Sunulan çalışma ESM1/ Endocan üzerine küçük hayvan çalışmaları içerinde alanında ikinci çalışmadır. Dolayısıyla bu alanda halen kontrollü, farklı hastalıklarda, sepsis kriterlerinin doğrulandığı, hastalık süresince Endocan takiplerinin yapıldığı, damar endotel hasarının immünohistokimyasal yöntemlerle ve kan bulguları ile saptandığı daha ileri çalışmalara ihtiyaç bulunmaktadır.

Teşekkür

TYL-2021-9233 kodlu proje ile bu tez çalışmasının yapılmasındaki katkılarından dolayı Erciyes Üniversitesi Bilimsel Araştırma Projeleri Birimi'ne teşekkür ederiz.

Kaynaklar

Başbuğ O, Yurdakul I, Yüksel M. Evaluation of serum amyloid A and procalcitonin in some inflammatory diseases of cattle. Kafkas Univ Vet Fak Derg 2020; 26(3): 397-402.

Bathen-Noethen A, Carlson R, Menzel D, Mischke R, Tipold A. Concentrations of acute-phase proteins in dogs with steroid responsive meningitis-arteritis. J Vet Intern Med 2008; 22(5): 1149-56.

Büyüktiryaki M, Okur N, Tayman C, Serkant U, Çakır U, Halil H, Öncel MY, Oğuz SŞ. Endocan geç neonatalsepsisi öngörebiliyor mu? Yirmi Beşinci Ulusal Neonatoloji Kongresi (UNEKO 25), Nisan, 24-28, 2017, Antalya; Türkiye.

Castell JV, Gómez-Lechón MJ, David M, Hirano T,

Kishimoto T, Heinrich PC. Recombinant human interleukin-6 (IL-6/BSF-2/HSF) regulates the synthesis of acute phase proteins in human hepatocytes. FEBS Lett 1988; 232(2): 347-50.

Charoenkul K, Janetanakit T, Chaiyawong S, Bunpapong N, Boonyapisitsopa S, Tangwangvivat R, Amonsin A. First detection and genetic characterization of canine Kobuvirus in domestic dogs in Thailand. BMC Vet Res 2019; 15(1): 254.

Christensen MB, Langhorn R, Goddard A, Andreasen EB, Moldal E, Tvarijonaviciute A, Kirpensteijn J, Jakobsen S, Persson F, Kjelgaard-Hansen M. Comparison of serum amyloid A and C-reactive protein as diagnostic markers of systemic inflammation in dogs. Can Vet J 2014; 55(2): 161-8.

DeClue AE, Sharp CR, Harmon M. Plasma inflammatory mediator concentrations at ICU admission in dogs with naturally developing sepsis. J Vet Intern Med 2012; 26(3): 624-30.

Ford J, McEndaffer L, Renshaw R, Molesan A, Kelly K. Parvovirus infection is associated with myocarditis and myocardial fibrosis in young dogs. Vet Pathol 2017; 54(6): 964-71.

Goddard A, Leisewitz A. Canine Parvovirus. Vet Clin NA Small Anim Pract. 2010; 40: 1041-53.

Hillström A, Hagman R, Tvedten H, Kjelgaard-Hansen M. Validation of a commercially available automated canine-specific immunoturbidimetric method for measuring canine C-reactive protein. Vet Clin Pathol 2014; 43(2): 235-43.

Hindenberg S, Keßler M, Zielinsky S, Langenstein J, Moritz A, Bauer N. Evaluation of a novel quantitative canine species-specific point-of-care assay for C-reactive protein. BMC Vet Res 2018; 14: 99.

Hsu T, Nguyen-Tran HH, Trojanowska M. Active roles of dysfunctional vascular endothelium in fibrosis and cancer. J Biomed Sci 2019; 26: 86.

Kocaturk M, Martinez S, Eralp O, Tvarijonaviciute A, Cero J, Yılmaz Z. Prognostic value of serum acute-phase proteins in dogs with parvoviral enteritis. J Small Anim Pract 2010; 51: 478-83.

Lamm CG, Rezabek GB. Parvovirus infection in domestic companion animals. Vet Clin North Am Small Anim Pract 2008; 38(4): 837-50.

Mazzaferro EM. Update on Canine Parvoviral Enteritis. Vet Clin North Am Small Anim Pract 2020; 50 (6): 1307-25.

McClure V, van Schoor M, Thompson PN, Kjelgaard-Hansen M, Goddard A. Evaluation of the use of serum C-reactive protein concentration to predict outcome in puppies infected with canine parvovirus. J Am Vet Med Assoc 2013; 243(3): 361-6.

Miao LH, Song Q, Liu H, Pan L, Zhou FH. Clinical study of prognostic value of interleukin-6 in severe acute pancreatitis. Zhonghua Wei Zhong Bing Ji Jiu Yi Xue 2013; 25(4): 238-41.

Mitchell KD, Kruth SA, Wood RD, Jefferson B. Serum acute phase protein concentrations in dogs with autoimmune hemolytic anemia. J Vet Intern Med 2009; 23(3): 585-91.

Nandi S, Kumar M. Canine parvovirus: current perspective. Indian J Virol 2010; 21(1): 31-44.

Naseri A, Gulersoy E, Ider M, Durgut MK, Erturk A, Avci C, Koral E, Sevinc M, Ok M. Serum biomarkers of endothelial glycocalyx injury in canine parvoviral infection. Austral J Vet Sci 2020 52(3): 52.

Naugler WE, Karin M. The wolf in sheep's clothing: the role of interleukin-6 in immunity, inflammation and cancer. Trends Mol Med 2008; 14(3): 109-19.

Nelson A, Berkestedt I, Schmidtchen A, Ljunggren L, Bodelsson M. Increased levels of glycosaminoglycans during septic shock: relation to mortality and the antibacterial actions of plasma. Shock 2008; 30 (6): 623-7.

Nielsen L, Toft N, Eckersall PD, Mellor DJ, Morris JS. Serum C-reactive protein concentration as an indicator of remission status in dogs with multicentric lymphoma. J Vet Intern Med 2007; 21(6): 1231-6.

Ok M, Er C, Yıldız R. Evaluation of acute phase proteins and cytokines in dogs with parvoviral enteritis Eurasian J Vet Sci 2015; 31(3): 143-7.

Planellas M, Bassols A, Siracusa C, Saco Y, Giménez M, Pato R, Pastor J. Evaluation of serum haptoglobin and C-reactive protein in dogs with mammary tumors. Vet Clin Pathol 2009; 38(3): 348 -52.

Polat PF, Şahan A, Aksoy G, Timurkan MO, Dinçer E. Molecular and restriction fragment length polymorphism analysis of canine parvovirus 2 (CPV-2) in dogs in southeast Anatolia, Turkey. OJVR 2019; 86(1): 1-8.

Prittie J. Canine parvoviral enteritis: a review of diagnosis, management, and prevention. J Vet Emerg Crit Care 2004; 14: 167-76.

Rallis TS, Papazoglou LG, Adamama-Moraitou KK, Prassinos NN. Acute enteritis or gastroenteritis in young dogs as a predisposing factor for intestinal intussusception: A retrospective study. J Vet Med A Physiol Pathol Clin Med 2000; 47(8): 507-11.

Shida T. Characteristics of C-reactive protein (CRP), a1-acid glycoprotein (AAG) and serum amyloid a (SAA) in dogs and cats with malignant Cancer. Intern J Appl Res Vet Med 2011; 9: 376-81.

Uchimido R, Schmidt EP, Shapiro NI. The glycocalyx: a novel diagnostic and therapeutic target in sepsis. Crit Care 2019; 23(1): 16.

ERCİYES ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ
Journal of Faculty of Veterinary Medicine, Erciyes University
Research Article / Araştırma Makalesi
21(3), 184-193, 2024
DOI: 10.32707/ercivet.1587451

The Effect of Light Intensity and Temperature-Humidity Index on Egg Performance and Growth Rate in Laying Hens Raised in Different Cage Tiers*

Uğur ÖZENTÜRK[1,a], Ömer ÇOBAN[1,b], Ali Osman KESEN[1,c], Ayşe UYSAL[1,d]

[1]Atatürk University, Faculty of Veterinary Medicine, Department of Animal Science, Erzurum-TÜRKİYE
ORCID: [a]0000-0002-2037-9340; [b]0000-0003-2368-6247; [c]0000-0001-9638-7649, [d]0000-0003-2803-2410

Corresponding author: Uğur ÖZENTÜRK; E-mail: ugur.ozenturk@atauni.edu.tr
How to cite: Özentürk U, Çoban Ö, Kesen AO, Uysal A. The effect of light intensity and temperature-humidity index on egg performance and growth rate in laying hens raised in different cage tiers. Erciyes Univ Vet Fak Derg 2024; 21 (3):184-193

Abstract: The aim of this research is to evaluate the effects of light intensity and the temperature-humidity index (THI) on egg performance and growth rate in laying hens reared on different cage tiers. Additionally, the study seeks to understand the sensitivity and efficiency of chickens' comfort conditions under various climatic environmental conditions. Brown layer Hyline Brown hens were used as the animal material. In the experiment, 392 hens were housed in a total of 56 cages, with 7 hens per cage in a 4-tier cage system. During the experiment, live weights, growth rates, egg production, and egg weights of the hens were recorded and correlated with the THI and light intensity values measured in front of each cage. The results indicated that hens on the upper tiers had higher live weights, body weight gains, growth rates, egg production, and egg weights compared to those on the lower tiers. A negative relationship was found between the THI value and both growth rate and egg production. Increasing THI values negatively affected both body weight and egg production. Significant positive relationships were observed between light intensity and egg weight, egg production, and average egg production. In conclusion, environmental management and physiological factors are crucial in optimizing the performance of laying hens. Appropriate housing conditions, including optimal light intensity, and temperature management, are essential for maximizing both growth and reproductive performance. Additionally, achieving an ideal body weight at the beginning of the productive period is a crucial for egg production.
Keywords: Cage tier, egg production, growth rate, light intensity, temperature-humidity index

Farklı Kafes Katlarında Yetiştirilen Yumurtacı Tavuklarda Işık Şiddeti ve Sıcaklık-Nem İndeksinin Yumurta Performansı ve Büyüme Hızına Etkisi

Öz: Bu araştırmanın amacı, farklı kafes katlarında yetiştirilen yumurtacı tavuklarda ışık şiddeti ve sıcaklık-nem indeksinin yumurta performansı ve büyüme hızı üzerine etkilerini değerlendirmektir. Ayrıca çalışma, çeşitli iklimsel çevre koşulları altında tavukların konfor koşullarının hassasiyetini ve verimliliğini anlamayı amaçlamaktadır. Hayvan materyali olarak kahverengi yumurtacı Hyline Brown tavukları kullanıldı. Denemede 4 katlı kafes sisteminde kafes başına 7 tavuk olacak şekilde toplam 56 kafese 392 tavuk yerleştirildi. Tavukların canlı ağırlıkları, büyüme oranları, yumurta üretimleri ve yumurta ağırlıkları kaydedilerek her kafesin önünden ölçülen THI ve ışık şiddeti değerleri ile ilişkilendirildi. Araştırmada, üst kattaki tavukların canlı ağırlık, büyüme hızı, yumurta üretimi ve yumurta ağırlığı değerleri alt kattakilere göre daha yüksekti. THI değeri ile hem büyüme hızı hem de yumurta üretimi arasında negatif bir ilişki bulundu. THI değerinin artması hem canlı ağırlığı hem de yumurta üretimini olumsuz etkiledi. Işık şiddeti ile yumurta ağırlığı, yumurta üretimi ve ortalama yumurta üretimi arasında önemli pozitif ilişkiler gözlendi. Sonuç olarak, çevre yönetimi ve fizyolojik faktörler yumurtacı tavukların performansının optimize edilmesinde çok önemlidir. Optimum ışık şiddeti ve sıcaklık yönetimi de dâhil olmak üzere uygun barınma koşulları, hem büyüme hem de üreme performansını en üst düzeye çıkarmak için gereklidir. Ayrıca, üretim dönemi başında ideal canlı ağırlığa ulaşılması yumurta üretimi için çok önemlidir.
Anahtar kelimeler: Büyüme hızı, ışık şiddeti, kafes katı, sıcaklık-nem indeksi, yumurta verimi

Introduction

Chickens are sensitive to environmental conditions (Anderle et al., 2023). Therefore, understanding the optimal environmental requirements for chickens is crucial for enhancing both productivity and animal welfare (Kim and Lee, 2023; Qi et al., 2023; Küçüktopçu et al., 2024). Moreover, providing ideal environmental conditions is also vital for effective care-management and health protection practices (Qi et al., 2023). This necessitates rigorous control and monitoring of the climatic environment in poultry houses (Küçüktopçu et al., 2024).

Temperature and humidity are critical environmental conditions in poultry production (Kim et al., 2021; Amaripadath et al., 2023). As homothermic organ-

Geliş Tarihi/Submission Date : 12.06.2024
Kabul Tarihi/Accepted Date : 30.09.2024

*: This study utilized animals purchased under project code TAD-2020-8778, supported by the Atatürk University Scientific Research Projects Unit.

isms, poultry must maintain a stable body temperature (Kim and Lee, 2023). The effort to regulate body temperature is directly related to the environmental temperature (Kim et al., 2021). Significant deviations in environmental temperature can cause metabolic and physiological changes aimed at preserving heat balance (Kim and Lee, 2023). The thermal comfort zone for adult chickens is reported to be 18-22°C (Pawar et al., 2016; Sarıca et al., 2018). Chickens can stabilize their body temperature at ambient temperatures between 10-27°C (the homoeothermic zone) through metabolic and physiological adjustments (Sarıca et al., 2018). Outside this range, chickens may experience hypothermia or hyperthermia, leading to yield losses, health deterioration, and even mortality (Farag and Alagawany, 2018; He et al., 2018).

Poultry exhibit greater tolerance to humidity than heat (Sarıca et al., 2018). However, humidity tolerance is influenced by temperature. Ideal humidity in poultry houses is between 40-70% (Oloyo, 2018; Sarıca et al., 2018). Low humidity can cause dust and respiratory diseases, while high humidity can lead to damp litter, increased ammonia levels, and condensation on the roof and walls during winter. In summer, high humidity combined with high temperatures impedes heat dissipation through evaporation, increases perceived temperature, and exacerbates heat stress effects (Oloyo, 2018; Kim et al., 2021; Elghardouf et al., 2023). Therefore, evaluating temperature and humidity together is crucial for maintaining an optimal environment in poultry houses. The Temperature-Humidity Index (THI) is a valuable tool for assessing the combined effects of temperature and humidity on animals (Kim and Lee, 2023; Loengbudnark et al., 2023). THI quantitatively evaluates environmental factors affecting animal health, productivity, and welfare, thereby playing a critical role in effective environmental management strategies (Shin et al., 2024). Different THI formulas exist for various animal species and environments, with values classified into normal, alert, danger, and emergency zones for poultry (Zulovich and DeShazer, 1990; Kim and Lee, 2023).

Lighting is another crucial environmental factor in poultry houses. Light stimulates ovarian activity in hens, promoting sexual maturity and egg production (Mohammed, 2019; Nega, 2024). It also influences growth, behaviour, physiological processes, and immune health (Erensoy et al., 2021; Bahuti et al., 2023). Key lighting factors include photoperiod and light intensity (Barros et al, 2020; England and Ruhnke, 2020). In extensive farming, hormonal stimulation typically occurs in spring when natural light duration is 10-12 hours, prompting egg-laying. In commercial intensive farming, a daily lighting duration of 14 hours or more is desired (Sarıca et al, 2018; Nega, 2024). Light intensity plays a crucial role in chicken welfare (Mohammed, 2019); low intensities can reduce activity levels and productivity, while high intensities can increase aggression (Mohammed, 2019; Barros et al., 2020; Erensoy et al., 2021).

Maintaining temperature balance and providing uniform illumination are essential to prevent negative environmental impacts and ensure optimal production (Küçüktopçu et al., 2024). Modern poultry houses are equipped with systems for climatic control. Despite welfare concerns, cage systems are frequently used in commercial egg production for economic and sustainability reasons (Şekeroğlu et al., 2014; Erensoy et al., 2021; Majewski et al., 2024). These cages can have 3-8 tiers (Adegbenro et al., 2023). Despite efforts to provide homogeneous environmental conditions (Sarıca et al., 2018; Özentürk and Yildiz, 2021; Özentürk et al., 2023), it is challenging to maintain uniform conditions across all cage tiers (Yildiz et al., 2006; Şekeroğlu et al., 2014; Türker et al., 2021). Therefore, understanding the environmental differences between cage tiers is crucial to ensuring equal conditions for all chickens and achieving optimal production.

This study aimed to evaluate the effects of light intensity and the Temperature-Humidity Index on egg performance and growth rate in laying hens reared in different cage tiers. Additionally, the study sought to understand the sensitivity of hens to comfort conditions and their productivity, providing insights into the climatic conditions under which hens are most productive.

Materials and Methods

The research was ethically approved by the Atatürk University Faculty of Veterinary Medicine Unit Ethics Committee (Protocol no: 2024/10, dated 28.03.2024).

Study Location and Design

The research was conducted at the Poultry Unit of Atatürk University Food and Livestock Application and Research Centre. The poultry house utilized a battery cage system with 4 tiers (numbered 4, 3, 2 and 1 from top to bottom) and 2 rows. Each tier contained 14 cage compartments, symmetrically arranged into right (7 compartments) and left (7 compartments) sides, making a total of 56 cage compartments. The right side of the cage unit faced the windows, while the left side faced the aisle. All cage compartments have identical dimensions, made of galvanized sheet and wire, allowing for light and air circulation. The 7° inclined base wires allowed eggs to roll to the egg collection band easily. The cage dimensions are as follows: depth 60 cm, width 62.5 cm, back height 46 cm, front height 51 cm, and feeder length 62.5 cm. Each cage compartment has two water nipple systems, and feed and water were provided ad libitum. Ventilation was managed via win-

dows on the side walls, ventilation shafts on the ceiling, and a 140 cm x 140 cm electric negative pressure fan. Lighting was provided by white fluorescent lamps on the ceiling, with a lighting program of 16 hours of light and 8 hours of darkness per day.

Animal Material

The study used Hyline Brown layers. Each of the 56 cage compartments housed 7 birds, resulting in a total of 392 birds.

During the productive period, the hens were fed ad libitum with granule form feed: 1st Term egg feed at 20-45 weeks (2750 ME, 16.26% HP), 2nd Term egg feed at 46-60 weeks (2720 ME, 15.83% HP), and 3rd Term egg feed at 61-72 weeks (2720 ME, 15.65% CP).

Determination of Body Weights and Growth Rate

The hens were weighed before the production period (at 18 weeks of age) and divided into two body weight groups based on high uniformity: high body weight (≥1700 g) and low body weight (<1500 g). Starting at 24 weeks of age, body weights were measured every four weeks until 72 weeks of age, and the average weight per cage was recorded. The Gompertz growth model was used to determine the growth rate (k) using average body weights (Gonzalez Ariza et al., 2021; Mancinelli et al., 2023).

The Gompertz growth model is given by:
Wt = Wmax ((exp-b) (exp(-kt)))

The terms in the mathematical model are:

- W_t observed weight at *t* weeks of age
- *t:* weekly age
- W_{max}*:* asymptotic body weight
- *b:* initial weight
- *k:* growth rate

The *W*max was determined as 2.17 and *b* as 3.43, with *k* estimated by keeping *W*max and *b* constant. The coefficient of determination (R^2) of the functions averaged 0.97 ± 0.02.

Determination of Egg Performance

Eggs from each cage were collected and recorded daily from 24 to 72 weeks of age. Weekly average egg weights were determined by weighing the eggs each week. Average daily egg production (g) per cage was calculated based on egg production and average egg weights.

Determination of Light Intensity and Temperature Humidity Index (THI)

Light intensity, temperature, and humidity values were measured in front of the cages at 09:00, 12:00, and 15:00 once a week. The average light intensity and THI values were recorded. The THI formula used was (Elshafaei et al., 2020):

$THI=0.8T_{db} + (RH\ (T_{db}-14.3)/100) + 46.3$

where:

- T_{db} = air dry-bulb temperature (ºC)

RH = relative humidity of air (%)

Statistical Analyses

The effects of cage side, cage tier, and body weight group on average daily egg production (g), egg yield (%), egg weight (g), body weight at the start and end of the experiment, percentage of body weight change, and growth rate (k) per cage were analyzed using the General Linear Model (GLM). Duncan multiple comparison was used for multiple comparison test. Pearson correlation was used to calculate the relationship between these values and THI and light intensity. Multiple linear regression analysis with a forward approach was used to analyze the effects of light intensity and THI on average egg production (g), egg yield (%), and growth rate (k) per cage. The variables were normally distributed and demonstrated a linear relationship. No multicollinearity was detected among the independent variables, with the highest correlation coefficient being r = 0.572. The collinearity was further assessed using the Variance Inflation Factor (VIF), which ranged from 1.195 to 1.959. The standardized residuals fell within the acceptable range, with the lowest value being -2.204 and the highest 1.911. Additionally, the errors of the estimates were normally distributed. For regression analysis, the assumptions of collinearity, VIF, and normal distribution of errors were verified. Statistical analyses were performed using SPSS software.

Results

Temperature and humidity values were measured in front of each cage and THI value and light intensity were determined for each cage (Table 1). The light intensity was higher on the window side and on the upper tiers. The average THI value was 72.36 (min: 71.60- max 73.17) and the THI values of the aisle side and middle (2 and 3) tiers were found to be higher.

Table 1. Light intensity and THI values of the cage tiers

		Light intensity (lux)			THI		
			95% Confidence Interval			95% Confidence Interval	
Cage side	Cage tier	Mean	Lower Bound	Upper Bound	Mean	Lower Bound	Upper Bound
Window	Tier 4	460.66	434.194	487.134	71.95	71.753	72.148
	Tier 3	225.61	199.140	252.080	71.97	71.777	72.171
	Tier 2	108.52	82.053	134.993	72.00	71.803	72.197
	Tier 1	62.52	36.053	88.993	72.07	71.870	72.264
Aisle	Tier 4	106.52	80.046	132.986	72.64	72.447	72.841
	Tier 3	78.80	52.333	105.273	72.86	72.662	73.056
	Tier 2	52.39	25.923	78.863	72.80	72.604	72.998
	Tier 1	37.05	10.576	63.516	72.56	72.360	72.754
	SEM	13.16			0.10		

The average egg production (g), egg yield (%), and average egg weight (g) values according to cage tier, cage side and body weight groups are presented in Table 2. The highest average egg weight was obtained from hens reared on the upper tiers (P<0.01). Egg yield was lower in the hens reared on the lower tier (P<0.05). For average daily egg production (g) per hen, which is a function of average egg weight

Table 2. Mean egg production (g), egg yield (%), and egg weight (g) values of the groups ($\bar{x}$ +SE)

Cage side	Cage tier	Body weight group	Average egg production (g)	Egg yield (%)	Average egg weight (g)
Window	Tier 4	High	57.32±0.98	87.61±0.71	65.27±0.49
		Low	56.63±0.85	86.19±0.67	64.94±0.47
	Tier 3	High	55.93±0.97	86.75±0.71	63.65±0.49
		Low	53.46±0.86	85.33±0.68	63.32±0.47
	Tier 2	High	53.91±0.98	86.12±0.71	63.48±0.49
		Low	53.87±0.85	84.71±0.67	63.15±0.47
	Tier 1	High	53.44±1.00	84.67±0.71	63.06±0.49
		Low	51.30±0.84	83.26±0.67	62.73±0.47
Aisle	Tier 4	High	56.03±0.85	87.62±0.67	65.57±0.47
		Low	56.76±0.98	86.21±0.71	65.24±0.49
	Tier 3	High	56.09±0.85	86.76±0.67	63.95±0.47
		Low	53.15±0.98	85.35±0.71	63.62±0.49
	Tier 2	High	54.12±0.85	86.13±0.67	63.78±0.47
		Low	54.71±0.98	84.72±0.71	63.45±0.49
	Tier 1	High	54.77±0.86	84.68±0.68	63.36±0.47
		Low	52.40±0.97	83.27±0.70	63.03±0.49
	Tier 4		56.69±0.46^{a}	86.91±0.56^{a}	65.25±0.39^{a}
	Tier 3		54.66±0.46^{b}	86.05±0.56^{a}	63.64±0.39^{b}
	Tier 2		54.15±0.46^{bc}	85.42±0.56^{ab}	63.47±0.39^{b}
	Tier 1		52.98±0.46^{c}	83.97±0.56^{b}	63.05±0.39^{b}
		High	55.20±0.33	86.29±0.40	64.02±0.28
		Low	54.04±0.33	84.88±0.40	63.68±0.28
			P Value		
			Average egg production (g)	**Egg yield (%)**	**Average egg weight (g)**
Cage side			0.562	0.984	0.447
Cage tier			<0.001	0.020	0.001
Body weight group			0.011	0.013	0.402

$^{a-c}$: Different letters within one column are significantly different (P<0.05).

and yield, the lowest values were obtained from hens reared on the 1st and 2nd tier, followed by the 3rd tier, with the highest egg production observed in hens reared on the 4th tier (P<0.001). No significant difference was observed in egg production and egg weight in hens reared on the window and aisle sides (P>0.05). Although hens from different body weight groups produced eggs with similar weights, hens with lower body weight had lower egg production (P<0.05), resulting in fewer eggs throughout their production life in terms of average egg production (g) (P<0.05).

Growth rate and body weight values according to cage tier, cage side and body weight groups are presented in Table 3. The hens that started the laying period with low body weight had lower body weight at the end of the period (P<0.001). The rate of weight gain was higher in the hens with low body weight (P<0.001). During this period, it was observed that hens with low body weight performed compensatory growth. The k value of the hens in the high body weight group was calculated as higher because they reached the targeted weight faster (P<0.001). The rate of weight gain (P<0.05) and k value (P<0.01) of the hens reared on the upper tiers were determined to be the highest. The final body weight (P<0.001), weight gain rate (P<0.001) and k value (P<0.05) of the hens reared on the window side were lower (P<0.001).

The correlation coefficients between light intensity and THI with egg production and growth characteristics are given in Table 4. There was a moderate negative correlation (r=-0.399) between THI value and growth rate (k) (P<0.01). It was determined that those with higher body weight at the beginning of the experiment had higher egg production (r=0.552; P<0.01), therefore those with higher k value had higher egg production (r=0.582; P<0.01). It was determined that the average egg weight (r=0.531), egg yield (r=0.500) and therefore the average egg production per cage (r=0.572) increased with increasing light intensity (P<0.01). The increase in THI value caused a decrease in egg production (r = -0.268) as well as k value (P<0.05).

Table 3. Growth rate and body weight values of the groups ($\bar{x}$ +SE)

Cage side	Cage tier	Body weight group	Initial body weight (g)	Final body weight (g)	Weight gain rate (%)	Growth rate (k value)
Window	Tier 4	High	1728.82±12.16	2210.02±28.75	29.39±1.77	0.152±0.003
		Low	1398.81±14.04	2087.05±30.17	50.26±1.86	0.116±0.003
	Tier 3	High	1693.39±12.16	2160.84±28.75	27.43±1.77	0.144±0.003
		Low	1372.33±14.04	2037.86±30.17	48.31±1.86	0.108±0.003
	Tier 2	High	1672.14±12.16	2108.13±28.75	24.72±1.77	0.142±0.003
		Low	1376.90±14.04	1985.16±30.17	45.60±1.86	0.106±0.003
	Tier 1	High	1689.64±12.16	2076.78±28.75	23.04±1.77	0.143±0.003
		Low	1336.19±14.04	1953.81±30.17	43.92±1.86	0.107±0.003
Aisle	Tier 4	High	1700.05±14.04	2341.83±30.17	36.62±1.86	0.157±0.003
		Low	1392.29±12.16	2218.86±28.75	57.50±1.77	0.121±0.003
	Tier 3	High	1699.76±14.04	2292.65±30.17	34.67±1.86	0.149±0.003
		Low	1395.75±12.16	2169.67±28.75	55.54±1.77	0.113±0.003
	Tier 2	High	1706.67±14.04	2239.94±30.17	31.96±1.86	0.147±0.003
		Low	1385.89±12.16	2116.97±28.75	52.83±1.77	0.111±0.003
	Tier 1	High	1701.19±14.04	2208.60±30.17	30.27±1.86	0.148±0.003
		Low	1396.54±12.16	2085.62±28.75	51.15±1.77	0.112±0.003
	Tier 4		1555.80±6.87	2214.44±23.98^{a}	43.44±1.48^{a}	0.137±0.002^{a}
	Tier 3		1540.92±6.87	2165.26±23.98^{ab}	41.50±1.48^{ab}	0.128±0.002^{b}
	Tier 2		1534.49±6.87	2112.55±23.98^{bc}	38.78±1.48^{b}	0.127±0.002^{b}
	Tier 1		1532.63±6.87	2081.20±23.98^{c}	37.10±1.48^{b}	0.127±0.002^{b}
		High	1699.52±4.89	2204.85±17.04	29.78±1.05	0.148±0.002
		Low	1382.40±4.89	2081.88±17.04	50.64±1.05	0.112±0.002

P Value

	Initial body weight (g)	Final body weight (g)	Weight gain rate (%)	Growth rate (k value)
Cage side	0.053	<0.001	<0.001	0.027
Cage tier	0.085	0.001	0.019	0.006
Body weight group	<0.001	<0.001	<0.001	<0.001

$^{a-c}$: Different letters within one column are significantly different (P<0.05).

Table 4. Relationship between egg production and growth characteristics with light intensity and THI

	Growth rate (k)	Weight gain rate (%)	Initial body weight (g)	Final body weight (g)	Avg. egg weight (g)	Egg yield (%)	Avg. egg production (g)	Light intensity (lux)
Weight gain rate (%)	-0.631**							
Initial body weight (g)	0.922**	-0.810**						
Final body weight (g)	0.638**	0.090	0.510**					
Average egg weight (g)	0.222	0.021	0.213	0.388**				
Egg yield (%)	0.582**	-0.288*	0.552**	0.515**	0.647**			
Average egg production (g)	0.432**	-0.137	0.410**	0.493**	0.919**	0.896**		
Light intensity (lux)	0.140	-0.082	0.091	0.042	0.531**	0.500**	0.572**	
Total THI	-0.399**	0.645**	-0.454**	0.164	-0.025	-0.268*	-0.155	-0.487**

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

The coefficient of determination of the multiple regression model for growth rate (k value) was calculated as 0.499. In the model, the beta coefficient (b) of egg yield to growth rate was 0.007 and the coefficient of THI value was -0.012 (Table 5).

Table 6 shows the result of multiple regression analysis for daily egg production (R^2 =0.544). It was determined that light intensity, k and THI values were effective on average daily egg production.

Table 5. Estimated parameter and significance levels in multiple linear regression analysis for k value

Predictor	Coefficient	SE of coefficient	t	P-value
Constant	0.349	0.413	0.844	0.402
Egg yield	0.007	0.002	4.646	<0.001
THI	-0.012	0.005	-2.375	0.021

R^2= 0.499

Table 6. Estimated parameter and significance levels in multiple linear regression analysis for average egg production (g) value

Predictor	Coefficient	SE of coefficient	t	P-value
Constant	-18.432	10.986	-1.678	0.099
Light intensity (lux)	0.003	<0.001	6.375	<0.001
Growth rate (k)	13.945	2.950	4.727	<0.001
THI	0.478	0.149	3.202	0.002

R^2= 0.544

In the multiple regression model for egg yield (R^2 = 0.250) and average egg weight (R^2 = 0.354), light intensity was found to be the effective factor (Tables 7 and 8).

Table 7. Estimated parameter and significance levels in multiple linear regression analysis for egg yield (%) value

Predictor	Coefficient	SE of coefficient	t	P-value
Constant	84.874	0.234	362.800	<0.001
Light intensity (lux)	0.005	0.001	4.247	<0.001

R^2= 0.250

Table 8. Estimated parameter and significance levels in multiple linear regression analysis for average egg weight (g) value

Predictor	Coefficient	SE of coefficient	t	P-value
Constant	5.415	23.857	0.227	0.821
Light intensity (lux)	0.006	0.001	5.384	<0.001

R^2= 0.354

Discussion and Conclusion

The results indicate a significant effect of cage tier on body weights. Hens on the top tier exhibited the highest final body weights, while those on the bottom tier had the lowest. Consequently, the body weight gain rate and growth rate were higher for hens on the upper tier. There was also a notable difference between cage sides in terms of body weight gain. Similarly, Karaman et al. (2013) reported body weight gains of 8.81%, 11.06%, and 15.39% for tiers 1, 2, and 3, respectively, with a significant difference among tiers (Karaman et al., 2013). Sogunle et al. (2022) also noted higher body weight gain in chickens on the upper tier, although this was not statistically significant (Sogunle et al., 2022). This may be attributed to the higher feed intake observed in chickens on the upper tier (Adegbenro et al, 2023). Natural lighting, which can affect appetite and feeding behaviour, is more abundant on the top tier, potentially promoting feed intake and growth (Sogunle et al., 2022). Factors such as temperature, light intensity, ventilation, and stress levels may contribute to body weight differences between cage tiers and sides (Erensoy et al., 2021; Adegbenro et al., 2023). Upper tiers typically benefit from better temperature and ventilation conditions, while cages facing windows receive more natural light and potentially better airflow (Yildiz et al., 2006; Karaman et al., 2013). These conditions likely contribute to the higher growth rates and body weights in chickens on the upper tiers and window-facing sides of the cage units. Conversely, Şekeroğlu et al. (2014) found lower body weights on upper tiers compared to lower tiers at 30 weeks of age, though no significant difference was noted at 42 weeks (Şekeroğlu et al., 2014). Other studies have reported no significant differences in live weight between tiers at various ages (Onbaşılar and Aksoy, 2005). The experiment also revealed significant differences between body weight groups in terms of growth rate. Hens starting the yield period with higher body weight had a 29.78% weight gain, whereas those starting with lower body weight experienced a 50.64% weight gain, indicating compensatory growth in the lower weight group.

Differences in egg production and egg weight were observed between cage tiers, with the top tier yielding higher egg production and weight, and the lowest values recorded for hens on the bottom tier. These findings align with previous studies indicating that hens on the top tier produce more eggs than those on the bottom tier (Yildiz et al., 2006; Türker et al., 2021; Sogunle et al., 2022; Adegbenro et al., 2023). The better light illumination in upper tiers likely stimulates egg production through hormonal mechanisms. Additionally, environmental factors such as temperature, ventilation, and stress levels may influence these differences (Akkuş and Yıldırım, 2018; Adegbenro et al., 2023). Hens on upper tiers benefit from optimal environmental conditions, enhancing metabolic activity and hormonal regulation, leading to higher egg production and greater egg weight (Karaman et al., 2013; Eleroğlu, 2019; Erensoy et al, 2021). In contrast, sub-optimal conditions in lower tiers may reduce productivity and egg weight. Some studies, however, reported no effect of cage tiers on egg production (Durmuş and Kamanlı, 2012; Sahin, 2012; Karaman et al., 2013; Şekeroğlu et al., 2014). Yıldırım et al. (2008) noted a negative impact of excessive light intensity on egg production, highlighting

the importance of optimal lighting conditions (Yıldırım et al., 2008). Consistent with our results, Dereli Fidan and Nazlıgül (2012) observed that egg weight was significantly influenced by cage tier, with the highest weights recorded on the top and middle tiers (Dereli Fidan and Nazligul, 2012). Similarly, Onbaşılar and Aksoy (2005) found higher egg weights on the top tier compared to the bottom tier (Onbaşılar and Aksoy, 2005). Eleroğlu (2019) also reported higher egg weights from the upper tier at 24 week of age (Eleroğlu, 2019). However, some studies found no difference in egg weights across cage tiers (Yıldırım et al., 2008; Durmuş and Kamanlı, 2012; Sahin, 2012; Karaman et al., 2013; Şekeroğlu et al., 2014; Türker et al., 2021; Sogunle et al., 2022; Adegbenro et al., 2023).

In our study, the cage side did not significantly affect egg production or egg weight. Sahin (2012) also found no significant differences in these parameters between window-facing and aisle-facing cages (Sahin, 2012). However, Yildiz et al. (2006) reported heavier eggs from window-facing hens, possibly due to increased light intensity (Yildiz et al., 2006). Variations in study results regarding the effect of cage tiers on egg production, egg weight, and body weight are often attributed to differing environmental conditions such as light, temperature, humidity, and ventilation (Karaman et al., 2013; Akkuş and Yıldırım, 2018; Eleroğlu, 2019; Erensoy et al., 2021; Türker et al., 2021). However, the relationship between these conditions and performance parameters remains inconsistent in the literature.

In our study, THI values, calculated from temperature and humidity data, averaged 72.36 (range: 71.60-73.17), with higher values on the aisle side and middle tiers. The THI chart classifies stress into four levels: comfort (THI < 70), alert (THI 70–75), danger (THI 76–81), and emergency (THI > 81) zones (Zulovich and DeShazer, 1990; Kim and Lee, 2023). These THI values fall into the alert category (THI 70-75), indicating potential heat stress. A moderate negative correlation between THI and growth rate and a negative correlation between THI and egg production were observed, suggesting that higher THI negatively impacts body weight and egg production. Multiple regression models explained 49.9% of the variability in growth rate and 54.4% of the variability in egg production, indicating moderate to high predictability. High THI levels challenge thermoregulation in hens, leading to heat stress and various physiological disturbances (Amaripadath et al., 2023; Kim and Lee, 2023; Loengbudnark et al., 2023; Shin et al., 2024). These disturbances reduce feed intake, impair nutrient absorption, cause hormonal imbalances, and decrease metabolic efficiency (Kim et al., 2021; Elghardouf et al., 2023). Consequently, hens experience slower growth rates and reduced egg production. Temperature and humidity differences between cage tiers were also significant, with higher temperatures and humidity recorded in the middle tier (Kılıç and Şimşek, 2008; Eleroğlu, 2019). These environmental variations may explain differences in egg performance and quality (Akkuş and Yıldırım, 2018).

Light intensity significantly influenced egg weight, egg yield, and average egg production. It emerged as a key factor in multiple regression models for both egg production and average egg weight. Light intensity regulates photoperiod, affecting reproductive hormones in poultry (England and Ruhnke, 2020; Erensoy et al., 2021). Adequate light intensity ensures proper secretion of hormones essential for ovulation and egg production, such as LH and FSH, leading to increased egg production and egg size (Bahuti et al., 2023). Proper light intensity also promotes feeding, providing necessary nutrients for egg formation (Adegbenro et al., 2023; Nega, 2024) and helps maintain the circadian rhythm, essential for optimal laying cycles (Saad et al., 2024). These factors might explain the relationship between light intensity, egg production, and egg weight observed in the study. Studies support that, variations in lighting conditions between cage floors and blocks, due to proximity to light sources, can affect performance (Yildiz et al., 2006; Kılıç and Şimşek, 2008; Karaman et al., 2013; Erensoy et al., 2021). Light intensity did not affect the growth rate of the chickens. This may be because the chickens were already in the laying period, where the physiological and hormonal effects of light intensity are aimed at reproduction rather than growth.

Higher body weights at the start of the experiment were correlated with higher egg production, indicating that hens with higher growth rates also had higher egg production. This is consistent with another study linking body weight to egg weight and production (Durmuş and Kamanlı, 2012). Higher body weights may reflect better nutrient reserves, physiological maturity, and advantageous genetics, contributing to increased egg production. Additionally, efficient feed conversion and metabolic health in hens with higher growth rates may result in higher egg production. A significant positive correlation between egg production and egg weight was observed, indicating efficient resource allocation and hormonal regulation. This finding contradicts another study, which reported a negative relationship between these parameters (Durmuş and Kamanlı, 2012).

In conclusion, this study has shown that pullets failing to achieve the required growth rates before the laying period exhibit lower yields during production periods. Therefore, improved management practices are necessary to achieve optimal growth targets during the pullet phase. Despite efforts to maintain controlled environmental conditions for laying hens, similar conditions are not always present in all cages due to the influence of light from windows and ventilation sys-

tems. Consequently, block trial designs should be employed in scientific studies to account for these variations. Although the THI showed minor variations throughout the poultry house, it significantly impacted the growth of the chickens. High THI resulted in slower growth, likely due to less efficient feed utilization, and led to decreased egg production in smaller hens. More efficient use of ventilation systems can substantially increase productivity in multiple ways. Appropriate housing conditions encompassing optimal light intensity, ventilation, and temperature management are essential for maximizing both growth and reproductive performance. Addressing these factors allows poultry producers to enhance the productivity and welfare of their flocks, ultimately leading to improved overall performance and economic benefits.

Acknowledgements

We extend our thanks to the Atatürk University Scientific Research Projects Unit for their contribution to the completion of this study under project code TAD-2020-8778.

References

Adegbenro M, Omole TR, Jongbo AO, Osho IB. Influence of cage tiers height on cloaca temperature, performance, and egg quality of Isa-Brown laying hens reared in a humid tropical climate. Arch Zootech 2023; 26(2): 116-34.

Akkuş B, Yıldırım İ. Beyaz ve kahverengi ticari yumurtacı tavuklarda tavuk yaşı ve kafes katının yumurta dış kalite parametreleri üzerine etkileri. Akad. Ziraat Derg 2018; 7(2): 211-8.

Amaripadath D, Rahif R, Velickovic M, Attia S. A systematic review on role of humidity as an indoor thermal comfort parameter in humid climates. J Build Eng 2023; 68: 106039.

Anderle M, Brambilla M, Hilpold A, Matabishi JG, Paniccia C, Rocchini D, et al. Habitat heterogeneity promotes bird diversity in agricultural landscapes: Insights from remote sensing data. Basic Appl Ecol 2023; 70: 38-49.

Bahuti M, Junior TY, Fassani ÉJ, Ribeiro BPVB, de Lima RR, Campos AT. Evaluation of different light intensities on the well-being, productivity, and eggs quality of laying hens. Comput Electron Agr 2023; 215: 108423.

Barros JSG, Barros TADS, Sartor K, Raimundo JA, Rossi LA. The effect of linear lighting systems on the productive performance and egg quality of laying hens. Poult Sci 2020; 99(3): 1369-78.

Dereli Fidan E, Nazligul A. The effect of cage position and density on some production traits in Denizli chickens. Anim Health Prod Hyg 2012; 1: 31-7.

Durmuş İ, Kamanlı S. Yumurtacı tavuklarda kafes katlarının bazı verim özelliklerine etkisi ile verimler arasındaki ilişkilerin belirlenmesi. Akad. Ziraat Derg 2012; 2: 77-82.

Eleroğlu H. The effect of breeding age end cages tiers on egg quality of white layer pure line. Turjaf 2019; 7: 173-82.

Elghardouf N, Lahlouh I, Elakkary A, Sefiani N. Towards modelling, and analysis of differential pressure and air velocity in a mechanical ventilation poultry house: Application for hot climates. Heliyon 2023; 9; e12936.

Elshafaei HE, Rashed RR, Goma AA, El-Kazaz SE, Kerr MJ, Smith M, et al. Use of water electrolyte supplementation for three days prior to processing helps alleviate the consequences of a severe thermal challenge on performance in meat chickens. Livest Sci 2020; 242: 104-260.

England A, Ruhnke I. The influence of light of different wavelengths on laying hen production and egg quality. J World's Poult Sci 2020; 76(3): 443-58.

Erensoy K, Sarıca M, Noubandiguim M, Dur M, Aslan R. Effect of light intensity and stocking density on the performance, egg quality, and feather condition of laying hens reared in a battery cage system over the first laying period. Trop Anim Health Prod 2021; 53(2): 320.

Farag MR, Alagawany M. Physiological alterations of poultry to the high environmental temperature. J Therm Biol 2018; 76: 101-6.

Gonzalez Ariza A, Arando Arbulu A, Navas Gonzalez FJ, Nogales Baena S, Delgado Bermejo JV, Camacho Vallejo ME. The study of growth and performance in local chicken breeds and varieties: A review of methods and scientific transference. Animals 2021; 11(9): 24-92.

He SP, Arowolo MA, Medrano RF, Li S, Yu QF, Chen JY, et al. Impact of heat stress and nutritional interventions on poultry production. J World's Poult Sci 2018; 74(4): 647-64.

Karaman S, Sekeroglu A, Duman M. Physical characteristics and performance of laying hens caged in different tiers and environmental parameters of each tier. J Asabe Trans 2013; 56(1): 321-8.

Kılıç İ, Şimşek E. Derin tabanlı bir yumurta tavuğu kümesinde iç ortam çevre koşullarının yeterliliğinin belirlenmesi üzerine bir araştırma. Bursa Uludag Üniv Ziraat Fak Derg 2008; 22(1): 17-29.

Kim DH, Lee KW. An update on heat stress in laying hens. J World's Poult Sci 2023; 79(4): 689-712.

Kim DH, Lee YK, Kim SH, Lee KW. The Impact of Temperature and humidity on the performance and physiology of laying hens. Animals 2021; 11(1): 56.

Küçüktopçu E, Cemek B, Simsek H. Modeling environmental conditions in poultry production: computational fluid dynamics approach. Animals 2024; 14 (3): 501.

Loengbudnark W, Chankitisakul V, Boonkum W. The genetic impact of heat stress on the egg production of Thai native chickens (Pradu Hang dum). Plos one 2023; 18(2): e0281328.

Majewski E, Potori N, Sulewski P, Wąs A, Mórawska M, Gębska M, et al. End of the cage age? A study on the impacts of the transition from cages on the EU laying hen sector. Agriculture 2024; 14(1): 111.

Mancinelli AC, Menchetti L, Birolo M, Bittante G, Chiattelli D, Castellini C. Crossbreeding to improve local chicken breeds: predicting growth performance of the crosses using the Gompertz model and estimated heterosis. Poult Sci 2023; 102(8): 102783.

Mohammed HH. Assessment of the role of light in welfare of layers. SVU-IJVS 2019; 2(1): 36-50.

Nega TE. Influence of day length, light color, light intensity, and sources on the performance of growers, and layers of different strains of chicken: A Review. EC Nu 2024; 19: 1-17.

Oloyo A. The use of housing system in the management of heat stress in poultry production in hot and humid climate: A Review. Poult Sci 2018; 6 (1): 1-9.

Onbaşılar EE, Aksoy FT. Stress parameters and immune response of layers under different cage floor and density conditions. Livest Prod Sci 2005; 95(3): 255-63.

Özentürk U, Yildiz A, Genç M. Assessment of the feather score and health score in laying hens reared at different cage densities. Ankara Univ Vet Fak Derg 2023; 70(1): 1-8.

Özentürk U, Yildiz A. Comparison of performance parameters, stress, and immunity levels of native andcommercial layers reared in different cage densities in Turkey. Turk J Vet Anim Sci 2021; 45(6): 1052-64.

Pawar SS, Sajjanar B, Lonkar VD, Kurade NP, Kadam AS, Nirmal AV, et al. Assessing and mitigating the impact of heat stress in poultry. Adv Anim Vet Sci 2016; 4(6): 332–41.

Qi F, Zhao X, Shi Z, Li H, Zhao W. Environmental factor detection and analysis technologies in livestock and poultry houses: A Review. Agriculture 2023; 13(8): 1489.

Saad HF, AL-hummod SK, Karomy AS. Lighting systems in the fields of laying hens (their importance and impact on the productive and immunological) performance of laying hens. EJARE 2024; 5(2); 1-6.

Sahin S. Effects of cage location and tier level on performance and egg quality traits of laying hens. J Anim Vet Adv 2012; 11(14): 2380-3.

Sarıca M, Türkoğlu M, Yamak US. Tavukçuluktaki gelişmeler ve Türkiye tavukçuluğu. Tavukçuluk bilimi, yetiştirme, besleme, hastalıklar (Editörler M Türkoğlu, M Sarıca). 2018; 1(36): 5.

Shin H, Lee S yeon, Kim J gyu, Park DH, Jo SK, Kwak Y. Applicability evaluation of a temperature humidity index-controlled ventilation system in livestock using a building energy simulation model. Case Stud Therm Eng 2024; 57: 104335.

Sogunle OM, Fagbamila OE, Ayo-Ajasa OY, Sanda AJ, Odutayo OJ, Olatunbosun OB, et al. Age and Cage Tiers: Effects on productive performance, blood profile and egg quality traits of egg-type chickens. NJAS 2022; 24(3): 28-42.

Şekeroğlu A, Duman M, Tahtalı Y, Yıldırım A, Eleroğlu H. Effect of cage tier and age on performance, egg quality and stress parameters of laying hens. S Afr J Anim Sci 2014; 44(3): 288-97.

Türker İ, Kamanlı S, Çelen MF. Batarya tipi kafeslerde ışığın geliş açısının yumurta verimi ve kalitesi üzerine etkisi. Ordu Üniv Bil Tek Derg 2021; 11(2): 152-8.

Yıldırım İ, Parlat SS, Aygün A, Yetişir R. Apartman tipi kafeste uygulanan askılı aydınlatma sisteminin kahverengi yumurtacı hibritlerin performans, yumurta kalite özellikleri ve stres düzeyine etkileri. Selcuk J Agric Food Sci 2008; 22(44): 7-11.

Yildiz A, Lacin E, Hayirli A, Macit M. Effects of cage location and tier level with respect to light intensity in semiconfined housing on egg production and quality during the late laying period. JAPR 2006; 15 (3): 355-61.

Zulovich JM, DeShazer JA. Estimating egg production declines at high environmental temperatures and humidities. Paper-American Society of Agricultural Engineers. 1990; 90: 4021.

ERCİYES ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ
Journal of Faculty of Veterinary Medicine, Erciyes University
Research Article / Araştırma Makalesi
21(3), 194-201, 2024
DOI: 10.32707/ercivet.1592078

Diagnostic Significance of Mean Platelet Volume and Erythrocyte Distribution Width in Calves with Sepsis

Murat UZTİMÜR[1,a]

[1]Bingöl University, Faculty of Veterinary Medicine, Department of Internal Medicine, Bingöl-TÜRKIYE
ORCID No: [a]0000-0001-9294-1825

Corresponding author; Murat UZTİMÜR, E-mail: muratuztimur@yahoo.com
How to cite: Diagnostic significance of mean platelet volume and erythrocyte distribution volume in calves with sepsis. Erciyes Univ Vet Fak Derg 2024; 21(3):194-201

Abstract: Medical studies conducted on humans have revealed significant changes in hematological parameters during sepsis and used these changes for diagnostic and prognostic purposes. However, there are very few studies on the diagnostic and prognostic utility of hematological parameters in the field of veterinary medicine. The objective of this study was to assess whether the parameters mean platelet volume (MPV), erythrocyte distribution width standard deviation/coefficient of variation (RDW-SD/CV), platelet distribution width (PDW) and plateletcrit (PCT) hold diagnostic significance in identifying sepsis in calves, while also investigating the interrelationships of these parameters within these sepsis group. The study included 45 calves diagnosed with sepsis and healthy 15 calves as control group. In calves with sepsis, MPV, PCT, RDW-SD, RDW-CV and total leukocyte count were found to be significantly higher than the control group. In their analysis for the diagnosis of sepsis in calves, the sensitivity of MPV was 86.67, the specificity was 84.44, the area under the curve (AUC) was 0.91, and the cut-off point value was 5.95 fL. In addition, the AUC values for other parameters were found as PCT 0.79 (P=0.009), RDW-SD 0.68, RDW-CV 0.75 and WBC 0.80, respectively. In the correlation analysis between MPV and other parameters, it was determined that there was a significant relationship between PCT 0.630, PDW 0.310, WBC 0.271, RDW-SD 0.383 and RDW-CV 0.-643. In conclusion, MPV may be a useful biomarker in calves with sepsis due to its favorable diagnostic performance in the early detection of sepsis in new born calves with diarrhea. In order to determine the effects of the results in this study on sepsis very well, it is necessary to work with populations with large sample numbers in the future.
Keywords: Calf, diarrhea, MPV, RDW, sepsis

Sepsisli Buzağılarda Ortalama Trombosit Hacmi ve Eritrosit Dağılım Hacminin Diyagnostik Önemi

Öz: İnsanlarda yapılan tıbbi çalışmalarda sepsis sırasında hematolojik parametrelerde anlamlı değişiklikler ortaya konmuş ve bu değişiklikler tanı ve prognoz amaçlı kullanılmıştır. Ancak veteriner hekimliği alanında hematolojik parametrelerin tanı ve prognozdaki faydasına ilişkin çok az çalışma bulunmaktadır. Bu çalışmanın amacı buzağılarda sepsisin belirlenmesinde ortalama trombosit hacmi (MPV), eritrosit dağılım genişliği-standart sapma/varyasyon katsayısı (RDW-SD/CV), trombosit dağılım genişliği (PDW) ve plateletkrit (PCT) parametrelerinin tanısal önem taşıyıp taşımadığını değerlendirmek ve ayrıca sepsis grubunda bu parametrelerin birbirleriyle olan ilişkilerini araştırmaktır. Çalışmaya sepsis tanısı konulan 45 buzağı dahil edildi. Sağlıklı olduğu belirlenen 15 buzağı kontrol grubunu oluşturdu. Sepsisli buzağılarda MPV, trombosit sayısı, RDW-SD, RDW-CV ve toplam lökosit sayısının kontrol grubuna göre anlamlı derecede yüksek olduğu bulundu. Buzağılarda sepsis tanısı için yapılan ROC analizinde MPV'nin duyarlılığı 86.67, özgüllüğü 84.44, eğri altında kalan alan 0.91 ve kesme noktası değeri 5.95 fL olarak bulundu. Ayrıca diğer parametreler için AUC değerleri sırasıyla PCT 0.79 (P=0.009), RDW-SD 0.68, RDW-CV 0.75 ve WBC 0.80 olarak bulundu. MPV ile diğer parametreler arasındaki korelasyon analizinde PCT 0.630, PDW 0.310, WBC 0.271, RDW-SD 0.383 ve RDW-CV 0.-643 arasında anlamlı ilişki olduğu belirlendi. Sonuç olarak MPV, ishalli yenidoğan buzağılarda sepsisin erken tespitinde iyi tanı performansı nedeniyle sepsisli buzağılarda yararlı bir biyobelirteç olabilir. Bu çalışmadaki sonuçların sepsis üzerine etkilerinin çok iyi belirlenebilmesi için gelecekte geniş örneklem sayılarına sahip popülasyonlarla çalışılması gerekmektedir.
Anahtar kelimeler: Buzağı, ishal, MPV, RDW, sepsis

Introduction

Sepsis arising from neonatal calf diarrhea elicits a substantial burden of morbidity and mortality (Panda et al., 2022; Milas et al., 2022). Moreover, sepsis engenders noteworthy economic ramifications for corporate entities. Analogous to numerous maladies, the early and precise diagnosis of sepsis assumes paramount significance for the efficacy of treatment protocols and the principles of preventive medical practice. Blood culture stands as the definitive criterion for sepsis diagnosis. Nevertheless, the necessity for novel biomarkers is underscored by reasons such

Geliş Tarihi/Submission Date : 02.08.2024
Kabul Tarihi/Accepted Date : 10.10.2024

as the extended duration requisite for blood culture application-typically spanning 48-72 hours-coupled with its susceptibility to yielding false positive outcomes and demonstrating reduced sensitivity (Pugni et al., 2015; Sağıroğlu et al., 2023). Despite the emergence of novel therapeutic interventions and early diagnostic methodologies in the domain of veterinary medicine in recent years, sepsis continues to uphold its clinical significance as a pivotal cause of mortality (Llewellyn et al., 2017). Automated hematology analyzers scrutinize indices associated with erythrocytes and platelets, encompassing metrics like erythrocyte distribution width standard deviation/ coefficient of variation (RDW-SD/VC), platelet count, platelet distribution width (PDW), plateletcrit (PCT), and mean platelet volume (MPV). Evaluation of these indices related to erythrocytes and platelets bestows clinicians with the capacity to proffer observations concerning the processes of production and activities pertaining to these cellular elements (Phillips et al., 2022). MPV constitutes a parameter amenable to straightforward analysis through a complete blood count, revealing the platelet volume, while also demonstrating a substantial increase concomitant with the release of immature PLTs from the bone marrow (Goddard et al., 2015). It also serves as a reliable indicator of platelet production and thrombopoesis (Korniluk et al., 2019). PCT expresses the ratio of platelet volume to total blood volume as a percentage value. It is also calculated using PCT count, MPV, and platelet count (Goddard et al., 2015). A multitude of studies have attested to the diagnostic significance of the platelet index in sepsis within the realm of human medicine (Panda et al., 2022; Milas et al., 2022; Mangalesh et al., 2021). Furthermore, demonstrative evidence reveals that the platelet index undergoes significant alteration in dogs afflicted by sepsis, septic peritonitis, and systemic inflammation (Bommer et al., 2008; Pierini et al., 2020; Llewellyn et al., 2017). The causative factor behind the heightened platelet index during sepsis resides in the escalated turnover of platelets originating from the bone marrow and the subsequent release of nascent platelets into the circulation. Consequently, this intricate interplay gives rise to pathological modifications in the parameters encompassed within the platelet index (Korniluk et al., 2019).

RDW quantifies the coefficient of variation in the dimensions of erythrocytes within circulation, revealing the presence of anisocytosis within these red blood cells (Scalco et al., 2022). RDW holds significance across both human and veterinary medical domains for regenerative anemias. Additionally, within human medicine, it emerges as a biomarker with applicability in the identification of various conditions, including sepsis (Scalco et al., 2022; Hodeib et al., 2022), cardiovascular ailments (Chen et al., 2010; Förhécz., 2009), and critical illnesses (Bazick et al., 2011).

Numerous studies have documented substantial alterations in hematological parameters among septic calves, underscoring the relevance of these changes in the disease's progression (Naseri et al., 2018; Naseri et al., 2019). In parallel, human medical studies have similarly illuminated significant shifts in hematological parameters during sepsis, harnessing these changes for diagnostic and prognostic purposes (Hodeib et al., 2022). However, within the sphere of veterinary medicine, explorations into the diagnostic and prognostic utility of hematological parameters have primarily centered on felines (Gori et al., 2021), canines (Phillips et al., 2022; Pierini et al., 2020), and equine neonates (Scalco et al., 2022). Thus far, no study has been identified that delves into platelet and erythrocyte indices' determination and their diagnostic significance among septic calves. This study's objectives encompass: (i) ascertaining whether platelet and erythrocyte indices bear diagnostic relevance in both sepsis and healthy neonatal calves; (ii) elucidating the associations between these indices and sepsis; and (iii) unveiling novel avenues for the application of these traditional hematological parameters, widely entrenched within clinical practice.

Materials and Methods

Before the commencement of the study, ethical clearance was secured from the Bingöl University Animal Experiments Local Ethics Committee (B.U. AELEC Meeting Number: 2023/04 Decision No: 04/04).

Animals and etiological diagnosis

The study included a total of 60 calves of different breeds and gender and aged 1-28 days. Of these, the sepsis group included 45 calves with diarrhea whose, their etiological diagnosis [Rotavirus, Coronavirus*, Cryptosporidium parvum (C. parvum),* and *Giardia lamblia*] was based on the utilization of immuno chromatographic rapid test kits (Anigen Rapid BoviD-5 Ag Test Kit, Bionote, Inc. Korea). Within the sepsis group, calves manifesting clinical sign other than diarrhea (such as prematurity, pneumonia, omphalitis, arthritis, congenital anomalies, immunosuppressive drug administration, or antibiotic usage) were excluded from the study. The remaining 15 healthy calves served as control group. These calves were clinically healthy and were negative for any infectious agents of concern on the immunochromatographic rapid test kits.

Sepsis criteria and analyses

The calves included in the study underwent an initial physical examination, encompassing assessments of respiratory rate per minute, body temperature in degrees Celsius, and heart rate per minute. Criteria for diagnosis of SIRS in calves were as follow;

-body temperature was more than 39.5 °C or less

than 38.5 °C,

- heart rate was more than 160/min or less than 100/ min,

-respiratory rate was more than 36/min,

- total leukocyte count was more than 12,000/mm3 or less than 4000/mm3 (Fectau et al., 1997; Fectau et al., 2009).

The presence of at least two of the aforementioned criteria defines SIRS, and when coupled with an infection or suspicion of infection, it characterizes sepsis (Fectau, 2009). To assess the erythrocyte and platelet indices in all calves, 2 ml blood samples were collected from the jugular vein into K3-EDTA-containing anticoagulant tubes (BD Vacutainer®, Plymouth, UK). For the determination of the total leukocyte (WBC), MPV, RDW-CV, RDW-SD, PDW, and PCT counts for each calf, the blood samples in the anticoagulant tubes were gently mixed and read on a 3-part hematology device (Benesphera H-31, India) within a maximum of 5 minutes. Prior to analysis, the hematology analyzer was calibrated in accordance with the manufacturer's protocol, and the blood samples were processed. Samples with inappropriate PLT or RBC histogram indices were excluded from the study. Blood smears were checked for the presence of PLT aggregates and calf with abnormal feature were excluded from the study.

Statistical analysis

Data were analyzed using software [SPSS 26 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.) and Graph Pad Prism (Prism 9 for Windows, version 9)]. The data were presented in the form of descriptive statistics, including mean ± standard deviation, median, minimum, and maximum values. To assess the normal distribution of the data, the Shapiro-Wilk test was employed. When analyzing the distinctions between the sepsis and control group calves, the Mann-Whitney U test was utilized for data that did not meet the criteria for normal distribution. Conversely, the Independent Sample T test was applied to data that exhibited a normal distribution. The correlation between variables was examined through Spearman's rank correlation test. For the assessment of the diagnostic value of erythrocyte and platelet indices in calves with sepsis, sensitivity, specificity, AUC, and the cut off value were determined using ROC analysis. The interpretation of the AUC values was as follows: AUC>0.90 indicated high accuracy, AUC between 0.70 and 0.90 denoted moderate accuracy, AUC between 0.5 and 0.7 indicated low accuracy, and AUC<0.5 was considered a failure. The statistical significance between groups was determined as P<0.05.

Results

Basic characteristics of the calves in the study

The numerical distribution of etiological factors is shown in Table 1. Etiological agents identified in calves were rotavirus (n=18), coronavirus (n=10), *C. parvum* (n=4), *Giardia lamblia* (n=4), and mixed infections [Rotavirus + Coronavirus (n=3), Coronavirus + *C. parvum* + *Giardia lamblia* (n=1)]. The breed distribution of calves with sepsis were Simmental (n=32) and were Holstein calves (n=13). All controls were Simmental breed. The study population consisted of 40 male and 20 female calves.

Table 1. Numerical distribution of etiological factors

Etiological Factors	Number
Rotavirus	18
Coronavirus	10
C. parvum	4
Giardia lamblia	4
Rotavirus+Coronavirus	3
Coronavirus+*C.parvum*+*Giardia lamblia*	1

Comparison of hematological variables between study groups

Table 2, 3 and Figure 1 present the descriptive statistics of MPV, RDW-SD, RDW-CV, PDW, PCT, WBC, body temperature, heart rate, and respiratory rate for calves with sepsis and calves in the control group. Statistically significant differences were observed between the sepsis and control groups. Specifically, in calves with sepsis, MPV (P<0.001), WBC (P<0.001), PCT (P<0.001), RDW-SD (P<0.034), and respiratory rate (P<0.025) were significantly higher compared to the control group. Conversely, RDW-CV was notably lower in calves with sepsis compared to the control group (P<0.004). While PDW and heart rate showed higher values in calves with sepsis, these differences were not statistically significant (PDW: P>0.061; heart rate: P>0.972).

Table 2. MPV, RDW-SD, RDW-CV, PLT, PDW, PCT, WBC, mean±standard deviation, median, minimum and maximum values of calves with sepsis and those in the control group

	Sepsis Group	**Control Group**	
Variables	**Mean±SDMean±SD Median (IQR)**	**Median (IQR)**	**P value**
MPV (fL)	12.96±4.42[a] 15 (4.40-17.90)	5.26±0.50[b] 5.10 (4.6-6)	0.001
RDW-SD (fL)	42.30±10.55[a] 44.4 (12.80-73)	40.92±4.20[b] 40.5 (33.3-49.70)	0.034
RDW-CV (%)	22.14±5.19[a] 24.1 (7.60-27.20)	30.39±8.23[b] 32.8 (16-44.60)	0.004
PDW (fL)	13.66±6.63 18.3 (1.8-20.70)	11.09±4.69 14.1 (4.2-14.5)	0.061
PCT (%)	5.51±2.86[a] 6.38 (0.05-9.79)	3.04±2.2[b] 3.64 (0.19-5.43)	0.001
WBC (x10^9)	18.52±11.66[a] 16.61 (2.5-55)	9.52±2.86[b] 8.64 (6.94-18.7)	0.001

RDW-SD/VC: Erythrocyte distribution width standard deviation/coefficient of variation; PDW: platelet distribution width; PCT: plateletcrit; MPV: Mean platelet volume; WBC: White blood cell. Statistical significance differences between groups P<0.05.

Table 3. Body temperature, heart and respiratory frequency values of calves with sepsis and those in the control group

Variables	**Sepsis Group mean±SD min-max**	**Control Group mean±SD min-max**	**P value**
Heart Frequency (min)	124.89±34.7 35-200	121.87±20.12 68-140	0.972
Respiratory Frequency (min)	38.96±14.5[a] 12-80	30.13±4.64[b] 20-40	0.025
Body Temperature (°C)	37.95±1.47 35-40.3	38.07±2.25 30-39.2	0.476

Statistical significance differences between groups P<0.05.

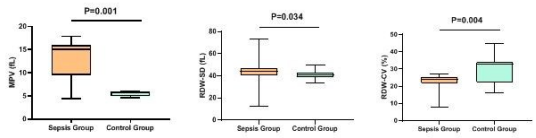


Figure 1. Box plots show MPV, RDW-SD and RDW-CV values of calves with sepsis and control group calves. Statistical significance differences between groups P<0.05. MPV: mean platelet volume; RDW-SD: erythrocyte distribution width standard deviation; RDW-CV, erythrocyte distribution width coefficient of variation.

The Value of hematological variables in the diagnosis of sepsis

Table 4 and Figure 2 present the sensitivity, specificity, AUC, and cut-off point values for the parameters MPV, RDW-SD, RDW-CV, and PCT in the context of diagnosing sepsis using the ROC curve. For the diagnosis of sepsis, the MPV parameter exhibited a sensitivity of 86.67% and specificity of 84.44%. The AUC was determined to be 0.91, with a cut-off point value of less than 5.95. Similarly, the PCT parameter displayed a sensitivity of 73.33% and specificity of 68.89%, yielding an AUC of 0.79. The corresponding cut-off point value was less than 4.91. Regarding RDW-SD, its sensitivity was calculated as 73.33% and specificity as 73.33%, resulting in an AUC of 0.68. The cut-off point value was determined to be less than 41.65. On the other hand, RDW-CV showed a sensitivity of 66.67% and specificity of 66.67%, with an AUC of 0.75. The optimal cut-off point value was greater than 24.85.

Table 4. Sensitivity, specificity, AUC and cut-off point values of variables in the diagnosis of sepsis in calves

Variables	Sensitivity	Specificity	AUC	Cutt-off	P value
MPV (fL)	86.67	84.44	0.91	>5.95	0.001
RDW-SD (fL)	73.33	73.33	0.68	>41.65	0.034
RDW-CV (%)	66.67	66.67	0.75	<24.85	0.003
PCT (%)	66.67	68.89	0.79	>4.91	0.009
WBC (x10^9)	80	80	0.80	>9.68	0.006

AUC: area under the curve; RDW-SD/VC: Erythrocyte distribution width standard deviation/coefficient of variation; PDW: platelet distribution width; PCT: plateletcrit, MPV: Mean platelet volume; WBC: White blood cell. Statistical significance differences between groups P<0.05

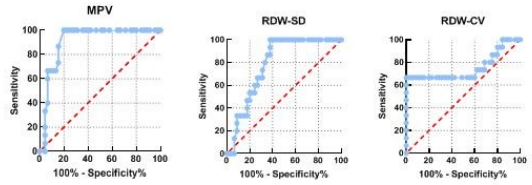


Figure 2. Receiver operating characteristic curves of MPV, RDW-SD and RDW-CV values of calves with sepsis. MPV: mean platelet volume; RDW-SD: erythrocyte distribution width standard deviation; RDW-CV: erythrocyte distribution width coefficient of variation.

Relationship between hematological variables in sepsis

Correlation analysis was performed between MPV value and PLT, PCT, RDW-SD, PDW and WBC values in calves diagnosed with sepsis. In the septic calves, a statistically significant positive correlation was determined between MPV and PCT (r=0.630, P=0.005), MPV and RDW-SD (r=0.383, P=0.001), MPV and PDW (r=0.310, P=0.005) and MPV and WBC (r=0.271, P=0.001).

Discussion

The primary hypothesis of this study asserts that the erythrocyte and platelet indices measured by accessible, cost-effective, user-friendly hematology analyzers, which require minimal labor and technical expertise and are readily available in most clinical settings, can serve as potential biomarkers for diagnosing sepsis in calves. As such, the objective of this study was to assess whether the parameters MPV, RDW-SD, RDW-CV, PDW, and PCT hold diagnostic significance in identifying sepsis in calves, while also investigating the interrelationships of these parameters within the sepsis group. The findings indicated statistically significant elevations in MPV, WBC, PCT, RDW-CV, and RDW-SD levels among septic calves compared to the control group. The sensitivity, specificity, AUC (0.91), and cut off point (>5.95) for MPV were 86.67%, 84.44%, and 0.91, respectively. Furthermore, this study unveiled significant alterations in platelet activation and production within calves experiencing sepsis triggered by diarrhea. This underscores the potential utility of MPV as a valuable biomarker for sepsis diagnosis.

Timely recognition of sepsis-related problems in calves, prior to developing into irreversible condition is a critical in calf survival as it is of utmost importance in minimizing sepsis-related mortality. Consequently, the necessity for diagnostic biomarkers exhibiting high sensitivity and specificity is evident (Pugni et al., 2015; Schwartz et al., 2014; Uztimür et al., 2024). Novel diagnostic biomarkers can significantly contribute to the early identification and prognosis monitoring of infected newborns, ultimately reducing both morbidity and mortality rates, and preventing the progression towards septic shock (Pierini et al., 2020; Scalco et al., 2022).

Notable studies on MPV, a pivotal parameter of platelet index, have disclosed a significant increase in its value among dogs infected with canine parvovirus compared to control groups (Engelbrecht et al., 2021). Correspondingly, investigations conducted by Bommer et al. (2008), Schwartz et al. (2014), and Moritz et al. (2005), involving dogs afflicted by inflammatory thrombocytopenia, have reported considerable MPV elevation vis-à-vis healthy cohorts. This phenomenon of MPV increase has been attributed to the presence of larger, immature platelets resultant from regenerative processes (Bommer et al., 2008; Schwartz et al., 2014; Moritz et al., 2005). In parallel, studies have noted elevated MPV in dogs with sepsis (Pierini et al., 2020) and septic peritonitis (Llewellyn et al., 2017), underscoring its diagnostic potential. Consistent with these findings, Panda et al. (2022) observed a substantial MPV elevation in 43 neonates with sepsis. The recorded MPV sensitivity was 63.4%, specificity was 53.8%, and the cut-off point value was ≥9 fL, all indicating its utility in sepsis diagnosis. A meta-analysis by Milas et al. (2022) concurred that MPV has diagnostic significance in neonatal sepsis. Its sensitivity and specificity were reported as 0.675 and 0.733, respectively, with a cut-off point value of 9.28 fL. Another study concerning neonates with sepsis demonstrated MPV sensitivity of 93.9%, specificity of 60.9%, an AUC of 0.825, and a cut-off point >10.25 fL (Mangalesh et al., 2021). Consistent with prior studies, our study found a significant

elevation in MPV (12.96±4.42 fL) among septic calves compared to controls (5.26±0.50 fL). The ROC analysis yielded an AUC of 0.91, sensitivity of 86.67%, specificity of 84.44%, and a cut-off point value exceeding 5.95 fL, all reflecting robust diagnostic capability for sepsis. The upsurge in MPV during sepsis is attributed to the presence of larger, immature platelets and augmented activation, aggregation, and adhesion due to platelet regeneration (Khadka et al., 2022; Bommer et al., 2008).

PCT, an integral component of the platelet index, emerges as a parameter significantly impacted by sepsis (Khadka et al., 2022; Phillips et al., 2022). Two distinct studies involving neonates with sepsis disclosed marked increases in PCT values relative to healthy subjects, with this parameter exhibiting substantial correlation with disease severity (Zhang et al., 2015; Khadka et al., 2022). The ensuing ROC analysis for early sepsis prediction yielded a sensitivity of 75.9%, specificity of 67.6%, and a cut-off point exceeding 0.19%. Correspondingly, Phillips et al. (2022) observed a significant increase in PCT values within a study involving dogs with hematological neoplasia. In the present study, PCT levels were notably higher in calves afflicted with neonatal sepsis compared to the control group. Furthermore, the diagnostic capacity of PCT in sepsis was established with a sensitivity of 73.33%, specificity of 68.89%, AUC of 0.79, and a cut-off point exceeding 4.98%. These findings resonate with the outcomes of aforementioned studies.

RDW is a parameter inherent to the whole blood profile, illustrating the variance and heterogeneity among erythrocytes (Kim et al., 2020). Recent years have witnessed an expanded recognition of RDW beyond its traditional role in anemia, emphasizing its significance as a biomarker in conditions involving respiratory, cardiovascular diseases, inflammation, infection, and sepsis-related contexts (Lippi et al., 2009; Hodeib et al., 2022). Empirical studies on dogs with heartworm disease (Kim et al., 2020) and pulmonary hypertension (Swann et al., 2014), encompassing 86 and 44 subjects respectively, unveiled substantial elevations in RDW count. This led to suggestions that RDW serves as a crucial parameter for disease monitoring. These observations underline the importance of RDW in determining disease prognosis. In sepsis, profound alterations transpire within the hematopoietic system. Among these changes, the presence of pro-inflammatory cytokines inhibits erythrocyte maturation, contributing to heightened erythrocyte heterogeneity and, consequently, elevated RDW values (Pierce et al., 2005; Tóth et al., 2017). For sepsis diagnosis, RDW-SD demonstrated a sensitivity of 73.33%, specificity of 73.33%, AUC of 0.68, and a cut-off point exceeding 41.65 fL. Conversely, RDW-CV value (22.14±5.19%) was lower in septic calves than the control group (30.39±8.23%). The sensitivity and specificity of RDW-CV in sepsis diagnosis were 66.67% each, with an AUC of 0.75, and a cut-off point below 24.85. The elevation of RDW might be attributed to heightened inflammatory reactions in sepsis, impacting bone marrow and iron metabolism (Förhécz et al., 2009). Additionally, endocrine and neuro-hormonal factors stimulate erythrocyte proliferation, thereby enhancing erythropoietin production and consequently elevating RDW (Chen et al., 2010).

In conclusion, the acquired values of MPV, RDW-SD, RDW-CV, PDW, and PCT from automated hematology analyzers, readily accessible in nearly all clinical settings, requiring minimal additional labor and technical expertise, and offering cost-effective and swift results, hold potential for deployment in the diagnosis of sepsis in calves. In this study, discernible elevations were observed in MPV, RDW-SD, RDW-CV, PDW, PCT, and WBC counts among calves afflicted with sepsis in comparison to the control group. Notably, MPV, RDW, PLT, and PCT levels in calves exhibited diagnostic significance for sepsis, with an evident interrelation between these indices. The findings of this study firmly establish the diagnostic utility of MPV values as a viable biomarker for sepsis diagnosis.

REFERENCE

Bazick HS, Chang D, Mahadevappa K, Gibbons FK, Christopher KB. Red cell distribution width and all-cause mortality in critically ill patients. Crit Care Med 2011; 39(8): 1913-21.

Bommer NX, Shaw DJ, Milne EM, Ridyard, AE. Platelet distribution width and mean platelet volume in the interpretation of thrombocytopenia in dogs. J Small Anim Pract 2008; 49(10): 518-24.

Chen PC, Sung FC, Chien KL, Hsu HC, Su TC, Lee YT. Red blood cell distribution width and risk of cardiovascular events and mortality in a community cohort in Taiwan. Am J Epidemiol 2010; 171(2):214-20.

Engelbrecht M, Atkinson B, Goddard A, Pazzi P, Mcclure V. Mean platelet volume and platelet volume distribution width in canine parvoviral enteritis. Front Vet Sci 2021; 8: 722280.

Fecteau G, Pare J, Van Metre DC, Smith BP, Holmberg CA, GuterbockW, Jang S. Use of a clinical sepsis score for predicting bacteremia in neonatal dairy calves on a calf rearing farm. Can Vet J 1997; 38(2): 101.

Fecteau G, Smith BP, George, LW. Septicemia and meningitis in the newborn calf. Vet Clin North Am Food Anim 2009; 25(1): 195-208.

Förhécz Z, Gombos T, Borgulya G, Pozsonyi Z, Prohaszka Z, Janoskuti L. Red cell distribution width in heart failure: prediction of clinical events and relationship with markers of in effective erythropoiesis, inflammation, renal function, and nutritional state. Am Heart J 2009; 158(4): 659-66.

Goddard A, Leisewitz AL, Kristensen AT, Schoeman JP. Platelet indices in dogs with Babesia rossi infection. Vet Clin Pathol 2015; 44(4): 493-7.

Gori E, Pierini A, Lippi I, Lubas G, Marchetti V. Leukocytes ratios in feline systemic inflammatory response syndrome and sepsis: A retrospective analysis of 209 cases. Animals 2021; 11(6): 1644.

Hodeib M, Morgan D, Hedaya A, Waked N. A study of elevated red cell distribution width (RDW) in early -onset neonatal sepsis. Gaz Egypt Paediatr Assoc 2022; 70(1): 1-6.

Khadka P, Maharjan G, Chapagain G, Paudyal P. Economic and diagnostic biomarker tests of neonatal sepsis: A prospective study from a tertiary care hospital in a low-income country. Biomed Res Int 2022; 2022: 5166380.

Kim SJ, Suh SI, Hyun C. Evaluation of red blood cell profiles in dogs with heartworm disease. Can J Vet Res 2020; 84(4): 265-71.

Korniluk A, Koper-Lenkiewicz OM, Kamińska J, Kemona H, Piekarska VD. Mean platelet volume (MPV): new perspectives for an old marker in the course and prognosis of inflammatory conditions. Mediators Inflamm 2019;17(2019): 921307.

Llewellyn EA, Todd JM, Sharkey LC, Rendahl A. A pilot study evaluating the prognostic utility of platelet indices in dogs with septic peritonitis. J Vet Emerg Crit Care 2017; 27(5): 569-78.

Lippi G, Targher G, Montagnana M, Salvagno GL, Zoppini G, Guidi GC. Relation between red blood cell distribution width and inflammatory biomarkers in a large cohort of unselected outpatients. Arch Path Lab 2009; 133(4): 628-32.

Mangalesh S, Dudani S, Malik A. Platelet indices and their kinetics predict mortality in patients of sepsis. Indian J Hematol Blood Transfus 2021; 37(4): 600-8.

Milas GP, Karageorgiou V, Bellos I. Mean platelet volume and neonatal sepsis: a systematic review and meta-analysis of diagnostic accuracy. J Matern Fetal Neonatal Med 2022; 35(25): 5324-36.

Moritz A, Walcheck BK, Weiss DJ. Evaluation of flow cytometric and automated methods for detection of activated platelets in dogs with inflammatory disease. Am J Vet Res 2005; 66(2): 325-9.

Naseri A, Sen I, Turgut K, Guzelbektes H, Constable PD. Echocardiographic assessment of left ventricular systolic function in neonatal calves with naturally occurring sepsis or septic shock due to diarrhea. Res Vet Sci 2019; 126: 103-12.

Naseri A, Turgut K, Sen I, Ider M, Akar A. Myocardial depression in a calf with septic shock. Vet Rec Case Rep 2018;6(1): e000513.

Panda SK, Nayak MK, Thangaraj J, Das P, Pugalia R. Platelet parameters as a diagnostic marker in early diagnosis of neonatal sepsis-seeking newer answers for older problems. Fam Med Prim Care Rev 2022; 11(5): 1748.

Phillips C, Naskou MC, Spangler E. Investigation of platelet measurands in dogs with hematologic neoplasia. Vet Clin Pathol 2022; 51(2): 216-24.

Pierce CN, Larson DF. Inflammatory cytokine inhibition of erythropoiesis in patients implanted with a mechanical circulatory assist device. Perfusion 2005; 20(2): 83-90.

Pierini A, Gori E, Lippi I, Lubas G, Marchetti V. Are leukocyte and platelet abnormalities and complete blood count ratios potential prognostic markers in canine sepsis? Front Vet Sci 2020; 7: 578846.

Pugni L, Pietrasanta C, Milani S, Vener C, Ronchi A, Falbo M, Arghittu M, Mosca F. Presepsin (soluble CD14 subtype): reference ranges of a new sepsis marker in term and preterm neonates. PloS one 2015; 10(12): e0146020.

Sağıroğlu M, Uztimur M, Kızıl Ö. Sepsisli buzağılarda serum heparan sülfat, sürfaktant protein-D ve sRAGE düzeylerinin diyagnostik önemi. FÜ Sağ Bil Vet Derg 2023; 37(2): 122-6.

Scalco R, Aleman M, Nogueira CE, Freitas N, Curcio BR. Red cell distribution width values and red cell distribution width-to-platelet ratio in Thoroughbred foals in the first 24 hours of life. J Vet Emerg Crit Care 2022; 1-6.

Schwartz D, Sharkey L, Armstrong PJ, Knudson C, Kelley J. Platelet volume and plateletcrit in dogs with presumed primary immune-mediated thrombocytopenia. J Vet Intern Med 2014; 28(5): 1575-79.

Swann JW, Sudunagunta S, Covey HL, English K, Hendricks A, Connolly DJ. Evaluation of red cell distribution width in dogs with pulmonary hypertension. J Vet Cardiol 2014; 16(4): 227-35.

Tóth J, Debreceni IB, Berhés M, Hajdú E, Deák Á, Pető K, Kappelmayer J. Red blood cell and platelet parameters are sepsis predictors in an Escherichia coli induced lethal porcine model. Clin Hemorheol Microcirc 2017; 66(3): 249-59.

Uztimür M, Kizil Ö, Akbulut HH. Immunophenotyping of peripheral circulating lymphocytes and serum selenium levels in calves with neonatal diarrhea. Vet Immunol Immunopathol 2024; 269: 110728.

Zhang S, Cui YL, Diao MY, Chen DC, Lin ZF. Use of platelet indices for determining illness severity and predicting prognosis in critically ill patients. Chin Med J 2015; 128: 2012-2018.

ERCİYES ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ
Journal of Faculty of Veterinary Medicine, Erciyes University
Review/Derleme
21(3), 202-208, 2024
DOI: 10.32707/ercivet.1587170

Keratitis and Current Treatment Methods in Pets

Nevzat Emre ASLAN[1a], Hanifi EROL[2b]

[1]Yozgat Bozok University, Faculty of Veterinary Medicine, Department of Surgery, Yozgat-TURKİYE
[2]Erciyes University, Faculty of Veterinary Medicine, Department of Surgery, Kayseri-TURKİYE
ORCID: [a]0000-0001-8970-7763; [b]0000-0001-8140-3108

Corresponding author: Hanifi EROL; E-mail: drhaneroll@yahoo.com
How to cite: Aslan NE, Erol H. Keratitis and current treatment methods in pets. Erciyes Univ Vet Fak Derg 2024; 21 (3):202-208

Abstract: Keratitis is defined as infectious and non-infectious eye disease in human medicine and ulcerative and non-ulcerative disease in veterinary medicine. The corneal epithelium is the transparent anterior part of the eye that covers the iris and pupil. It acts as a transparent membrane that allows light to pass through the eye. A healthy cornea prevents bacteria invasion of the eye via anatomical, mechanical, immunological, and microbiological mechanisms. Failure of these defenses and trauma, immunosuppression, and neurological or iatrogenic factors predispose the cornea to ulcerative keratitis and bacterial eye infections. Inadequate tear secretion and eyelid dysfunction lead to insufficient corneal protection, and the combination of these reasons with endogenous causes triggers excessive epithelial damage.
Keywords: Cornea, keratitis, ocular surface disease

Evcil Hayvanlarda Keratit ve Güncel Tedavi Yöntemleri

Öz: Keratit, insan hekimliğinde enfeksiyöz ve non-enfeksiyöz göz hastalığı, veteriner hekimliğinde ise ülseratif ve non-ülseratif göz hastalığı olarak tanımlanır. Kornea epiteli, iris ve pupillayı örten gözün şeffaf ön kısmıdır. Işığın gözden geçmesini sağlayan şeffaf bir zar görevi görür. Sağlıklı bir kornea anatomik, mekanik, immunolojik ve mikrobiyolojik mekanizmalarla çeşitli bakterilerin istilasını önler. Hastalıklar, travma ve immunsupresyon, nörolojik veya iyatrojenik nedenlerle bu savunmanın yetersiz kalması korneayı ülseratif keratit ve bakteriyel göz enfeksiyonlarına yatkın hale getirir. Eksik gözyaşı salgısı ve göz kapağı disfonksiyonu yetersiz kornea korumasına yol açar ve bu nedenlerin endojen nedenlerle birleşmesi aşırı epitel hasarını tetikler.
Anahtar kelimeler: Keratit, kornea, oküler yüzey hastalığı

Introduction

The transparent front layer of the eye known as the corneal epithelium serves as a transparent membrane to let light pass through while covering the iris and pupil (Leong and Tong, 2015). A healthy cornea is reported to prevent the invasion of various bacteria via anatomical, mechanical, immunological and microbiological mechanisms. Failure of these defenses due to disease, trauma, immunosuppression, neurological or iatrogenic causes has been reported to predispose the cornea to ulcerative keratitis and bacterial infections (Hindley et al., 2015). Ocular surface infections, which can develop due to corneal abrasion, could pose a severe threat to vision by causing ulceration and tissue destruction (Cappiello et al., 2023). The corneal infection could be seen due to contamination of the traumatic ulcer or the introduction of microorganisms from the environment by traumatically micro puncture of the corneal stroma. However, epithelial nonulcerative keratomycosis associated with precorneal tear film instability occurs without predisposing corneal damage. Therefore, a cornea infected with fungi may not initially stain with fluorescein (Mustikka et al., 2020).

Geliş Tarihi/Submission Date : 22.04.2024
Kabul Tarihi/Accepted Date : 03.06.2024

Types of Keratitis

Bacterial keratitis

Bacterial keratitis caused by infectious organisms is a sight-threatening disease. These organisms should be identified early and their antibiotic susceptibility should be known (Hall and Franzco, 2004). Bacterial infections can cause rapid disease progression and loss of the eye due to both bacterial and host-derived factors, including toxins and proteinases (Hindley et al., 2015). It is reported that bacterial keratitis is a clinically significant disease, although it is less common in cats compared to other domestic animals (Goldreich et al., 2020). Refractive corneal surgery and immunosuppression trigger bacterial keratitis (Fleiszig and Evans, 2002; Carion et al., 2018). A combination of direct microscopy and culture on a bacteriological plate medium is recommended for

diagnosing bacterial keratitis (Schonheyder et al., 1997). Gram-negative and gram-positive bacteria such as *Staphylococcus* spp., *Streptococcus* spp., *Pseudomonas* spp., and *Serratia* spp. are mostly isolated from patients with bacterial keratitis (Fleiszig and Evans, 2002). The traditional clinical protocol for bacterial keratitis is the topical application of broad-spectrum antibiotic eye drops. However, delay in laboratory testing for appropriate antibiotic selection may alter the course of the disease and high doses of antibiotics may cause toxicity. Therefore, new therapeutic approaches such as metal nanoparticles, cationic species, photothermal and photodynamic therapy are being investigated (Fan et al., 2023). *Pseudomonas aeruginosa* is an opportunistic, gram-negative pathogen commonly associated with bacterial keratitis (Carion et al., 2018). This pathogen, which causes significant destruction and loss of stromal tissue, typically presents as a rapidly progressive, suppurative stromal infiltrate and prominent mucopurulent exudate. Necrosis surrounded by inflammatory epithelial edema and stromal ulceration is reported to be characteristic of this disease (Hazlett, 2004). In *Pseudomonas aeruginosa* infections, glucocorticoids with potent strong anti-inflammatory and immunosuppressive properties are recommended to be used with intensive topical antibiotic use (Murugan et al., 2016; Yang et al., 2018).

Zhu et al. (2020) created four groups with the *P. aeruginosa* keratitis model in their study. They administered TobraDex (0.3% tobramycin + 0.1% dexamethasone) in the first group, Tobrex (0.3% tobramycin) in the second group, 0.1% dexamethasone in the third group and standard saline solution in the fourth group four times in a day. As a result of the study, they reported that neutrophil infiltration decreased in the Tobrex group, severe neutrophil infiltration and bacterial load decreased in the Dexamethasone group, and the amount of neutrophils and bacterial load decreased more in the TobraDex group than in other groups. In conclusion, they emphasized that the mice treated with TobraDex and Tobrex exhibited mild corneal damage; converserly, the mice treated with dexamethasone exhibited very severe corneal damage, and clinical findings supported these conditions.

Clinically, small abscess-like lesions with a mostly grey-white appearance, minimal epithelial edema and stromal infiltrates had been reported in *Staphylococcus spp.* in keratitis. It is emphasized that intrastromal abscesses and perforation may occur in chronic *Staphylococcus* keratitis (Shrestha et al., 2020). In their study, Bello et al. (2023) compared the effects of Genipin extract which was obtained from *Gardenia Jasminoides Ellis* in *S. auerus* and *P. aeruginosa* keratitis in their study. They reported that Genipin treatment reduced the bacterial load and alleviated the severity of keratitis by suppressing neutrophil infiltration. In addition, they reported that Interleukin-1, Interleukin-6, Interleukin-8, Interleukin-15, and Tumour Necrosis Factor- α values decreased significantly in Genipin treatment. As a result of the study, they offered that Genipin could be used in bacterial keratitis.

Ulcerative keratitis

Ulcerative keratitis, which exposes the corneal stroma and causes blepharospasm, photophobia, lacrimation, conjunctival hyperemia, and corneal edema, is one of the most common ocular surface diseases (Iwashita et al., 2020). The inadequate corneal protections due to the lack of tear volume and eyelid dysfunction with endogenous causes trigger excessive epithelial damage. Eyelid, eyelash, and tear film dysfunctions are more common in dogs, especially in brachycephalic breeds (Iwashita et al., 2020; Packer et al., 2015). Numerous etiologies, including morphological and neurological abnormalities of the eyelids, abnormal eyelashes or facial hair, quantitative or qualitative tear film abnormalities, corneal innervation deficiencies, foreign bodies, and bacterial infections, can cause corneal ulcers (Ledbetter et al., 2006). The eye can be examined with white or cobalt blue light to diagnose ulcerative keratitis after fluorescein staining. In addition, it is reported that bacterial and fungal cell culture, cytology and Polymerase Chain Reaction (PCR) can be used to determine the causative agent of ulcers (Edman et al., 2019). It is reported that most superficial ulcerative keratitis can heal rapidly, but progressed keratitis to the stroma may cause visual loss. Corneal stroma damage is typically attributed to bacterial infection by the presence of proteases and collagenases and is reported to be associated with anterior uveitis. Therefore, deep ulcerative keratitis is an ocular disease that requires intensive treatment with antibiotics, and cycloplegic and proteinase inhibitors (Bustamente et al., 2018). Topical corticosteroids are reported to be contraindicated (Hartley, 2010).

Deepika et al. (2023) randomly divided 20 dogs with deep ulcerative keratitis into two groups and treated the first group with 0.1% Tacrolimus, 0.5% moxifloxacin and oral doxycycline and dietary nutritional supplements. The second group was treated with 0.1% cyclosporine, 0.5% moxifloxacin, oral doxycycline and dietary nutritional supplements. At the end of the one month they reported that there was a significant difference between clinical findings and ulcer healing in Group 1 compared to Group 2. They reported that tacrolimus is more effective in the treatment of ulcerative keratitis. It has immunomodulatory effects similar to cyclosporine, suppresses T-cell proliferation and is a good lacrimomimetic.

Bayley et al. (2018) investigated the effect of superficial keratectomy in non-healing corneal ulcers associated with primary corneal endothelial degeneration.

They reported that superficial keratectomy was effective in 47 of 89 dogs with painful, non-healing corneal ulcers associated with primary corneal endothelial degeneration. However, Dalmatians had a high risk for corneal ulcer development. Mezzadri et al. (2021) performed surgical treatment in descamatocele, perforated corneal ulcer, and deep corneal ulcer with autologous buccal mucosa membrane graft in cats and dogs in their study. In 12 cats (13 eyes) and 14 dogs (14 eyes), autologous buccal mucosa grafts were applied; they reported that there were no intraoperative complications, 24 of the 27 eyes healed, and 22 eyes regained effective visual function. They emphasized that an otology buccal mucosa graft can be considered an alternative treatment method for ulcerative keratitis.

Mycotic keratitis

Mycotic keratitis, which is usually a result of corneal injury in farm environments or environments with plant materials, is a slowly developing ocular disease that occurs in immunosuppressed conditions such as overuse of broad-spectrum antibiotics, indiscriminate use of corticosteroids, and diabetes (Shukla et al., 2008). *Aspergillus* spp. and *Fusarium* spp. are the most common causative agents of mycotic keratitis. *Alternaria, Curvularia, Helminthosporium, Penicillium,* and *Candida* are also reported to cause mycotic keratitis. (Raj et al., 2021). It is reported that topical use of 5% natamycin is effective in treatment, and topical amphotericin B 0.3-0.5% can be used as an alternative, but its use is limited because of toxicity (Austin et al., 2017). In addition, voriconazole, which has high ocular penetration capability, has gained popularity in treating fungal keratitis (Hariprasad et al., 2008). Many limitations in treating fungal keratitis, include delayed diagnosis, limited availability of systemic and topical agents, poor drug penetration, toxicity, corneal thinning, recurrence, and corneal perforation (Ler et al., 2022). Wei et al. (2022) investigated the efficacy of standard corneal cross-linking. They accelerated corneal cross-linking in treating an experimental fungal keratitis model caused by *Aspergillus fumigatus* in 26 New Zealand rabbits. In Group 1, the cross-linking time was set as 10 min, the irradiation parameters were 9 mW/cm^2, and 0.1% riboflavin was added every 3 min for 30 min. In Group 2, the cross-linking time was set as 30 min, irradiation parameters were 3 mW/cm^2, and 0.1% riboflavin was added every 5 min. They treated the rabbits in both groups medically with 1% voriconazole and concluded that both cross-linking models can prevent ulcer progression and promote ulcer healing. At the same time, they emphasized that the rapid cross-linking model can control infection faster and is superior to standard cross-linking in ulcer healing.

Parasitic keratitis

Acanthamoeba keratitis is a rare parasitic disease characterized by acute infection with trophozoites of the opportunistic protozoan *Acanthamoeba castellani* (Cooper et al., 2021). In vivo confocal microscopy (IVCM) is generally accepted as the first method to confirm the diagnosis of *Acanthamoeba* keratitis cases, because it is rapid and has high specificity and sensitivity for amoeba detection (Ledbetter, 2021). Although eye pain and photophobia are usually seen as clinical symptoms, they may cause blindness (Morales et al., 2015). In the IVCM image of *Acanthamoeba* keratitis, cysts and trophozoites are seen in the corneal stroma in clusters or in a chain arrangement (Ledbetter, 2021). There is no standard treatment option for *Acanthamoeba* keratitis. However, diamidine (propamidine-isethionate, hexamidine-isethionate), biguanide (polyhexanide, 0.02% chlorhexidine) and neomycin sulfate that can show antiamoebic effect was indicated in literature (Larkin et al., 1992; Reinhard and Baumans, 2006; Szentmary et al., 2020). At the same time, the compound of 1% povidone-iodine, antileischmaniatic (miltefosine), antifungal (miconazole, clotrimazole, voriconazole, natamycin) can be used for the treatment was recorded (Szentmary et al., 2020). *Onchocerca spp.* and other microfilarial parasites cause keratitis in humans, horses, and dogs. The inflammatory response due to the migration of microfilariae into the cornea and their subsequent death causes keratitis in most cases (Edelmann et al., 2017).

Non-ulcerative keratitis

Keratitis is classified as infectious and non-infectious in human medicine and ulcerative and non-ulcerative in veterinary medicine. Non-ulcerative keratitis is usually caused by mechanical irritation (pigmentary keratitis) or immune-mediated (Kecova et al., 2004). In pigmentary keratitis, corneal inflammation, vascularization and corneal edema are observed with progressive pigmentation on the corneal surface (Sebbag and Sanchez, 2022).

Superficial pigmentary keratitis

Pigmentary keratitis, which is described as the development of corneal pigmentation associated with chronic inflammation, is an ocular surface disease. It causes significant visual impairment and blindness in severe cases. Pigmentary keratitis is caused by migrating melanocytes from the limbal and perilimbal regions and their accumulation in the corneal epithelium and anterior stroma (Azoulay, 2013). Corneal pigmentation has also been reported as a feature of inflammatory corneal pathologies such as keratoconjunctivitis sicca, chronic superficial keratitis, and ulcerative/non-ulcerative keratitis. It is reported that pigmentary keratitis develops more rapidly and efficiently in brachycephalic breeds, especially in pugs

(Maini et al., 2019). The factors causing pigmentary keratitis are chronic distichiasis, nasal fold trichiasis, medial entropion and macroblepharon (Labelle et al., 2013). In addition, limbal stem cell deficiency or genetic factors may cause pigmentary keratitis in pugs (Maini et al., 2019).

Azoulay (2013) applied cryogen consisting of 95% dimethyl ether, 3% isobutane, and 2% propane to the pigmented area of the cornea under anesthesia in 9 dogs with unilateral or bilateral corneal pigmentation and investigated its effect. The pigmented area mostly healed within 5-15 days after cryosurgery in three dogs, as well as corneal edema, inflammation in the cornea and conjunctiva, and superficial corneal ulceration were recorded as postoperative complications. In conclusion, they emphasized that cryotherapy is a suitable adjunctive treatment method for severe corneal pigmentation and that further studies are needed to evaluate its safety and effectiveness.

Chronic superficial keratitis

Chronic superficial keratitis, also known as Uberreiter Syndrome is a common idiopathic non-ulcerative corneal disease characterized by progressive lymphoplasmacytic infiltration of the anterior corneal stroma (Balicki et al., 2021; Pereira et al., 2022). Although the etiology of chronic superficial keratitis is unknown, immune-mediated etiology is suspected to be the reason for it (Jokinen et al., 2011). Chronic superficial keratitis is most common in German shepherd dogs (82%), although it is also seen in other breeds (Balicki et al., 2021). The main symptom of chronic superficial keratitis is depigmentation of the margin of the membrane nictitans and rarely erosion and thickening of the medial central third eyelid (Balicki, 2012). Pereira et al. (2022) conducted a pilot study on the subconjunctival effect of allogeneic mesenchymal stem cells in eight German shepherd dogs with chronic superficial keratitis. They formed a conventional treatment group with topical 1% prednisolone and an experimental group with allogeneic mesenchymal stem cell transplantation. At the end of the 110 days, they reported no local or systemic side effects in the mesenchymal stem cell group, However, the healing was better in the conventional treatment group than the mesenchymal group. They also emphasized that more studies are needed to evaluate the efficacy of stem cells in ulcerative keratitis treatment.

Neurogenic keratitis

Neurogenic keratitis is a degenerative corneal injury due to damage to the trigeminal innervation. Corneal nerves play an essential role in tear production and maintenance of normal metabolism and function of the ocular surface (Versura et al., 2018). Various ocular and systemic diseases can cause damage to the fifth cranial nerve at different levels, from the trigeminal nucleus to the corneal nerve endings. Common causes include herpetic keratitis, diabetes, chemical or surgical damage and neurosurgical procedures (Bonini et al., 2000; Hsu and Modi, 2015). Among these common causes, viral infections especially herpetic infections cause damage to ganglion cells and ganglion sensory fibers (Hsu and Modi, 2015). Acyclovir, a thymidine nucleoside analog is a widely available antiviral agent used to treat of Herpes simplex virus infections (Williams et al., 2005). Jegou et al. (2014) investigated the effectiveness of superficial keratectomy as a surgical procedure in 36 cats with chronic ulcerative keratitis. They did superficial lamellar keratectomy to 41 eyes in 36 cats with ulcerative keratitis due to feline herpes virus-1, calicivirus, *Chlamidophylae felis* and *psittaci*, and they said that 32.5% of the ulcers were cured. They stated that most patients recovered within two weeks after the operation, and 85% recovered completely within four weeks. They explained that the mean healing time was 22.1 days and excellent corneal transparency was gained in a mean follow-up period of 8.9 months. However, recurrence was seen in nine cases, and superficial keratectomy was performed again. In conclusion, they emphasized that superficial keratectomy is an effective treatment method for treating chronic ulcerative keratitis that is resistant to medical treatment in cats.

Superficial spotted keratitis

Superficial spotted keratitis is an ocular disease characterized by punctate staining on fluorescein staining due to the loss of individual cells of the superficial cell layer of the corneal epithelium, including corneal epithelial defects such as corneal erosion and permanent epithelial defects (Kagawa et al., 2013). In a study conducted by Kim et al. (2023), the effect of 0.03% tacrolimus (an immunosuppressant) on the treatment of a dog with superficial spotted keratitis was investigated by spectral domain-optical coherence tomography (SD-OCT). They emphasized that use of 0.2% cyclosporine for an extended period in the treatment of immune-mediated superficial spotted keratitis, is insufficient for treatment. In conclusion, they said that tacrolimus is effective in providing corneal clarity early and continuous treatment with topical immunosuppressants required, and to obtain useful structural information in immune-mediated keratitis and monitor the response of treatment SD-OCT can be used.

Conclusion

Keratitis is still a common disease in domestic animals. Ocular surface infections, which may develop due to corneal abrasion, cause ulceration and tissue destruction and pose a severe visual threat unless untreated. For this reason, it was concluded that this

review would be helpful to determine the etiology of keratitis and providing effective and updated the treatment options.

References

Austin A, Lietman T, Rose-Nussbaumer J. Update on the management of infectious keratitis. Am Academy Opht 2017; 124(11): 1678-89.

Azoulay T. Adjunctive cryotherapy for pigmentary keratitis in dogs: A study of 16 corneas. Vet Opth 2013; 17(4): 241-9.

Balicki I. Clinical study on the application of tacrolimus and DMSO in the treatment of chronic superficial keratitis in dogs. Pol J Vet Sci 2012; 15(4): 667-76.

Balicki I, Szadkowski M, Balicka A, Zwolska J. Clinical study on the application of dexamethasone and cyclosporine/dimethylsulfoxide combination eye drops in the initial therapy of chronic superficial keratitis in dogs. Pol J Vet Sci 2021; 24(3): 415-23.

Bayley KD, Read RA, Gates MC. Superficial keratectomy as a treatment for non-healing corneal ulceration associated with primary corneal endothelial degeneration. Vet Opth 2018; 22(4): 485-92.

Bello MH, Cuellar-Saenz JA, Rodriguez CN, Cortes-Vecino JA, Navarrete ML, Avila MY, Koudouna E. A pilot study to evaluate genipin in *Staphylococcus aureus* and *Pseudomonas aeruginosa* keratitis models: Modulation of pro-inflammatory cytokines and matrix metalloproteinases. Int J Mol Sci 2023; 24(8): 6904.

Bonini S, Lambiase A, Rama P, Caprioglio G, Aloe L. Topical treatment with nerve growth factor for neurotrophic keratitis. Am Academy Opth 2000; 107 (7): 1347-51.

Bustamente MGM, Good KL, Leonard BC, Hollingsworth SR, Edwards SG, Knickelbein KE, Cooper AE, Thomasy SM, Maggs DJ. Medical management of deep ulcerative keratitis in cats: 13 cases. J Feline Med Surg 2018; 21(4): 387-93.

Cappiello F, Verma S, Lin X, Moreno IY, Casciaro B, Dutta D, McDermott AM, Willcox M, Coulson-Thomas VJ, Mangoni ML. Novel peptides with dual properties for treating *Pseudomonas aeruginosa* keratitis: Antibacterial and corneal wound healing. Biomolecules 2023; 13(7): 1028.

Carion TW, Greenwood M, Ebrahim AS, Jerome A, Suvas S, Gronert K, Berger EA. Immunoregulatory role of 15-lipoxygenase in the pathogenesis of bacterial keratitis. FASEB J 2018; 32(9): 1-13.

Cooper E, Cowmeadow W, Elsheikha HM. Should veterinary practitioners be concerned about *Acanthomoeba* keratitis? Parasitologia 2021; 1(1): 12-9.

Deepika A, Nagaraj P, Kumar VVVA, Rani MU, Kumar KS. Diagnostic and therapeutic management of deep corneal ulcers in dogs. Pharma Inn J 2023; 12(9): 1866-9.

Edelmann ML, Jager M, Espinheira F, Ledbetter EC. In vivo confocal microscopy for detection of subconjunctival *Oncocerca lupi* infection in a dog. Vet Opth 2017; 21(6): 632-37.

Edman AH, Ström L, Ekesten B. Corneal cross-linking (CXL)- A clinical study to evaluate CXL as a treatment in comparison with medical treatment for ulcerative keratitis in horses. Vet Opth 2019; 22(4): 1-11.

Fan W, Han H, Lu Z, Huang Y, Zhang Y, Chen Y, Zhang X, Ji J, Yao K. ε-poly-L-lysine-modified polydopamine nanoparticles for targeted photothermal therapy of drug-resistant bacterial keratitis. Bioeng Transl Med 2023; 8(1): e10380.

Fleiszig SMJ, Evans DJ. The pathogenesis of bacterial keratitis: Studies with *Pseudomonas aeruginosa.* Clin Exp Optom 2002; 85(5): 271-78.

Goldreich JE, Franklin-Guild RJ, Ledbetter EC. Feline bacterial keratitis: Clinical features, bacterial isolates, and in vitro antimicrobial susceptibility patterns. Vet Opht 2020; 23(1): 90-6.

Hall RC, Franzco MJM. Bacterial keratitis in Christchurch, New Zealand, 1997-2001. Clin Exp Opth 2004; 32(5): 478-81.

Hartley C. Treatment of corneal ulcers what are the medical options? J Feline Med Surg 2010; 12(5): 384-97.

Hariprasad SM, Mieler WF, Lin TK, Sponsel WE, Graybill JR. Voriconazole in the treatment of fungal eye infections: A review of current literature. Br J Ophtahalmol 2008; 92(7): 871-78.

Hazlett LD. Corneal response to *Pseudomonas aeruginosa* infection. Prog Ret Eye Res 2004; 23(1): 1-30.

Hindley KE, Groth AD, King M, Graham K, Billson FM. Bacterial isolates, antimicrobial susceptibility, and clinical characteristics of bacterial keratitis in dogs presenting to referral practice in Australia. Vet Opth 2015; 19(5): 1-9.

Hsu HY, Modi D. Etiologies, Quantitative hypoesthesia, and clinical outcomes of neurotrophic keratopathy. Eye & Contact Lens 2015; 41(5): 314-17.

Iwashita H, Wakaiki S, Kazama Y, Saito A. Breed

prevalence of canine ulcerative keratitis according to depth of corneal involvement. Vet Opth 2020; 23(5): 849-55.

Jegou JP, Tromeur F. Superficial keratectomy for chronic corneal ulcers refractory to medical treatment in 36 cats. Vet Opth 2014; 18(4): 335-40.

Jokinen P, Rusanen EM, Kennedy LJ, Lohi H. MHC class II risk haplotype associated with canine chronic superficial keratitis in German shepherd dogs. Vet Immunol Immunopathol 2011; 140(1-2): 37-41.

Kagawa Y, Itoh S, Shinohara H. Investigation of capsaicin-induced superficial punctate keratopathy model due to reduced tear secretion in rats. Curr Eye Res 2013; 38(7): 729-35.

Kecova H, Hlinomazova Z, Rauser P, Necas A. Corneal ınflammatory diseases- infectious keratitis in dogs. Acta Vet Brno 2004; 73(3): 359-63.

Kim H, Jeong Y, Lee E, Seo K, Kang S. Treatment of immüne-mediated keratitis (IMMK) in dogs with immunosuppressants observed with spectral domain optical coherence tomography (SD-OCT). J Vet Sci 2023; 24(5): e66.

Labelle AL, Dresser CB, Hamor RE, Allender MC, Disney JL. Characteristics of, prevalence of, and risk factors for corneal pigmentation (pigmentary keratopathy) in pugs. J Am Vet Med Assoc 2013; 243(5): 667-74.

Larkin DFP, Kilvington S, Dart JKG. Treatment of Acanthamoeba keratitis with polyhexamethylene biguanide. Ophtalmol. 1992; 99(2): 185-91.

Ledbetter EC, Munger RJ, Ring RD, Scarlett JM. Efficacy of two chondroitin sulfate ophthalmic solutions in the therapy of spontaneous chronic corneal epithelial defects and ulcerative keratitis associated with bullous keratopathy in dogs. Vet Opth 2006; 9(2): 77-87.

Ledbetter EC. Applications of in vivo confocal microscopy in the management of infectious keratitis in veterinary ophtalmology. Vet Opth 2021; 25: 5-16.

Ler D, Pidro A, Miokovic AP. Challenging case of treating fungal keratitis. Rom J Opth 2022; 66(1): 69-74.

Leong YY, Tong L. Barrier function in the ocular surface: from conventional paradigms to new opportunities. Ocul Sur 2015; 13(2): 103-9.

Maini S, Everson R, Dawson C, Chang YM, Hartley C, Sanchez RF. Pigmentary keratitis in pugs in the united kingdom: prevalence and associated features. BMC Vet Res 2019; 15: 1-11.

Mezzadri V, Crotti A, Nardi S, Barsotti G. Surgical treatment of canine and feline descemetoceles, deep and perforated corneal ulcers with autologous buccal mucous membrane grafts. Vet Opth 2021; 24(6): 599-609.

Morales JL, Khan NA, Walochnik J. An update on *Acanthomoeba* keratitis: Diagnosis, pathogenesis and treatment. Parasite 2015; 22(10): 1-20.

Murugan N, Malathi J, Umashankar V, Madhavan HN. Unraveling genomic and phenotypic nature of multidrug-resistant (MDR) *Pseudomonas aeruginosa* VRFPA04 isolated from keratitis patient. Microbiol Res 2016; 193: 140-9.

Mustikka MP, Grönthal TSC, Pietila EM. Equine infectious keratitis in Finland: Associated microbial isolates and susceptibility profiles. Vet Opth 2020; 23(1): 148-59.

Packer RMA, Hendricks A, Tivers MS, Burn CC. Impact of facial conformation on canine health: brachycephalic obstructive airway syndrome. PLoS ONE 2015; 10(10): e0137496.

Pereira AL, Bittencourt MKW, Baaros MA, Malago R, Panattoni JFM, Morais BP, Ferreira FM, Vasconcellos JPC. Subconjunctival use of allogeneic mesenchymal stem cells to treat chronic superficial keratitis in German shepherd dogs: Pilot study. Open Vet J 2022; 12(5): 744-53.

Raj N, Vanathi M, Ahmed NH, Gupta N, Lomi N, Tandon R. Recent perspectives in the management of fungal keratitis. J Fungi 2021; 7(11): 907.

Reinhard T, Bauman WB. Anti-infective drug therapy in ophtalmology-part 4: Acanthamoeba keratitis. Klin Monatsbl Augenheilkd 2006; 223(6): 485-92.

Schonheyder HC, Pederson JK, Naeser K. Experience with a broth culture technique for diagnosis of bacterial keratitis. Acta Opth Scan 1997; 75(5): 592-4.

Sebbag L, Sanchez RF. The pandemic of ocular surface disease in brachycephalic dogs: The brachycephalic ocular syndrome. Vet Opht 2022; 26: 1-22.

Shrestha GS, Vijay AK, Stapleton F, Henriquez FL, Carnt N. Understanding clinical and immunological features associated with *Pseudomonas* and *Staphylococcus* keratitis. Contact Lens Ant Eye 2020; 44(1): 3-13.

Shukla PK, Kumar M, Keshava GBS. Mycotic keratitis: An overview of diagnosis and therapy. Mycoses 2008; 51(3): 183-99.

Szentmary N, Shi L, Daas L, Seitz B. Diagnostic and

management approaches for *Acanthamoeba* keratitis. Exp Opinion Orp Drugs 2020;8(7): 227-36.

Versura P, Giannaccare G, Pellegrini M, Sebastiani S, Campos EC. Neurotrophic keratitis: current challenges and future prospects. Eye and Brain 2018; 10: 37-45.

Wei A, Zhao Z, Kong X, Shao T. Comparison of accelerated and standard corneal collagen cross-linking treatments in experimental fungal keratitis for *Aspergillus fumigatus*. Hindawi J Opth 2022; 1-9.

Williams DL, Robinson JC, Lay E, Field H. Efficacy of topical aciclovir for the treatment of feline herpetic keratitis: Results of a prospective clinical trial and data from in vitro investigations. Vet Rec 2005; 157(9): 254-7.

Yang SS, Hung CT, Li SF, Lee HM, Chung YC, Chen HH, Chang SC. Hepatitis B virüs-related mortality in rheumatoid arthritis patients undergoing long-term low-dose glucocorticoid treatment: A population-based study. J Form Med Assoc 2018; 117(7): 566-71.

Zhu B, Zhang L, Yuan K, Huang X, Hu R, Jin X. Neutrophil extracellular traps may have a dual role in *Pseudomonas aeruginosa* keratitis. Eur J Clin Microbiol Infect Dis 2020; 40(1): 169-80.

Yazım Kuralları

1. Erciyes Üniversitesi Veteriner Fakültesi Dergisi'nde veteriner bilimlerini ilgilendiren alanlarda orijinal araştırmalar, olgu sunumları, araştırma notları, kısa bildiri, derleme ve editöre mektup yayımlanır.
2. Dergide yayımlanacak yayınlar için resmi dil Türkçe'dir. İngilizce yazılmış eserler de yayımlanabilir. **İngilizce hazırlanmış makalelerin yayımlanmasına öncelik verilir**.
3. Yayınlar A4 tipi formatta, çift aralık, Arial, 10 punto ve iki yana yaslı olarak yazılmalıdır. Her kenardan 2.5 cm boşluk bırakılarak, sayfaların sağ altına numara verilmelidir. Resimler, şekiller ve kaynaklar dâhil orijinal makaleler ve derlemeler 14, olgu sunumları, araştırma notu ve kısa bildiriler 7 sayfayı geçmemelidir.
4. Yazılar, ercvet@gmail.com adresine gönderilmelidir. Yazışmalar için, makale kapak sayfasında, sorumlu yazarın yazar adı, unvanı, ORCID numarası ve E-posta adresi yazılmalıdır.
5. Daha önce kongrelerde tebliğ edilmiş ve özeti yayımlanmış çalışmalar, bu durum kapak sayfasında belirtilmek üzere kabul edilir.
6. Araştırma herhangi bir kuruluş tarafından desteklenmiş ise kapak sayfasında dipnot olarak belirtilir.
7. Kapak sayfasında Türkçe makale başlığı (koyu ve ilk harfleri büyük), İngilizce başlık (ilk harfler büyük), kısa başlık (40 karakteri geçmemeli ve ilk kelimenin ilk harfi büyük, diğerleri küçük olarak yazılmalıdır), yazar adları (unvansız), çalıştıkları kuruma ait bilgiler (soyadı üstüne numara konulup dipnot olarak) verilmelidir.
8. Türkçe ve İngilizce özetlerin bir sonraki sayfaya yazılması gerekir. Bu sayfa, paragrafsız olarak Türkçe ve İngilizce özetleri (en fazla 250 kelime) içermelidir. Anahtar kelimeler özetlerin altına alfabetik olarak (virgülle ayrılmış şekilde) yazılmalıdır. Yalnızca ilk anahtar kelime büyük harfle başlamalıdır. **Türkçe Bilmeyen yazarlar için Türkçe özet ve anahtar kelimeler yazma zorunluluğu bulunmamaktadır.**
9. Araştırma makalesi; Kapak Sayfası - Özet (Türkçe ve İngilizce) - Anahtar kelimeler (Türkçe ve İngilizce), Giriş, Gereç ve Yöntem, Bulgular, Tartışma ve Sonuç, Teşekkür, Kaynaklar, Tablo ve Şekiller, Sorumlu yazar (Correspondence Author) bölümlerini içerecek şekilde düzenlenmelidir. Metin içindeki tüm başlıklar koyu yazılmalıdır. Metin içinde paragraf girintisi yapılmamalı, devamlı satır numarası verilmelidir.
10. Derlemeler, orijinal olması, en son yenilikleri içermesi, yazarların konu ile doğrudan ilişkili **en az 3 adet** çalışmalarının olması ve bunların derleme içinde kullanılması durumunda yayınlanmak üzere kabul edilebilecektir. Derlemeler kapak sayfası, Özet (Türkçe ve İngilizce), Anahtar kelimeler (Türkçe ve İngilizce), Giriş, konunun kendine ait alt başlıkları, Sonuç, Kaynaklar, Tablo ve Şekiller ve Sorumlu yazar (Correspondence) bölümlerini içerecek şekilde düzenlenmelidir.
11. Olgu Sunumları, Özet (Türkçe ve İngilizce), Anahtar kelimeler (Türkçe ve İngilizce), Giriş, Olgu(lar), Tartışma ve Sonuç, Kaynaklar, Tablo ve Şekiller ve Sorumlu yazar bölümlerini içermelidir.
12. Etik kurul onayı gerektiren çalışmalarda Etik Kurul onayı alınan kurumun adı ve onay numarası, çalışmanın Gereç ve Yöntem kısmında belirtilmelidir.
13. Tablo ve şekillerin metinde geçeceği yer, altı ve üstü çizgili olarak belirtilmelidir.
14. Ondalık ifadelerde nokta kullanılmalıdır.
15. Tür isimleri ve anatomik terimler gibi Latince ifadeler *italik* karakterle yazılmalıdır. Tüm ölçü birimleri SI (*Systeme Internationale*)'e göre verilmelidir.
16. Tablolar kaynaklar kısmından sonra, her bir tablo ayrı sayfada olacak şekilde verilmelidir. Tablo başlıklarının yalnızca ilk harfleri büyük olmalıdır. Tablo başlıkları tablonun üzerinde bulunmalı ve **Tablo 1.** şeklinde numaralandırılmalıdır. Tablolarda iç ve yan kılavuz çizgiler kullanılmamalıdır. Tanımlayıcı bilgi ve açıklamalar tabloların altına yerleştirilmelidir.
17. Her resim, grafik ve çizim; şekil olarak kabul edilip **Şekil 1.** gibi yazılmalı, her biri ayrı sayfada olacak şekilde verilmelidir. Tanımlayıcı bilgi ve açıklamalar şekil ismi ile birlikte şeklin altına yerleştirilmelidir. Resimler 300dpi çözünürlükte olmalıdır.
18. Kaynaklar metin içinde cümle sonunda belirtilmelidir. Yazar soy isimleri ve tarihi yazı içinde her kaynağa ait yayın yılı yazar isminden hemen sonra parantez içinde belirtilmelidir. Kaynak iki isimli ise isimler belirtilmeli (örn; Kaldhone ve Nayak, 2008). Kaynakta yazar sayısı ikiden fazla ise sorumlu yazar "ve ark." şeklinde belirtilmelidir (örn, Kaldhone ve ark., 2008). Eğer kaynak cümlenin başında kullanılıyorsa yazar isimlerinden sonra parantez içinde yayın yılı belirtilmelidir.
19. Kaynaklar yazılırken alfabetik sıraya konulmalı, kaynaklar bölümünde 0.5 cm içeri doğru asılı halde yazılmalıdır. Noktalama işaretlerine örneklerde gösterildiği şekilde dikkat edilmelidir. Dergi kısaltmaları *Index Medicus* ile uyum içerisinde olmalıdır. **Orijinal araştırma makaleleri, derlemeler ve olgu sunumları sırasıyla 30, 45 ve 15'ten fazla kaynak içermemelidir.**
    Kaynaklar;

19.1. Kaynak süreli yayın ise;
Örnek: Kaldhone P, Nayak R, Lynne AM, Dvaid DE, McDermott PF. Characterisation of *Salmonella enterica* serovar Heidelberg from Turkey-associated sources. Appl Environ Microbiol 2008; 74(16): 5038-46.

19.2. Kaynak editörlü kitaptan bir bölüm ise;
Örnek: Hornbeck P. Assay for antibody production. Colign JE. Kruisbeek AM. Marguiles DH. eds. In: Current Protocols in Immunology. New York: Greene Publishing Associates, 1991; pp. 105-32.

19.3. Kaynak kitap ise;
Örnek: Fleiss JL. Statistical Methods for Rates and Proportions. Second Edition. New York: John Wiley and Sons,1981; p.103.

19.4. Kaynak editörlü kitap ise;
Örnek: Balows A, Mousier WJ, Herramafl KL, eds. Manual of Clinical Microbiology. Fifth Edition. Washington DC: IRL Press, 1990; p. 37.

19.5. Kaynak kongre bildirisi ise;
Örnek: Entrala E, Mascarp C. New structural findings in *Cryptosporidium parvum* oocysts. Eighth International Congress of Parasitology (ICOPA VIII). October, 10-14, 1994; İzmir-Türkiye.

19.6. Kaynak tez ise;
Örnek: Erdem V. Köpek göz hastalıklarında klinik oftalmoskopik ve ultrasonografik bulguların değerlendirilmesi, Doktora tezi, Ankara Üniv Sağ Bil Ens, Ankara 2003; s. 1-2.

19.7. Kaynak internette bulunan bir web sitesi ise;
Örnek: TUIK. Hayvancılık İstatistikleri. http://www.tuik.gov.tr/hayvancilik.app/hayvancilik.zul; Accessed Date: 14.03.2010.

20. Eserler dergide yayımlandıktan sonra, bütün sorumluluk sahiplerine aittir.
21. Yazılar gönderilirken son kontrol listesi izlenecek ve "Telif Hakkı Devir Formu" tüm yazarlarca isim sırasına göre imzalanacaktır. **Yazım kurallarına uygun olarak hazırlanmayan yayınlar işleme alınmayacaktır.**

Instructions to Authors

1. The Journal of Faculty of Veterinary Medicine, Erciyes University publishes original research articles, short communications, case reports, letter to editor and original review articles related to the field of Veterinary Medicine.
2. Formal language of manuscripts is Turkish. Manuscripts in English are also accepted. **The publication of English-language manuscripts is given priority.**
3. Publications should be in A4 format, double spacing and Arial 10 font size. With a margin of 2.5 cm from each edge, the page number should be placed at the bottom right of the pages. Original articles and reviews should not exceed 14 pages and case reports, research notes and short papers should not exceed 7 pages including illustrations, figures and references.,
4. Manuscripts should be sent to ercvet@gmail.com. For correspondence, author's name, title, ORCID number, and E-mail address should be written on cover page of the manuscripts.
5. Studies were presented in a meeting and published as an abstract can be published with indication of this status at the bottom of the cover page.
6. Information should be included on any institutions financially contributed to the study as a footnote on the cover page.
7. The cover page should be supplied as a separate page and include: Turkish running title (bold and first letters capital), English title (first letters capital), short title (max 40 characters and first letters of first word is capital, others should be written as small), author(s) names (without titles), author(s) affiliations (Superscript numbers should be given to the surnames of authors as affiliation information).
8. The summaries in Turkish and English should be written on the next page. The title page must contain the Turkish and English summaries (up to 250 words) with no paragraph and not more than five Key words in Turkish and English. Key words must be placed below summary with an alphabetical order (comma delimited). Only the first Key word must start with a capital letter. **For non-Turkish authors, there is no obligation to write summary and keywords in Turkish.**
9. Original research paper must be organized as follows: Cover page, Summary (Turkish and English), Key words (Turkish and English), Introduction, Material and Methods, Results, Discussion and Conclusion, Acknowledgements, References, Tables and Figures and Correspondence. All titles in the text should be written in bold. There should be no paragraph indent in the text and continuous line number should be given.
10. Review articles are considered for publications if they are original and contain recent developments and accepted for publication if the authors have **at least 3 papers** directly related to the subject. Reviews must be organized as follows: Summary (Turkish and English), Key Words (Turkish and English), Introduction, Sub-headings of the subject, Conclusion, Acknowledgements, References, Tables and Figures and Correspondence.
11. Case reports must be organized as follows: Summary (Turkish and English), Key Words (Turkish and English), Introduction, Case(s), Discussion and Conclusion, Acknowledgements, References, Tables and Figures and Correspondence.
12. In the studies requiring the ethics approval, the name and approval number of the institution of the Ethics Committee must be specified in the Materials and Methods section of manuscript.
13. The place where the tables and figures belong in the text should be indicated as underlined and upperlined.
14. Decimal expressions should be used in the dot.
15. Species names and anatomical terms in Latin should be italicized. All measurement specifications must follow the SI (Systeme Internationale) units.
16. Tables must be given in a separate page after the text. First letters of first word should be capital, others should be written as small in the headings of the tables. Title of tables and figures should be numbered in order as **Table 1**. Internal and lateral lines should not be used in the tables. Descriptive information and explanations should be placed below the tables.
17. Each picture, graphic and drawing; should be given as figure and should be written as **Figure 1**. Each one should be on a separate page. Descriptive information and explanations should be placed below the figures. Pictures should be the least 300dpi resolution.
18. References should be specified in the text at the end of the sentence. Author surnames and the date of publication should be specified in parentheses. If the reference has two names, the names should be given after the publication year (eg, Kaldhone and Nayak, 2008). If the reference has more than two names should be given as “et al.,” (eg, Kaldhone et al., 2008). If the source is used at the beginning of the sentence, the year of publication should be specified in parentheses after the names of the authors.
19. References should be placed in alphabetical order and hanging 0.5 cm inwards in the references section. Punctuation should be taken into consideration as shown in the examples, Journal abbreviations must be in line with *Index Medicus*. **The reference list must not contain more than 30, 45, and 15 references for original research articles, reviews and case reports, respectively.**
    References;

19.1. If the reference is a periodical, citation must be done as shown below;
    Example: Kaldhone P, Nayak R, Lynne AM, Dvaid DE, McDermott PF, Logue CM, Foley SL. Characterisation of *Salmonella enterica* serovar Heidelberg from turkey-associated sources. Appl Environ Microbiol 2008; 74(16): 5038-46.

19.2. If the reference is from chapter of a book with an editor, citation must be done as shown below;
    Example: Hornbeck P. Assay for antibody production. Colign JE. Kruisbeek AM. Marguiles DH. eds. In: Current Protocols in Immunology. New York: Greene Publishing Associates, 1991; pp. 105-32.

19.3. If the reference is a book, citation must be done as shown below;
    Example: Fleiss JL. Statistical Methods for Rates and Proportions. Second Edition. New York: John Wiley and Sons,1981; p.103.

19.4. If the reference is whole book with an editor, citation must be as below;
    Example: Balows A, Mousier WJ, Herramafl KL, eds. Manual of Clinical Microbiology. Fifth Edition. Washington DC: IRL Press, 1990; p. 37.

19.5. If the reference is from meeting, citation must be done as shown below;
    Example: Entrala E, Mascarp C. New structural findings in *Cryptosporidium parvum* oocysts. Eighth International Congress of Parasitology (ICOPA VIII). October, 10-14, 1994; İzmir-Türkiye.

19.6. If the reference is from a thesis, citation must be done as shown below;
    Example: Erakinci G. Investigation of Antibodies Against Parasites in Blood Donors. PhD Thesis. Ege Univ. Institute of Health Sciences. Parasitology Program, Izmir-Turkey, 1993.

19.7. The reference is a website on the internet, citation must be done as shown below;
    Example: TUIK. Hayvancılık İstatistikleri. http://www.tuik.gov.tr/hayvancilik.app/hayvancilik.zul; Accessed Date: 14.03.2010.

20. Once the studies one published in the journal, all the responsibility belongs to the authors.
21. The final checklist should be followed when submitting manuscripts and the “Copyright Release Form” must be signed by all authors in order. **Manuscripts which are not prepared in accordance with the “Instructions for authors” will not be processed.**

TELİF HAKKI DEVRİ / COPYRIGHT RELEASE

ERCİYES ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ / JOURNAL OF FACULTY OF VETERINARY MEDICINE, ERCIYES UNIVERSITY

Makale Türü/ Article Type: …/…/ 20..

(…) Araştırma / Research (…) Derleme / Review (…) Kısa Bilimsel Çalışma / Short Communication

(…) Olgu Sunumu / Case Report (…) Editöre Mektup / Letter to Editor

Makale Başlığı/Article

Entitled:……

……

……

Sayın Editör,

Yayınlanması dileğiyle Erciyes Üniversitesi Veteriner Fakültesi Dergisi'ne gönderdiğimiz makalenin yazarları olarak;

1- Derginizde yayımlanmak üzere yollamış olduğumuz makalenin orijinal olduğunu; bilimsel ve etik sorumluluğunun bize ait olduğunu,
2- Makalenin; daha önce yayımlanmadığını, derginizdeki değerlendirme sürecinde başka bir yayın organına yayımlanmak üzere gönderilmediğini ve gönderilmeyeceğini,
3- Makalenin; kişilik ve telif haklarına aykırı kanun dışı maddeler içermediğini,
4- Gerekli görülen düzeltmelerle birlikte her türlü yayın hakkını, yazının yayımlandığı günden itibaren Erciyes Üniversitesi Veteriner Fakültesi Dergisi'ne ait olduğunu kabul ve beyan ederiz.

Dear Editor,

Here we affirm and warranty as the Author(s) of this manuscript submitted to Journal of Faculty of Veterinary Medicine, Erciyes University that;

1- The manuscript /We submitted to the Bulletin is original and responsibilities belong to us ethically and scientifically,
2- The manuscript has not been previously published, being considered for publication by any other journal and will not be submitted to any other journal for such review while under evaluation by this bulletin,
3- The manuscript contains no unlawful statements and does not contain any materials that violate any personal or proprietary rights.
4- The Journal of Faculty of Veterinary Medicine, Erciyes University reserves all rights with due corrections from the date it has been published onwards.

Yazar/ Yazarların Adı
Author's/Authors' Printed Name

1)……İmza/Signature:……..

2)……İmza/Signature:……..…

3)……İmza/Signature:……..

4)……İmza/Signature:……..

5)……İmza/Signature:……..

Not/Note: Formu aşağıdaki adrese,e-mail ya da posta yolu ile gönderiniz veya elden teslim ediniz./ Please send this form to the address below by e-mail, post or deliver personally.

Erciyes Üniversitesi Veteriner Fakültesi Dergisi / Journal of Faculty of Veterinary Medicine, Erciyes University
Erciyes Üniversitesi Veteriner Fakültesi Dergisi Editörlüğü, 38039, Melikgazi-KAYSERİ / TÜRKİYE
Tel/Phone: 0352 339 94 84 Faks/Fax: 0352 337 27 40 e-posta/e-mail: ercvet@gmail.com

SON KONTROL LİSTESİ

Makalenizi göndermeden önce lütfen bu bölümdeki maddelerle karşılaştırma yapınız ve eksiklikleri gideriniz.

- ☐ Eksiksiz doldurulmuş ve bütün yazarlarca imzalanmış **"Telif Hakkı Devri Formu"** (http://ercvet.gmail.com adresinden ulaşabilirsiniz) makale ile birlikte gönderildi.
- ☐ Metnin tamamı çift aralıklı (5 mm) yazıldı (özetler, tablolar, şekil alt yazıları, kaynaklar v.d. dahil).
- ☐ Her bir kenarda 2,5 cm boşluk bırakıldı.
- ☐ Yazılar 10 punto (Arial) ile yazıldı.
- ☐ Satır numaraları verildi.
- ☐ Kapak sayfasında, makalenin başlığı (sadece yazım dilindeki) koyu (bold) yazıldı, kısa başlık eklendi.
- ☐ Kapak sayfasında, yazar isimleri açık olarak yazıldı (kısaltma yok).
- ☐ Kapak sayfasına dipnot (varsa) eklendi.
- ☐ Türkçe başlık yazıldı.
- ☐ Türkçe özet yazıldı.
- ☐ Türkçe anahtar kelimeler (alfabetik sıralı ve ilk kelimenin ilk harfi büyük diğerleri küçük harfle yazıldı) verildi.
- ☐ İngilizce başlık yazıldı.
- ☐ İngilizce özet yazıldı.
- ☐ İngilizce anahtar kelimeler verildi.
- ☐ Şekillerin orijinal halleri eklendi.
- ☐ Metin içinde şekiller ardışık numaralandı.
- ☐ Şekil boyutları min.=8x20; max.=16x20 cm.
- ☐ Metin içinde tablolar ardışık numaralandı.
- ☐ Tablo boyutları min.=8x20 cm; max.=16x20 cm.
- ☐ Şekil ve tabloların metin içinde gelmesi istenilen yer belirtildi.
- ☐ Şekiller listesi ayrı bir sayfa olarak hazırlandı.
- ☐ Her şekil ayrı sayfaya yerleştirildi.
- ☐ Tablolar listesi ayrı bir sayfa olarak hazırlandı.
- ☐ Her tablo ayrı sayfaya yerleştirildi.
- ☐ Kaynaklar yazım kurallarına uygun yazıldı.
- ☐ Yazışma adresi verildi.

FINAL CHECKLIST

Before you submit your work, please take the time to be certain that your paper (and other writings as applicable) is in the correct format and that you have included everything necessary by checking it against this checklist.

- ☐ Copyright Release Form has been enclosed, completed and signed by all authors (http://ercvet.gmail.com).
- ☐ Entire paper has been 5 mm double-spaced (abstract, tables, captions/legends, references).
- ☐ Margins have been 2,5 cm each side.
- ☐ Font size has been 10 pt (Arial).
- ☐ Lines have been numbered.
- ☐ Title of the manuscript has been written bold and short title added on the cover page.
- ☐ Author(s) names have been fully written (not abbreviated) on the cover page.
- ☐ Footnote has been given on the cover page (if necessary)
- ☐ English title has been given.
- ☐ English summary has been given.
- ☐ English keywords have been given alphabetically.
- ☐ Turkish title has been given.
- ☐ Turkish summary has been given.
- ☐ Turkish keywords have been given alphabetically.
- ☐ Orijinal figures have been enclosed.
- ☐ Orijinal figures have been prepared correctly according to instructions.
- ☐ Figures have been referred to consecutively in the paper.
- ☐ Dimensions of figures have been min =8x20 cm; max.=16x20 cm.
- ☐ Tables have been referred to consecutively in the paper.
- ☐ Dimensions of tables have been min =8x20 cm; max.=16x20 cm.
- ☐ Figures and tables have been stated requiring put on the manuscript.
- ☐ Names of figures have been given on a separate page as figure list.
- ☐ Each figure has been given on a separate page.
- ☐ Names of tables have been given in a separate page as table list.
- ☐ Each table has been given on a separate page.
- ☐ References has been typed according to instructions.
- ☐ Corresponding address has been given.