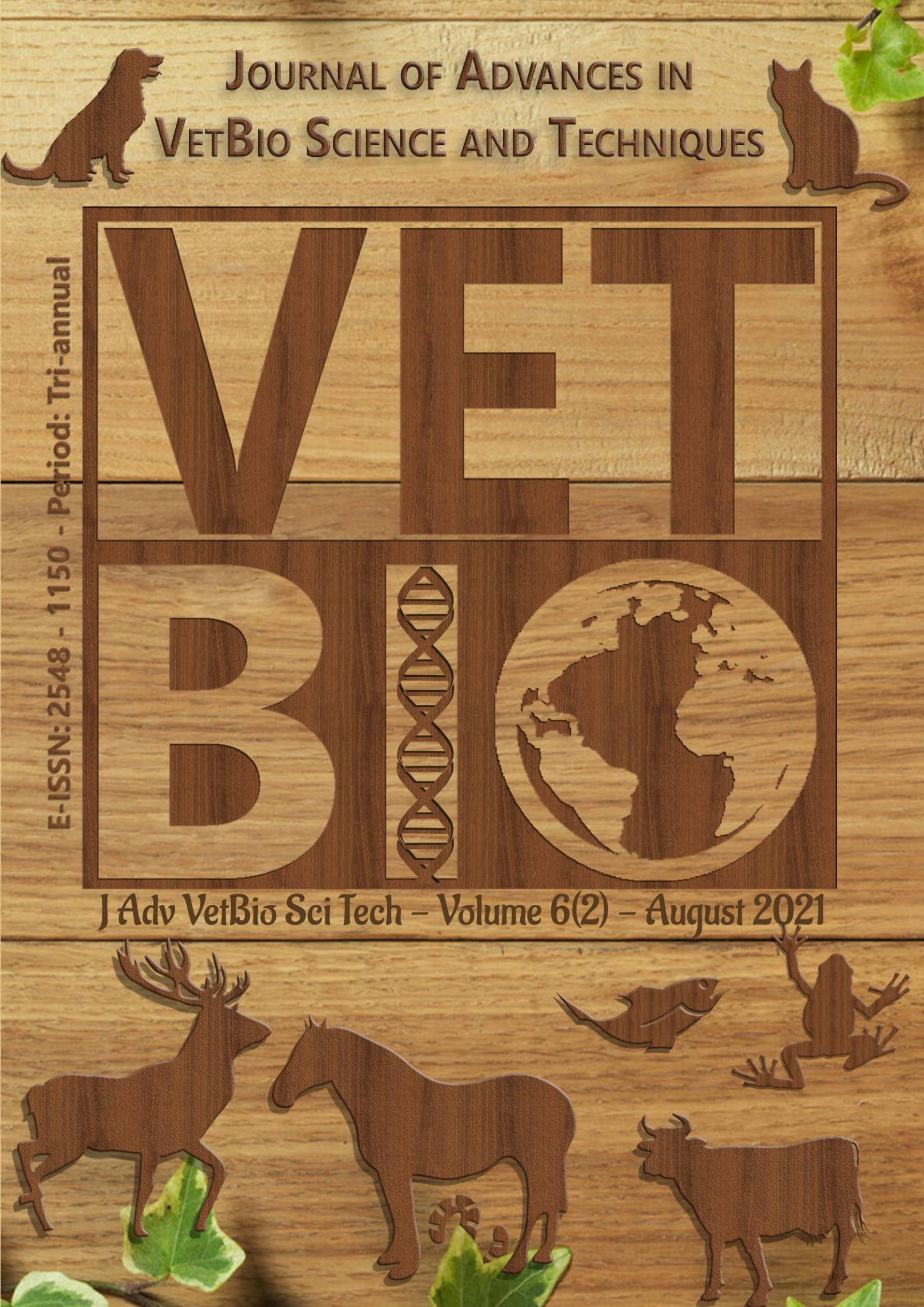
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Köpeklerde canine distemper virüs enfeksiyonunun klinik, patolojik ve real-time RT-PCR ile teşhisi

Clinical, pathological and real-time RT-PCR diagnosis of canine distemper virus infection in dogs

ÖZET

Klinik olarak canine distemper virüs (CDV) teşhisi konulan köpeklerde enfeksiyonun hem sürüntü örneklerinde ve hem de postmortem alınan doku örneklerinde hızlı teşhisine yönelik real-time RT-PCR metodu çalışılarak, viral nükleik asit tespitine dayalı real-time RT-PCR metodu ile histopatolojik yöntemin karşılaştırması amaçlanmıştır. Çalışmanın materyalini, ateş, gözyaşı akıntısı ile solunum, sindirim ve sinir sistemi enfeksiyonlarına ait klinik bulgulara sahip, CDV enfeksiyonu klinik tanısı ile tedaviye başlanmış, ancak tedaviye yanıt vermeden ölen köpeklerden histopatolojik bulgulara göre CDV teşhisi konulmuş köpeklere ait beyin, beyincik, karaciğer, akciğer, dalak, böbrek, taban yastığı ve kan ile tonsil, burun ve gözden alınan sürüntü örnekleri oluşturdu. Histopatolojik incelemelerde CDV enfeksiyonu için karakteristik sayılan intrasitoplazmik ve intranükleer inklüzyon cisimciği belirlenen köpeklerden nekropsi sırasında alınmış sürüntü örneklerinde viral nükleik asit varlığı incelendiğinde tonsillerden alınan sürüntülerin tamamında, burun ve konjunktivadan alınan örneklerin iki tanesinde CDV nükleik asit kopyalarının belirlendiği, kan örneklerinde ise viral nükleik asit belirlenemediği görüldü. Köpeklerde görülen klinik bulgular hastalığı düşündürse de kesin teşhis için kullanılan virolojik ve histopatolojik incelemeler uzun zaman almaktadır. Bu durum viral nükleik asitlerin çoğaltılması esasına dayalı PCR yöntemlerini ön plana çıkarmakta, PCR yöntemleri içerisinde de uygulayıcı hatasını minimize eden, hızlı sonuç veren ve virüs sayısının tahmin edilebildiği real-time RT-PCR uygulamalarının önemini artırmaktadır.

Anahtar Kelimeler: Canine distemper virüs (CDV), histopatoloji, real-time RT-PCR

ABSTRACT

It was aimed to compare the real-time RT-PCR based on nucleic acid detection and the histopathological method for viral nucleic acid detection by using the real-time RT-PCR method for rapid diagnosis of the disease in both swab samples and postmortem tissue samples in dogs with canine distemper virus (CDV) infection. The material of study was composed of brain, cerebellum, liver, lung, spleen, kidney, sole pad, blood and swab taken from tonsil, nose and eye samples of dogs with clinical signs of fever, tear discharge and respiratory, digestive and nervous system infections, who started treatment with a clinical diagnosis of CDV infection, but died without responding to treatment, but were diagnosed with CDV according to histopathological findings. When the presence of viral nucleic acid was examined in swab samples taken during necropsy from dogs with intracytoplasmic and intranuclear inclusion bodies that are considered characteristic for CDV infection in histopathological examinations, it was observed that CDV nucleic acid copies were detected in all swabs taken from tonsils, in two of samples taken from the nose and conjunctiva, and viral nucleic acid copies were not detected in blood samples. Although clinical findings in dogs suggest the disease, virological and histopathological examinations used for definitive diagnosis take a long time. This situation brings PCR methods to the fore based on the reproduction of viral nucleic acids and increases the importance of real-time RT-PCR applications that minimize operator error, give fast results and can predict the number of viruses among PCR methods.

Keywords: Canine distemper virus (CDV), histopathology, real-time RT-PCR

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Research Article

Nevin TUZCU[1a]
Mehmet TUZCU[2b]
Gökhan AKÇAKAVAK[2c]
Onur BAŞBUG[3d]
Mehmet KURUL[4e]

[1]Department of Biochemistry, Faculty of Pharmacy, Selçuk University, Konya, Turkey

[2]Department of Pathology, Faculty of Veterinary Medicine, Selcuk University Konya, Turkey

[3]Departmet of Internal Medicine, Faculty of Veterinary Medicine, Cumhuriyet University, Sivas, Turkey

[4]Kurul Laboratuvar, Konya, Türkiye

ORCİD-

[a]0000-0002-1017-718X

[b]0000-0003-3118-1054

[c]0000-0001-5949-4752

[d]0000-0003-3136-0589

[e]0000-0003-3075-0446

Correspondence

Nevin TUZCU

ntuzcu@hotmail.com

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GİRİŞ

Canine distemper virüs (CDV) enfeksiyonu köpek, kurt, tilki, çakal gibi canidea ailesi, vizon, gelincik, kokarca, samur, sansar, porsuk gibi mustelidae ailesi ve ayı, panda, rakun gibi procyonidae ailesinin üyelerinde görülmektedir (Beineke vd., 2009; Hoskins, 2010; Maclachlan ve Dubovi, 2011). CDV deniz memelilerinde de enfeksiyon oluşturduğu bilinmektedir (Goldstein vd., 2009).

Köpeklerde görülen CDV enfeksiyonunun kuduz hastalığından sonra en yüksek mortalite oranına sahip hastalık olduğu görülmektedir (Deem vd., 2000). Araştırmacılar maternal antikor alamamış yavruların CDV hastalığına karşı en duyarlı grubu oluşturduğunu rapor etmektedirler (Murphy vd., 1999). Virüs vücuda girdikten 1-2 gün sonra lenf yolu ile farengial, tonsillar ve bronşial lenf nodüllerine taşınır. Virüsün primer replikasyonu, solunum yolu lenf dokusu hücrelerinde gerçekleşir (Maclachlan ve Dubovi, 2011). Virüs enfeksiyonu takiben 3. ve 4. günlerde kan yolu ile kemik iliği, timus, servikal ve mezenteriyal lenf nodüllerine, mide ve barsak lamina propriyasındaki makrofajlara taşınır (Murphy vd., 1999; Maclachlan ve Dubovi, 2011). Epiteliyotropik karakterdeki virüs, enfekte lenfositler ve makrofajlar aracılığı ile solunum sistemi, gastro-intestinal sistem, genital sistem, ürogenital sistem ve MSS epitellerine taşınarak generalize enfeksiyon oluşturur (Hoskins, 2010).

CDV enfeksiyonunda karakteristik klinik belirti ateş ve kilo kaybı ile birlikte merkezi sinir sisteminin beyaz maddesinde görülen demiyelinizasyondan kaynaklanan myoklonustur (Amude vd., 2007). Bazı hayvanlarda nadiren idrar kaçırma görülebilir. İyileşen hayvanlarda lakrimal bezlerin keratinizasyonuna bağlı olarak keratokonjuktivitis sicca görülebilir (De Almeida vd., 2009; Hoskins, 2010). CDV enfeksiyonlarında deriye ilişkin en önemli bulgu ayak tabanlarında görülen hiperkeratotik alanlardır (Carvalho vd., 2012; Engelhardt vd., 2005). Karın altında şekillenen püstüler dermatit de dikkat çeken bulgular arasındadır (Jones vd., 1997).

CDV enfeksiyonundan kaynaklı ölen köpeklerde, ilk görülen makroskobik bulgu kaşeksi ve dehidrasyondur (Beineke vd., 2009). CDV enfeksiyonunda, sekonder bakteriyel enfeksiyonların eşlik ettiği durumlarda mukopurulent karakterdeki göz ve burun akıntısı görülmektedir (Demeter vd., 2009). İntersitisyel pnömoni şekillendiği durumlarda, akciğer loblarının kırmızıdan kahverengiye alacalı göründüğü, akciğer dokusunun kıvamının sertleştiği gözlenmektedir (Jones vd., 1997; Kubo vd., 2007).

Morbillivirüs enfeksiyonları için intrasitoplazmik ve intranükleer yerleşimli inklüzyon cisimcikleri karakteristiktir (Sato vd., 2006). Sindirim sistemindeki inklüzyon cisimciklerine mide mukozasının epitelyum hücrelerinde, nadiren de bağırsak epitel hücrelerinde rastlanır. İdrar yolunun transisyonel epitellerinde, böbreklerin renal epitellerinde intrasitoplazmik inklüzyon cisimcikleri görülebilir (Pardo vd., 2005). Merkezi sinir sisteminde demiyelinizasyon alanları belirgindir (Guo ve Lu, 2000; Moro vd., 2003; Vural ve Alcigir, 2010). Merkezi sinir sisteminde inklüzyon cisimciklerine ağırlıklı olarak astrositlerde, mikrogliyalarda ve nöronlarda rastlanır (Pardo vd., 2005).

Bu çalışmanın amacı, real -time RT-PCR tekniği ile CDV enfeksiyonundan ölmüş köpeklerde, virüsün organlara göre dağılımını belirlemek ve hastalığının teşhisinde kullanılabilecek viral nükleik asit tespitine dayalı real-time RT-PCR metodu ile histopatolojik yöntemin karşılaştırmasını yapmaktır.

MATERYAL VE METHOD

Hayvan materyali

Çalışmanın materyalini, Cumhuriyet Üniversitesi Veteriner Fakültesi Hayvan Hastanesi İç Hastalıkları kliniklerine yüksek ateş, göz yaşı akıntısı, solunum, sindirim ve sinir sistemine ait klinik bulgular ile müracaat eden, CDV enfeksiyonu klinik tanısı ile tedaviye başlanan ancak tedavi edilemeyip ölen köpeklerden histopatolojik bulgulara göre CDV enfeksiyonu teşhisi konulmuş 5 erkek 2 dişi, 7 adet 4-8 aylık köpeğe ait beyin, beyincik, karaciğer, akciğer (kranial loblar), dalak, böbrek, taban yastığı ve kan ile tonsil, burun ve gözden alınan sürüntü örnekleri oluşturdu.

Histopatolojik inceleme

Histopatolojik inceleme için organlardan alınan doku örnekleri bir gün boyunca %10 formol içinde tespit edildikten sonra rutin doku takip prosedürleri uygulandı. Elde edilen parafin bloklarından 5 mikron kalınlığında kesitler alınarak Hematoksilen-Eozin ile boyandı. Preparatlar ışık mikroskobu (Olympus BX51, Tokyo, Japonya) altında incelendi.

Real-time RT-PCR analizi

Real-time RT-PCR analizleri için alınan doku örnekleri nekropsi sırasında K3EDTA'lı tüplere ayrı ayrı alınarak standart yöntemlerle virüs RNA izolasyonu işlemi gerçekleştirilerek çalışmanın yapılacağı güne kadar -80°C'de derin dondurucuda saklandı.

RNA izolasyonu robotik olarak RNA izolasyon kiti MagNA Pure Compact Nucleic Acid Isolation Kit I (Roche Kat No: 03730964001) kullanılarak yapıldı.

cDNA Sentezi Transcriptor High Fidelity cDNA Synthesis Kit (Roche Kat No: 05081955001) kullanılarak yapıldı.

Real-time RT-PCR ile virüs tespiti için NP gene spesifik primer çiftleri olan

qCDVF4 (5'TCGGTAATCGAGGATTCGAG AG-3') ve ctctcgaatcctcgattaccga qCDVR3 (5'GCCGAAAGAATATCCCCAGT TAG-3') ve TaqMan probe

3CDV (5'-6-FAMATCTTCGCCAGAATCCT CAGTGCT-MGB-3') dizayn edilerek gerçekleştirildi (Scagliarini vd., 2003).

Oligo tool olarak PCR Mgrade ddH2O 3,0 µl, master olarak Light Cycler 480 Probe Master Kit (Roche Kat No: 04707494001) 10,0 µl, forward primer 0,5 µl, reverse primer 0,5 µl, probe 1,0 µl olarak 15,0 µL total reaksiyon karışımına ulaşıldı ve izole edilen virüs DNA ve kontrol olarak daha önceden CDV enfeksiyonu doğrulanmış pozitif kontrolden 5 µl eklenerek toplam 20 µl materyal hazırlandı. Negatif kontrol template DNA yerine 5 µl su eklenerek hazırlandı.

Reaksiyon karışımı (20µl) plate içine hazırlanarak Light Cycler 480 (Roche Diagnostic, GmBh, Germany) cihazı kullanılarak virüs tespiti gerçekleştirildi.

Pozitif örnekler ve pozitif kontroller için LC640 ve IPC için LC705 kanallarından elde edilen gerçek zamanlı PZR grafikleri ve Crossing Point (CP geçiş noktası) değerleri incelenerek sonuçlar değerlendirildi.

BULGULAR

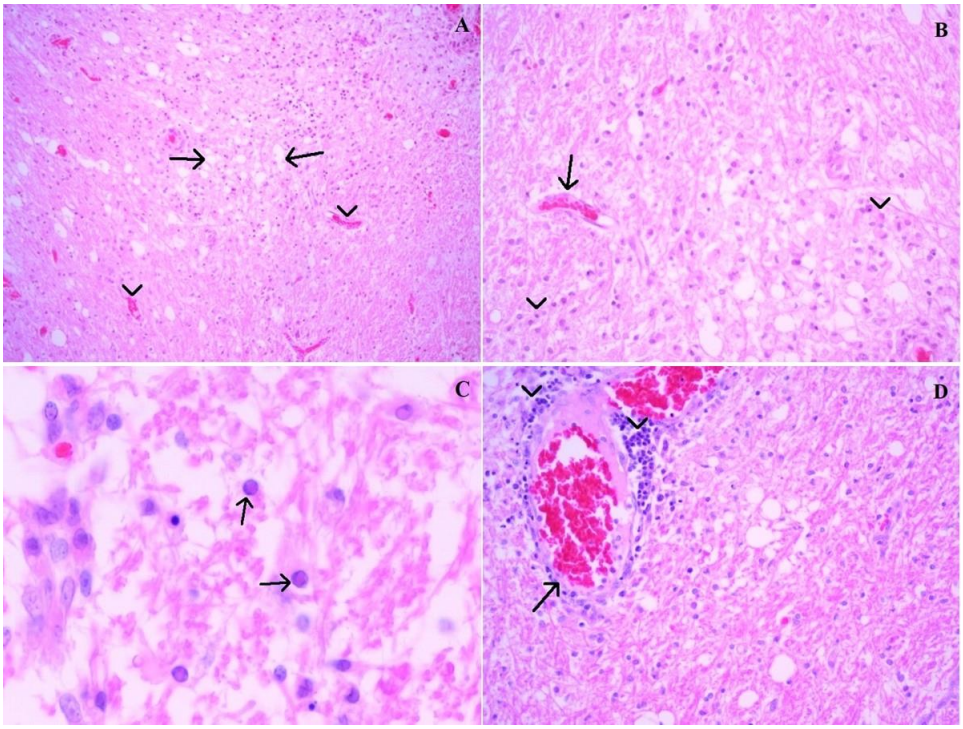
Çalışmanın materyalini oluşturan köpeklerde klinik olarak göz yaşı akıntısı ile birlikte solunum, sinir ve gastrointenstinal sisteme ait bulgulardan bir veya birkaçının bulunduğu belirlendi. En sık görülen klinik bulgunun, göz yaşı akıntısı, ateş, iştahsızlık ve ishal olduğu görüldü. Taban yastıklarında hiperkeratoz (hard pad) iki köpekte belirlendi. Çalışmada belirlenen klinik bulgular tablo 1'de verildi. Nekropsi yapılan köpeklerin tamamında karaciğerin büyümüş olduğu, renginin soluklaştığı tespit edildi. İki köpekte böbreklerin soluk renkli olduğu görüldü. Üç köpekte akciğerin kranial loblarında belirgin koyu kırmızı renkli pnömoni alanları ile amfizemli alanlar dikkati çekti. İki köpekte

endokartta peteşiyel alanlar görüldü. Bütün köpeklerin beyin ve beyinciklerinin ödemli olduğu ve meninkslerin hiperemik olduğu görüldü.

Tablo 1. Çalışmada belirlenen klinik bulgular

Köpek No	Cinsiyet	İştahsızlık	Kaşeksi	Kilo kaybı*	Ateş**	Gözde akıntı	Hiperkeratoz(Hard pad)	Öksürük	Myoklonus	İshal
1	Erkek	+***	-****	-	+	+	-	-	+	-
2	Erkek	+	-	-	+	+	-	+	-	+
3	Dişi	+	+	+	+	+	-	+	-	+
4	Erkek	+	+	+	+	+	+	-	-	+
5	Erkek	+	+	-	+	+	-	+	+	+
6	Erkek	+	-	-	+	+	-	-	-	+
7	Dişi	+	-	-	+	+	+	+	+	+

Mikroskobik olarak CDV ile enfekte köpeklerin serebellumlarında güve yeniği şeklinde görülen yoğun demiyelinizasyon alanlarına rastlandı (Şekil 1.A-B). Marjinal hiperkromazi ve intranükleer inklüzyon cisimciği sıklıkla gözlendi (Şekil 1.C). Demiyelinizasyon alanlarında astrositozis, astrogliozis ve mikroglial hücre proliferasyonları yanında ve gitter hücrelerine de rastlandı (Şekil 1.B). Bütün olgularda perivasküler mononüklear hücre infiltrasyonu görüldü. Beynin mikroskobik incelemesinde bütün köpeklerde, hiperemi, nöronal dejenerasyon ve substantia albada demiyelinizasyon belirlendi (Şekil 1.A-B-D).



Sekil 1. A. Beyincik, demiyelinizasyon alanları (oklar) ve hiperemi (ok başı), H.E, x100. B. Hiperemi (ok) ve gliozis (ok başı), H.E, x200. C. Demiyelinizasyon alanlarının çevresindeki glia hücrelerinde marjinal hiperkromazi ve intranükleer inklüzyon cisimcikleri (oklar), H.E, x1000. D. Hiperemi (ok) ve perivasküler mononükleer hücre infiltrasyonları (ok başı), H.E, x400.

Karaciğerlerin mikroskobik incelenmesinde sentrilobüler bölgedeki hepatositlerde hidropik dejenerasyonun bulunduğu bir olguda dejenerasyona birkaç hepatositin oluşturduğu fokal nekroz alanlarının eşlik ettiği belirlendi. Tüm olgularda kupffer hücrelerinin belirginleştiği üç köpekte ise kupffer hücrelerinde intrasitoplazmik inklüzyonların bulunduğu görüldü.

Böbreklerin mikroskobik muayenesinde tubulus epitellerinde hafiften orta dereceye kadar değişen vakuoler dejenerasyonlar ile üç olguda tubulus epitellerinde intrasitoplazmik inklüzyonlara rastlandı.

Akciğerlerin mikroskobik muayenesinde beş olguda mononükleer hücre artışına bağlı olarak interalveoler septumun kalınlaştığı, üç olguda alveol lümenlerinde ödemle birlikte amfizemli alveoller dikkati çekti. Alveollerde epitelizasyon ile birlikte lümenlerde tek tük makrofajların bulunduğu belirlendi. Bu olgularda bronşial epitellerde intrasitoplazmik inklüzyonların bulunduğu ve bazı bronş lümenlerini dökülmüş epitel hücreleri, nötrofil lökositler ve mukustan oluşan bir kitlenin doldurduğu görüldü.

Dalağın mikroskobik muayenesinde periarterioler lenfoid dokunun azaldığı, retikulum hücrelerinin sayısın arttığı belirlendi. Taban yastıklarının mikroskobik muayenesinde iki köpekte belirgin hiperkeratoz ile akantozis belirlendi. Perivasküler yerleşimli mononükleer hücre infiltrasyonları dikkat çekti.

Histopatolojik incelemelerde CDV enfeksiyonu için karakteristik sayılan intrasitoplazmik ve intranükleer inklüzyon cisimciği belirlenen köpeklerden nekropsi sırasında alınan sürüntü örneklerinde real-time RT-PCR ile belirlenen viral nükleik asit pozitiflikleri tablo 2'de verilmiştir.

Tablo 2. Sürüntü örneklerinde real-time RT-PCR ile belirlenen viral nükleik asit pozitiflikleri

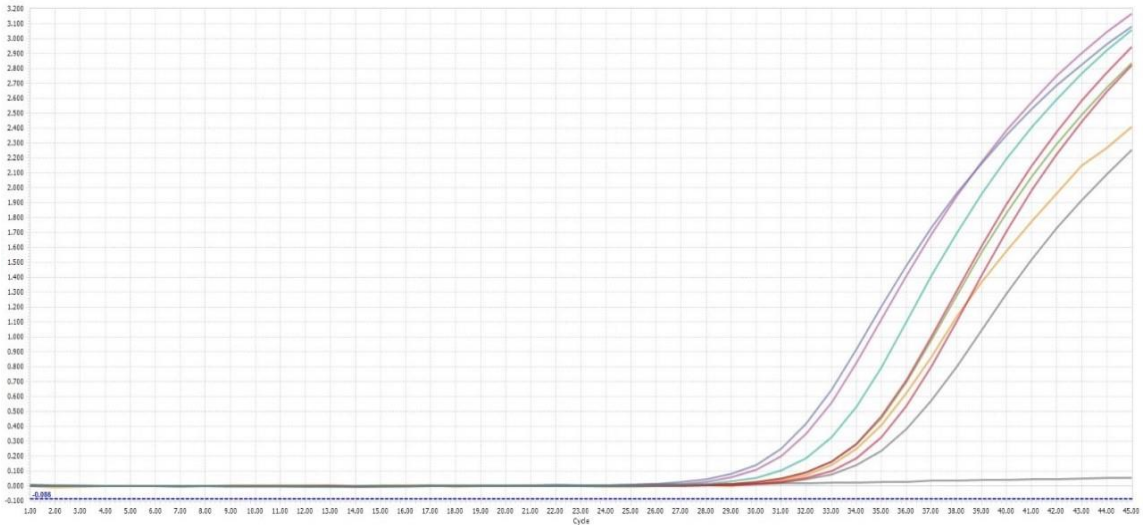
Köpek No	Sürüntü (tonsil)	Sürüntü (burun)	Sürüntü (konjuktiva)	Kan (Aorta)	İnklüzyon Cisimciği
1E	+	-	-	-	+
2E	+	-	-	-	+
3D	+	-	-	-	+
4E	+	+	+	-	+
5E	+	-	-	-	+
6E	+	-	-	-	+
7D	+	+	+	-	+

CDV enfeksiyonlu köpeklerden alınan dokularda real-time RT-PCR ile crossing point değerlerinin akciğer, serebellum ve böbrekte yüksek olduğu belirlendi. Dokularda real-time RT-PCR ile crossing point değerleri ve tablo 3 de verildi.

Tablo 3. Köpeklerden alınan doku örneklerinde CDV'a özgü crossing point değerleri

No	Beyin (kopya/µl)	Dalak (kopya/µl)	Karaciğer (kopya/µl)	Akciğer (kopya/µl)	Serebellum (kopya/µl)	Taban Yastığı (kopya/µl)	Böbrek (kopya/µl)
1E	-*	28	29	22	22	28	26
2E	-	-	29	22	22	28	26
3D	25	28	29	22	-	28	26
4E	22	26	29	22	22	28	26
5E	25	26	29	22	22	29	26
6E	-	26	29	22	22	29	-
7D	23	25	29	22	21	28	26

Çalışmada incelenen köpeklerden bir tanesinde elde edilen crossing point değerleri şekil 2'de görülmektedir.



Şekil 2. Çalışmada incelenen köpeklerden 3 No'lu köpeğe ait örneklerden elde edilen crossing point değerleri.

TARTIŞMA VE SONUÇ

Köpeklerin önemli viral hastalıklarından biri olan CDV enfeksiyonu, aşılamalara rağmen hala ülkemizde ve dünyanın birçok bölgesinde gözlenmekte ve köpeklerin toplu barındırıldığı barınaklarda oldukça fazla sayıda köpek ölümlerine neden olmaktadır (Myers vd., 1997; Leisewitz vd., 2001). Köpeklerde görülen iştahsızlık, kilo kaybı, yüksek ateş, öksürük, göz yaşı akıntısı ve myoklonus gibi klinik bulgular hastalığı düşündürse de kesin teşhis için kullanılan virolojik ve histopatolojik incelemeler uzun zaman almaktadır. Bu durum viral nükleik asitlerin çoğaltılması esasına dayalı PCR yöntemlerini ön plana çıkarmakla birlikte uygulayıcı hatasını minimize eden, hızlı sonuç veren ve virüs sayısın belirlenebildiği real-time RT-PCR uygulamalarının önemini daha da artırmaktadır.

Köpek distemper hastalığı virüsü merkezi sinir sisteminin beyaz ve gri maddesinde multifokal lezyonlara neden olmaktadır (Sumummers ve Appel, 1987). Beyaz maddedeki lezyonlar demiyelinizasyonla karakterizedir (Mutinelli vd., 1989). CDV tarafından şekillendirilen demiyelinizasyon oluşumunda akut ve kronik yangısal safha olmak üzere iki evre belirlenmiştir. Kronik yangısal evredeki demiyelinizasyonun mikroglialarla anti-viral antikorların etkileşimi sonrasında şekillenebileceği, akut demiyelinizasyon evresinin ise direkt virüs etkisiyle oluştuğu ileri sürülmüştür (Higgins vd., 1982). Yapılan bu çalışmada olguların tamamında demiyelinizasyonun görülmüş olması literatüre benzerdir. Ayrıca beyin ve beyincik dokularından real-time RT-PCR ile yapılan hesaplamalarda sırası ile 23-25 ve 21-22 crossing point değerlerinin belirlenmiş olması ve beyincikte belirlenen değerlerin, beyine kıyasla yüksek olması demiyelinizasyonun beyincikte daha şiddetli olmasını açıklamakla birlikte, demiyelinizasyonun virüs etkisiyle oluşmuş olabileceği görüşünü de desteklemektedir.

CDV enfeksiyonlu köpeklerde sıklıkla rapor edilen patolojik akciğer lezyonu intersitisyel pnömonidir (Appel, 1987; Guo ve Lu, 2000; Moro vd., 2003; Pardo vd., 2005; Vural ve Alcigir, 2010). Bu çalışmada da incelenen köpeklerin beşinde intersitisyel pnömoniye

ilgili bulguların görülmüş olması literatür ile örtüşmektedir. Ayrıca akciğerden real-time RT-PCR ile yapılan hesaplamalarda 22 crossing point değerinin belirlenmiş olması CDV enfeksiyonunun patogenezinde akciğerlerin önemli bir yeri olduğunu göstermektedir.

Köpeklerin CDV hastalığının önemli bulgularından bir tanesinin de taban yastıklarının "hard pad" olarak isimlendirilen hiperkeratotik lezyonları olduğu Carvalho vd. (2012) tarafından bildirilmiştir. Bu çalışmada da patolojik olarak iki köpekte hiperkeratoza eşlik eden yangısal değişikliklerin görülmüş olması ve bütün köpeklerden alınan taban yastığı doku örneklerinde 28-29 aralığında crossig point değeri belirlenmiş olması literatürü desteklemektedir.

İntrasitoplazmik ve intranükleer yerleşimli inklüzyon cisimcikleri morbillivirüs enfeksiyonları için karakteristiktir (Sato vd., 2006). Kubo vd. (2007) yaptıkları çalışmada 70 köpeğin akciğer dokusunda, 73 köpeğin idrar kesesi epitelinde, 77 köpeğin dalağında ve 81 köpeğin de lenf nodüllerinde inklüzyon cisimcikleri belirlediklerini rapor etmişlerdir. Appel (1987) intrasitoplazmik ve intranükleer inklüzyon cisimciklerinin hasta köpeklerin beyin ve omuriliğin glia hücrelerinde ve nöronlarında görüldüğünü bildirmiştir. Benzer şekilde CDV enfeksiyonun tanısında oldukça önemli olan intrasitoplazmik ve intranükleer inklüzyon cisimciklerinin lezyonlu dokularda yüksek oranda görüldüğünü belirten çok sayıda kaynak vardır (Beineke vd., 2009; Maxie, 2015). Literatüre benzer şekilde lezyonlu dokularda intrasitoplazmik ve intranükleer inklüzyon cisimcikleri bu çalışmada da köpeklerin tamamında farklı organlarda belirlenmiştir.

Histopatolojik incelemelerde CDV enfeksiyonu için karakteristik sayılan intrasitoplazmik ve intranükleer inklüzyon cisimciği belirlenen köpeklerden nekropsi sırasında alınmış sürüntü örneklerinde viral nükleik asit varlığı incelendiğinde, tonsillerden alınan sürüntülerin tamamında, burun ve konjunktivadan alınan örneklerde aynı olgular olmak üzere iki tanesinde CDV nükleik asit kopyalarının belirlenebildiği, kanda ise belirlenemediği görülmektedir. Bu sonuçlar hastalığın real-time RT-PCR ile hızlı tanısında tonsillerden alınacak sürüntü örneklerinin daha yararlı olacağı şeklinde yorumlanabilir. Ancak bu sonuçların hasta hayvanların antemortem klinik bulgularla desteklenmesi gerekmektedir.

Sonuç olarak bu çalışma ile CDV enfeksiyonlu köpeklerde hastalığın hem sürüntü örneklerinde ve hem de postmortem alınan doku örneklerinde hızlı teşhisine yönelik real-time RT-PCR metodu çalışılarak, viral nükleik asit tespitine dayalı real-time RT- PCR metodu ile histopatolojik yöntemin karşılaştırması yapıldı.

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Dip trolü ile avlanan mullidae familyası (Mullus barbatus ponticus, Mullus surmuletus, Upeneus moluccensis) türlerinin büyüme ve populasyon parametrelerinin tahmini

Estimation of growth and population parameters of mullidae family species (Mullus barbatus pontius, Mullus surmuletus, Upeneus moluccensis) captured by demersal trawl

ÖZET

Türkiye denizlerinde yaşayan barbunya (Mullus barbatus ponticus), tekir (Mullus surmuletus) ve Nil barbunyası (Upeneus moluccensis) balıklarının büyüme ve populasyon parametrelerinin tahmin edildiği araştırma 1-Eylül 2014-15 Nisan 2015 tarihleri arasında yürütülmüştür. Araştırma verileri Karadeniz (Samsun) ve Akdeniz (Mersin) kıyılarında demersal trol avcılığı yapan ticari balıkçı gemilerinden temin edilmiştir. Türlerin ortalama boyları sırasıyla12.67±0.09 cm, 11.55±0.11 cm ve 13.48 ±0.08 cm şeklinde belirlenmiştir. Boy ağırlık ilişkisi denklemleri ise barbunya balığı (Mullus barabatus ponticus) için W=0.0132L2.9066, tekir balığı (Mullus surmuletus) için W=0.0088L3.101 ve Nil barbunyası (Upeneus moluccensis) için W=0.0079L3.002 şeklinde hesaplanmıştır. Büyüme parametreleri sırasıyla asimptotik boyları (L_∞) 25.08 cm, 27.3 cm ve 23.5 cm, brody büyüme katsayısı (K) 0.24, 0.52 ve 0.61 şeklinde ve balığın doğmadan önceki yaşı (t_0) -1.82, -3.65 ve -1.05 olarak tahmin edilmiştir. Türlerin anlık ölüm oranları (Z), doğal ölüm oranları (M) ve balıkçılık ölüm oranları (F) sırasıyla 2,96; 4.57; 2.17, 0.48;0.80;0.85, 2.48; 3.52; 1.32 olarak saptanmıştır. İşletme oranı (E) ise barbunya balığı için 0.83, tekir balığı için 0.82 ve Nil barbunyası için 0.60 şeklinde bulunmuştur. Araştırmada türler için tahmin edilen tüm parametreler daha önceki yapılan çalışmalar ile karşılaştırılmıştır.

Anahtar Kelimeler: Barbunya balığı, Nil barbunyası, tekir balığı, ölüm oranları, işletme oranı

ABSTRACT

Growth and population parameters of red mullet (Mullus barbatus ponticus), surmullet (Mullus surmuletus) and goldband goatfish (Upeneus moluccensis) fishes living in Turkey sea were estimated. The study was carried out between 1 September 2014 and 15 April 2015 date. Study data were obtained from commercial fishing vessels engaged in demersal trawling in the Black Sea (Samsun) and Mediterranean (Mersin) coasts. The average length values of the species were determined as 12.67±0.09 cm, 11.55±0.11 cm and 13.48 ±0.08 cm, in order. The length-weight relationship equations were calculated as W=0.0132L2.9066 for red mullet (Mullus barabatus ponticus), W=0.0088L3.101 for surmullet (Mullus surmuletus) and W=0.0079L3.002 for goldband goatfish (Upeneus moluccensis). Growth parameters were estimated as asymptotic lengths (L_∞) 25.08 cm, 27.3 cm and 23.5 cm, brody growth coefficient (K) 0.24, 0.52 and 0.61 and the age before birth (t_0) of the fish -1.82, -3.65 and -1.05, respectively. Instantaneous death rates (Z), natural mortality (M) and fishery mortality rates (F) of the species were determined as 2,96; 4.57; 2.17, 0.48;0.80;0.85, 2.48; 3.52; 1.32, respectively. The exploitation ratio (E) was found as 0.83 for red mullet, 0.82 for surmullet and 0.60 for goldband goatfish. All the estimated parameters for the species in the study were compared with the previous studies.

Keywords: Red mullet, goldband goatfish, surmullet, mortalities rates, exploitation rate

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Research Article

Süleyman ÖZDEMİR[1a]
Baykal ARIDENİZ[2b]
Zekiye BİRİNCİ ÖZDEMİR[1c]
Uğur ÖZSANDIKÇI[1d]

[1]Sinop University, Fisheries Faculty, Sinop, Turkey

[2]Anamur Joint Health and Security Unit (OSGB), 33630 Anamur, Mersin-Turkey

ORCİD-

[a]0000-0002-2247-0703

[b]0000-0002-6496-6651

[c]0000-0002-7443-1298

[d]0000-0002-7246-5494

Correspondence
Süleyman ÖZDEMİR
suleymanozdemir57@gmail.com

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İRİŞ

Türkiye'de avcılığı yapılan deniz ürünlerinin %80' ni pelajik türler, %20' lik kısmını ise demersal türler oluşturmaktadır. Karadeniz diğer denizlerimiz içerisinde bu üretimdeki en önemli paya sahip olanıdır (TUİK, 2019).

Barbunya (Mullus barbatus ponticus) Karadeniz'in ekonomik demersal balık türlerinden biridir. Türkiye su ürünleri üretiminde mezgit (Merlangius merlangus euxinus) balığı 8940,5 ton ile ilk sırada yer alırken, barbunya balığı 1718,7 ton ile üçüncü sırada avlanan demersal balık türüdür. Tekir (Mullus surmuletus) ve Nil barbunyası (Upeneus moluccensis) Akdeniz'deki önemli demersal türlerin başında gelmektedir (TUİK, 2019). Hem küçük ölçekli balıkçılık hem de endüstriyel balıkçılıkta ekonomik olarak avlanan bu türlerin stokları iklim değişiklikleri, kirlilik, bilinçsiz ve aşırı avcılık gibi nedenlerden dolayı sürekli azalmaktadır. Bu amaçla balık stoklarının düzenli bir şekilde takip edilmesi ve populasyon özelliklerinin iyi bilinmesi, ekosistem yaklaşımlı sürdürülebilir balıkçılığa ve balıkçılık yönetimine katkı sağlayacaktır.

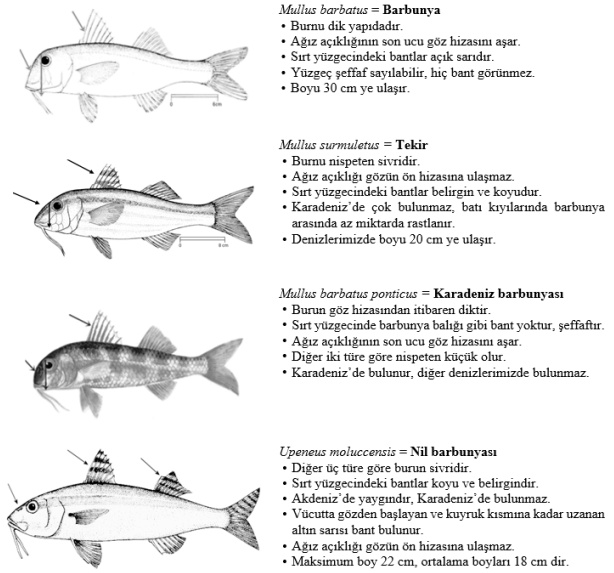
Barbunya türleri üzerine Karadeniz'in güney kıyılarında yapılan çalışmalar, Karadeniz'in kuzey kıyılarıyla, Akdeniz ve Ege Denizinde yapılan çalışmalara göre oldukça azdır. Karadeniz'de barbunya balığı çalışmalarına ait ilk güvenilir bilgiler Slastanenko (1956) ve Akşiray (1987) ye aittir. Slastanenko yaptığı çalışmada türün taksonomisi, dağılımı, beslenmesi ve daha önceki çalışmalara ait bilgiler sunmuştur. Sonraki yıllarda barbunya balığının balıkçılık biyolojisi ve populasyon dinamiği üzerine Karadeniz'de yapılan çalışmaların sayısı artmıştır (Samsun ve Özdamar, 1995; Genç, 2000; Süer, 2008; Aksu vd., 2011, Özdemir ve Erdem, 2011; Aydın ve Karadurmuş, 2013; Samsun, 2017; Erdem, 2018; Yılmaz vd., 2019). Akdeniz ve Ege denizinde barbunya balığı üzerine yapılan çok sayıda araştırma mevcuttur. Ayrıca Kızıldeniz göçmeni olan ve Türkiye'nin Akdeniz kıyılarında yoğun olarak dağılım gösteren Nil barbunyası üzerine yapılan araştırma sayısı da fazladır. (Toğulga ve Mater, 1992; Akyol vd., 2000; Çelik ve Torcu, 2000; Kınacıgil vd., 2001; Çiçek vd., 2002; Kökçü, 2004; Özbilgin vd., 2004; Metin, 2005; İşmen, 2006; Atar ve Mete; 2009; Özvarol vd., 2010; Arslan ve İşmen, 2014; Gündoğdu ve Baylan, 2014; İlkyaz vd., 2018). Ancak yapılan literatür incelemesinde yurt dışındaki araştırma sayısının fazla olmasına rağmen Türkiye denizlerinde tekir balığı üzerine yapılan çalışmaların sınırlı olduğu görülmektedir (Moldur, 1999; İlhan vd., 2009; Üstün, 2010; Arslan ve İşmen, 2013).

Ülkemiz denizlerinde; Mullidae familyasına ait iki cins (Mullus ve Upenus) ve dört türün (M. barbatus, M. surmuletus, U. moluccensis, U. pori) yaşadığı bilinmektedir (Mater vd., 2003; Bat vd., 2008; Atay ve Bekcan, 2000; Gündoğdu ve Baylan, 2016). Hint okyanusu kökenli olan Upeneus, Süveyş kanalının açılmasıyla Kızıldeniz' den Akdeniz'e giriş yapmıştır. Upeneus moluccensis (Blecker, 1855) ve Upeneus pori (Ben-Tuvia ve Golani, 1989) türleri sadece Akdeniz ve Güney Ege'de yayılım gösterirken Mullus surmuletus ve Mullus barbatus barbatus türleri tüm denizlerimize yayılır. Mullus barbatus ponticus ise Karadeniz'e özgü bir alt türdür (Şekil 1).

Barbunya balığının Karadeniz'de yaşayan Mullus barbatus ponticus türünün diğer denizlerimizde yaşayan barbunya türlerinden biyolojik ve morfolojik özellikleri bakımından farklılık gösterdiği çeşitli araştırmalarla tespit edilmiştir (Turan, 2006; Keskin ve Can, 2009; Vasiljeva, 2012; Erdem, 2018).

Barbunya türlerinin avcılığında küçük ölçekli kıyı balıkçılığında fanyalı ve sade uzatma ağları, endüstriyel düzeyde ise dip trol ağları kullanılmaktadır (Özdemir ve Erdem, 2006; Sümer vd., 2007; Özdemir ve Erdem, 2011).

Denizlerimizde uzatma ağlarının ağ göz açıklığına ilişkin herhangi bir yasal düzenleme bulunmazken, dip trol ağlarının torba göz açıklığı Karadeniz için 40 mm, Akdeniz ve Ege Denizi için 44 mm baklava veya 40 mm kare gözlü olarak belirlenmiştir (Anonim, 2020).



Şekil 1. Türkiye denizlerinde yaşayan barbunya balığı türleri ve ayırt edici bazı özellikleri (Fishbase, 2020)

Balıklardaki populasyon parametrelerinin tahmininde balığın boy ve yaş özelliklerinden yararlanılır. Bir populasyonun doğru örneklenerek balıkların boyunun ölçümü kolay ve basit bir yöntemken yaşın belirlenmesi daha hassas ve zor bir yöntemdir. Boy ölçümünde hatanın riski daha az iken yaş okuyan farklı kişiler arasında okumalar değişiklik gösterebilmektedir. Balıkta yaşın büyümesi hatanın gittikçe artmasına neden olmaktadır (Hightower, 1996). Bir balık populasyonunun boy kompozisyonunun doğru belirlenmesi için düzgün örnekleme yanında seçici olmayan avlanma yönteminin kullanılması yeterlidir. Bu şekilde avlanan balıkların tümünü ölçmek ve örnek miktarını artırmak mümkündür (Gulland, 1966).

Bu çalışmada Türkiye denizlerinin önemli barbunya balıklarından olan Barbunya (Mullus barbatus ponticus), Nil barbunyası (Upeneus moluccensis) ve Tekir (Mullus surmuletus) türlerinin boy kompozisyonundan büyüme ve populasyon parametreleri tahmin edilerek yapılan diğer çalışma sonuçları ile karşılaştırılmıştır.

MATERYAL VE METHOD

Araştırmada 2014–2015 avcılık sezonunda Karadeniz'in Samsun ili kıyılarından ticari dip trol gemileri ile avlanan Mullus barbatus ponticus, Doğu Akdeniz'in Mersin ili kıyılarından avlanan Upeneus moluccensis ve Mullus surmuletus türlerine ait veriler değerlendirilmiştir (Şekil 2).

Şekil 2. Barbunya türlerinin avlandığı sahalar

Araştırma verilerinin temin edilmesinde bu bölgelerdeki ticari balıkçı tekneleri ve teknedeki dip trol ağları kullanılmıştır. Kullanılan trol ağlarının torba göz açıklığı Karadeniz için 40 mm baklava, Akdeniz için 44 mm baklava şeklindedir. Araştırmada avlanan ve alt örnekleme ile barbunya için 292 adet, tekir için 278 adet, Nil barbunyası için 425 adet balığın total boy (cm) ve ağırlıkları (g) ölçülmüştür (Şekil 3).

Alınan verilerden türlerin boy kompozisyonu üzerinden yapılan hesaplamalar ile aşağıda verilen büyüme ve populasyon parametreleri tahmin edilmiştir.

L_∞ (Asimptotik boy): Balığın ulaşabileceği maksimum boy

K: Brody büyüme katsayısı

t_0: Balığın doğmadan önceki yaşı

Z: Anlık ölüm katsayısı

A: Gerçek ölüm oranı

S: Yaşama oranı

M: Doğal ölüm oranı

F: Balıkçılık ölüm oranı

E: İşletme (sömürülme) oranı

Şekil 3. Araştırmada avlanan barbunya türlerinin boy ve ağırlık ölçümleri

Von Bertalanffy Büyüme Denklemi (VBBD) parametrelerinden Maksimum (Asimptotik) Boy (L_∞) ve Büyüme Katsayısı (K) nın tahmininde Wetherall vd.,1987 (K) nın tahmininde ise (Pauly, 1980) yöntemi kullanılmıştır (Erkoyuncu, 1995). Anlık Ölüm Katsayısı (Z) nın boy kompozisyonu için Wetherall vd., 1987 yöntemi uygulanmıştır. Doğal Ölüm Katsayısı (M) ise çoklu regresyon kullanılarak bağımsız tahmin edilmiştir (Erkoyuncu, 1995; Sparre ve Venema, 1998). L_∞ ve Z nin boy kompozisyonundan tahmininde; Li(ort)=Σ(Li) / Σf olmak üzere, Li(ort)=a +b Li regresyon denklemi katsayıları kullanılarak L_∞ =a/(1-b) ve Z/K=b/(1-b) eşitliklerinden hesaplanmıştır. Burada kullanılan K değeri daha önceki araştırmalarda çeşitli yöntemlerle ele alınan tür için hesaplanmış K değerlerinin ortalamasıdır.

Doğal ölüm oranı (M) ise; Log M= -0.0066-0.279logL+0.6543logK+0.4634logT formülüyle tahmin edilmiştir. Burada; T: ilgilenilen balık stokunun yaşadığı ortalama su sıcaklığı olup barbunya balığı için +10 °C ve diğer iki tür için +12 °C olarak ölçülmüştür (Pauly, 1980; Sparre ve Venema, 1998). Balıkçılık ölüm oranı F=Z-M ve stoktan yararlanma oranı E=F/Z formülleri kullanılarak hesaplanmıştır.

Araştırmada ayrıca türlerin boy-ağırlık ilişkisi parametreleri de hesaplanmıştır. Hesaplamalarda Pauly (1984) W=aLb denkleminden yararlanılmıştır. Burada W balığın ağırlığını (g) L total boyunu (cm), a ve b regresyon katsayılarını ifade etmektedir. Balıkların içinde bulunduğu şartlara göre vücut şeklini gösteren "b" üssel değerinin izometrik değerden (b=3) farkının önem kontrolünde "t" testi uygulanmıştır.

BULGULAR

Araştırma süresince barbunya balığı familyasına ait toplam 292 adet Mullus barbatus ponticus, 278 adet Mullus surmuletus ve 425 adet Upeneus moluccensis türlerinin boy ve ağırlık ölçümleri yapılmıştır. Türlerin ortalama boyları sırasıyla 12.67±0.09 cm, 11.55±0.11 cm ve 13.48 ±0.08 cm olarak hesaplanmıştır.

Barbunya (Mullus barabatus ponticus), tekir (Mullus surmuletus) ve Nil barbunyası (Upeneus moluccensis) türlerinin boy-ağırlık ilişkisi denklemleri sırasıyla W=0.0132L2.9066, W=0.0088L3.101 ve W=0.0079L3.002 olarak tespit edilmiştir. Balığın içinde bulunduğu koşullara göre şeklini gösteren üssel "b" değeri Barbunya balığı için <3 ten küçük, tekir balığı için >3 ten büyük ve Nil barbunyası için 3 bulunmuştur. Bu sonuçlara göre barbunya balığının negatif (-) allometrik büyüme ($P<0.05$), tekir balığının pozitif allometrik büyüme ($P<0.05$) ve Nil barbunyasının izometrik büyüme ($P>0.05$) gösterdiği belirlenmiştir. Diğer boy ağılık ilişkisi parametresi olan "a" değeri ise sırasıyla 0.0132, 0.0088 ve 0.0079 şeklinde hesaplanmıştır.

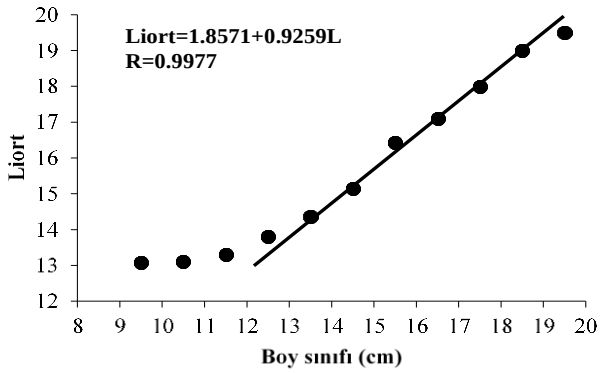
Karadeniz'de avlanan ve rastgele örnekleme sonucunda boyu ölçülen 292 adet barbunya balığının boy sınıflarına göre dağılımı ve bu

veriler kullanılarak hesaplanan % N ve Liort değerleri Tablo 1'de verilmiştir. Hesaplanan boy kompozisyonuna göre en fazla balığın % 29.973 oranla 13.5 cm'lik boy sınıfında avlandığı tespit edilirken, 18.5 cm ve 19.5 cm lik boy sınıfında en düşük avcılığın gerçekleştiği belirlenmiştir.

Tablo 1. Barbunya balığına ait boy kompozisyonu verileri

	Boy sınıfı	N	% N	Liort
	9.5	2	0.685	13.075
	10.5	21	7.192	13.100
	11.5	58	19.863	13.303
	12.5	64	21.918	13.799
Hesaplamada Kullanılan Boylar	13.5	70	23.973	14.364
	14.5	51	17.466	15.149
	15.5	11	3.767	16.423
	16.5	9	3.082	17.1
	17.5	4	1.370	18
	18.5	1	0.342	19
	19.5	1	0.342	19.5

Barbunya balığının boy sınıfı değerleri ile doğrusal dağılım gösteren değerler arasındaki ilişkiye ait regresyon denklemindeki a ve b değerleri (Şekil 4) kullanılarak maksimum boy (L_∞) 25.09 cm olarak tahmin edilmiştir.

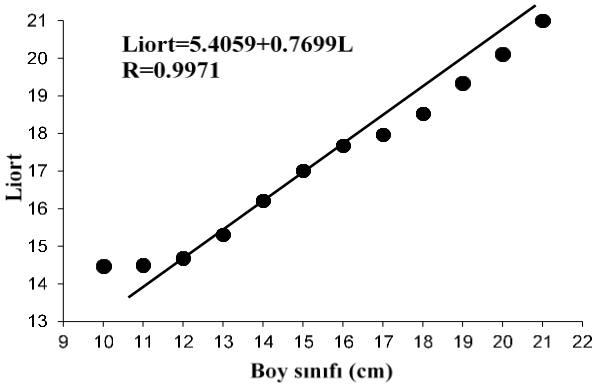


Şekil 4. Barbunya balığına ait L_∞ ve Z/K' nın hesaplanmasında kullanılan grafik

Boy kompozisyonu verilerine göre K=0.32 yıl-1 ve t_0= -1.82 yıl-1 olarak hesaplanmıştır. Anlık ölüm katsayısı Z= 2.96, doğal ölüm katsayısı M= 0.48, balıkçılık ölüm katsayısı F= 2.48 ve işletme oranı E= 0.83 olarak saptanmıştır.

Akdeniz'de avlanan ve boyu ölçülen 425 adet Nil barbunyasının boy sınıflarına göre dağılımı ve hesaplanan % N ve Liort değerleri Tablo 2'de gösterilmiştir. Boy kompozisyonuna göre en fazla balığın % 28.706 oranla 13 cm'lik boy sınıfında yakalandığı belirlenirken, en düşük avcılığın1 adet ile 21 cm lik boy sınıfında gerçekleştiği tespit edilmiştir.

Nil barbunyasının boy sınıfı değerleri ile doğrusal dağılım gösteren değerler arasındaki ilişkiye ait regresyon denklemindeki a ve b değerleri (Şekil 5) kullanılarak maksimum boy (L_∞) 23.49 cm olarak tahmin edilmiştir.



Şekil 5. Nil Barbunyası balığına ait L_∞ ve Z/K' nın hesaplanmasında kullanılan grafik

Tablo 2. Nil Barbunyası balığının boy kompozisyonu verileri

	Boy sınıfı	N	% N	Liort
	10	3	0.706	14.478
	11	20	4.706	14.509
Hesaplamada Kullanılan Boylar	12	77	18.118	14.684
	13	122	28.706	15.320
	14	37	8.706	16.714
	15	11	2.588	17.319
	16	38	8.941	17.484
	17	43	10.118	17.966
	18	45	10.588	18.527
	19	20	4.706	19.345
	20	8	1.882	20.111
	21	1	0.235	21

Bu türün boy kompozisyonu verilerine göre K=0.61 yıl-1 ve t_0=-1.05 yıl-1 olarak hesaplanmıştır. Anlık ölüm katsayısı Z= 2.17, doğal ölüm katsayısı M= 0.85, balıkçılık ölüm katsayısı F= 1.32 ve işletme oranı E= 0.60 olarak tespit edilmiştir.

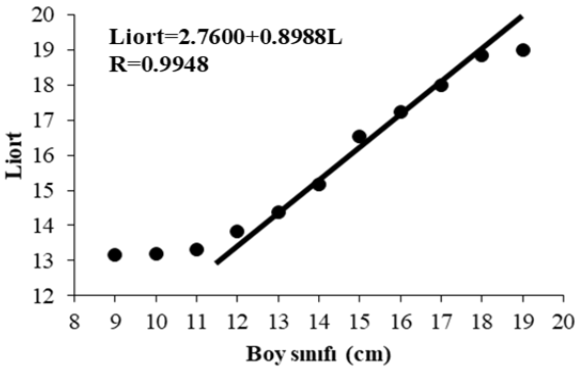
Akdeniz'de avlanan diğer bir tür olan Tekir balığı için 278 adet bireyden boy ölçümü gerçekleştirilmiştir. Tekir balığının boy sınıflarına göre dağılımı ve hesaplanan % N ve Liort değerleri Tablo 3'de gösterilmiştir. Boy kompozisyonuna göre en fazla balığın % 23.288 oranla 13 cm'lik boy sınıfında yakalandığı belirlenirken, en düşük avcılığın 1 adet ile 19 cm lik boy sınıfında gerçekleştiği tespit edilmiştir.

Tablo 3. Tekir balığının boy kompozisyonu verileri

	Boy sınıfı	N	% N	Liort
	9	2	0.685	13.155
	10	14	4.795	13.181
	11	57	19.521	13.324
	12	60	20.548	13.832
Hesaplamada Kullanılan Boylar	**13**	68	23.288	14.383
	14	52	17.808	15.162
	15	10	3.425	16.540
	16	8	2.740	17.233
	17	4	1.370	18
	18	2	0.685	18.833
	19	1	0.342	19

Tekir balığının boy sınıfı değerleri ile doğrusal dağılım gösteren değerler arasındaki ilişkiye ait regresyon denklemindeki a ve b değerleri (Şekil 6) kullanılarak maksimum boy (L_∞) 27.28 cm olarak tahmin edilmiştir. Bu türün boy kompozisyonu verilerine göre K=0.52 yıl-1

ve t_0=-3.65 yıl-1 olarak hesaplanmıştır. Anlık ölüm katsayısı Z= 4.57, doğal ölüm katsayısı M= 0.80, balıkçılık ölüm katsayısı F=3.52 ve işletme oranı E= 0.82 olarak belirlenmiştir.



Şekil 6. Tekir balığına ait L_∞ ve Z/K' nın hesaplanmasında kullanılan grafik

TARTIŞMA

Araştırmada Türkiye denizlerinde ekonomik olarak avlanan üç barbunya türünün bazı populasyon parametreleri tahmin edilmiştir. Bu parametrelerden LWR ilişkisine ait "b" değerleri üç tür için birebirinden farklı bulunmuştur. Karadeniz'de avlanan barbunya balığı için "b" değeri 3 den küçük ve türün büyümesi negatif (-) allometrik tespit edilmiştir. Akdeniz'de avlanan tekir balığı ve Nil barbunyası için "b" değerleri ve türlerin büyümeleri sırasıyla 3'den büyük pozitif (+) allometrik, b=3, izometrik olarak belirlenmiştir.

Türler üzerine yapılan çalışmalarda LWR ilişkisi parametrelerinden "b" değerleri benzerlikler ve farklılıklar göstermektedir. Karadeniz barbunyası için Ak vd. (2009) "b" değerini 3.139 olarak türün büyümesini pozitif allometrik olarak tespit ederken, Aksu vd. (2011) tür için "b" değerini 2.972, Özdemir ve Duyar (2013) 2.982 olarak belirlerken, türün büyümesini de negatif allometrik olarak saptamışlardır.

Türkiye denizlerinde yapılan birçok araştırmada tekir balığı için "b" değeri >3 ve türün büyümesi pozitif allometrik olarak belirlenmiştir (Karakulak vd., 2006; Özaydın vd., 2007; Ceyhan vd., 2009; İlhan vd., 2009; Keskin ve Gaygusuz, 2010). Bununla birlikte bu tür için Bilge vd. (2014) 2.796 ve Bök vd. (2011) 2.717 olarak "b" değerini <3 olarak bildirmiştir.

Nil barbunyası için b değeri Ceyhan vd., (2009) tarafından 2.782 olarak belirlenirken, diğer araştırma sonuçlarında 3.021 ile 3.564 arasında değişen değerlerle 3 den büyük bulunmuştur (Taşkavak ve Bilecenoğlu, 2001; Çiçek vd., 2006; Sangun vd., 2007; Ergüden vd., 2009; Bilge vd., 2014).

Araştırmalarda verinin alınma yöntemi, örnekleme sahası ve dönemindeki değişiklikler balığın yaş, cinsiyet, ağırlık ve boy kompozisyonlarında farklılıklara neden olabilmektedir (Gulland, 1966). Balığın beslenme aktivitesi öncesi veya sonrası yakalanması, midesinin doluluk seviyesi, tükettiği besin miktarı ve gonadlarının safhası balık şeklini ve ağırlığını doğrudan etkileyebilen bu faktörlere bağlı olarak balığın boy uzunluğunun değişimine bir etkisi olmamaktadır (Kohler vd., 1995). Balığın midesinin boş olması, beslenmesi ve doluluk oranının artması, gonadlarının olgunluk seviyesi, bunlara bağlı olarak üreme ve yumurtlama zamanın da "b" değeri üzerinde etkili olabileceği söylenebilir.

Bu araştırmada balıklarda genellikle yaş kompozisyonundan tahmini yapılabilen büyüme parametreleri, ölüm oranları ve işletme oranlarının balığın boy verilerinin de kullanılmasıyla tahmin edilebileceği ortaya konulmuştur. Yaş yöntemindeki hata riski boy kompozisyonunda daha düşük olmaktadır. Yaş okuması yapan kişilerdeki farklı okumalar, boy ölçümündeki standart nedeniyle araştırmacıya ve sonuçların doğruluğuna daha fazla avantaj sağlayabilmektedir. Bu konuda Karadeniz'de ekonomik olarak avcılığı yapılan birçok demersal ve pelajik balık türünün boy verileri kullanılarak birçok populasyon parametresinin tahmin edildiği araştırmalar bulunmaktadır (Özdemir vd., 2006; Özdemir vd., 2009; Özdemir vd., 2018).

Elde edilen sonuçlar diğer araştırma sonuçları ile karşılaştırıldığında benzerlikler ve farklılıklar dikkati çekmektedir. Özellikle Karadeniz barbunyası için L_∞ değerleri Süer, (2008) tarafından yapılan araştırmanın boy kompozisyonu ve Kasapoğlu (2018)'nin yaş kompozisyonu sonuçları ile benzerlik gösterirken, diğer araştırmalarla farklılık göstermektedir (Tablo 4). Nil barbunyası için elde edilen L_∞ değerleri diğer araştırma sonuçları ile yakınlık gösterirken Kökçü (2004) tarafından 27.9 cm olarak bulunan değerden farklı bulunmuştur. Diğer parametreler olan K brody büyüme katsayısı ve t0 değerleri her üç tür üzerine yapılan araştırmalarda benzerlik ve farklılıklar göstermektedir. L_∞ değerlerindeki değişkenlikler ya da yakınlıklar bu iki değeri etkileyeceğinden araştırma sonuçlarına da olduğu gibi yansımaktadır.

Tablo 4. Barbunya balığı üzerine yapılan çalışmalardan elde edilen parametreler

Araştırmacı	Bölge	Yöntem	Parametreler						
			L_∞	K	t_0	Z	M	F	E
Samsun ve Erkoyuncu (1992)	Orta Karadeniz (Samsun)	Yaş	29.49	0.104	-	-	-	-	-
Genç (2000)	Doğu Karadeniz	Yaş	23.83	0.227	-	-	-	-	-
Anonim (2002)	Karadeniz	Boy	24.22	0.218	-	2.30	0.37	1.93	0.84
Süer (2008)	Orta Karadeniz (Samsun)	Boy	25.25	0.154	-	-	-	-	-
		Yaş	39.36	0.082	-	-	-	-	-
Aksu vd. (2010)	Orta Karadeniz (Sinop)	Boy	20.15	0.011	-	1.28	0.68	0.60	0.47
Samsun (2017)	Karadeniz (Sinop)	Boy	-	-	-		0.34	0.40	0.54
Kasapoğlu (2018)	Karadeniz (Hopa-Sinop)	Yaş	24.60	0.220	-1.82	1.66	0.68	0.98	0.59
Mevcut Çalışma	Orta Karadeniz (Samsun)	Boy	25.09	0.237	-1.82	2.96	0.48	2.48	0.83

Türler için hesaplanan ölüm oranları sadece Karadeniz barbunyası için karşılaştırılabilmiştir (Tablo 4). Diğer türlerden tekir balığı için ölüm oranlarının hesaplandığı bir araştırmaya rastlanmamıştır (Tablo 6). Nil barbunyası için ise Dijabali vd. (1993) tarafından yapılan araştırmada hem yaş hem de boy kompozisyonundan hesaplanan doğal ölüm oranı (M) nin tahmini yapılmıştır (Tablo 5). Bu değerler de mevcut araştırma sonuçlarından oldukça farklı bulunmuştur.

Diğer parametreler gibi işletme oranı (E) üzerine sadece Karadeniz barbunyası türü için elde edilmiş değerler bulunmaktadır (Tablo 4). Araştırmada ele alınan türler içeresinde 0.60 işletme oranı ile Nil barbunyası optimum işletme oranına en yakın tür olarak karşımıza çıkmaktadır (Tablo 5). Diğer iki tür için ise durumun endişe verici boyuta ulaştığı görülmektedir (Tablo 4 ve Tablo 6). Karadeniz barbunyası için 2002'deki yüksek olan (E=0.84) işletme oranı 2010 yılında tekrar optimum seviyeye (E=0.47) gerilemiştir. Ancak sonraki yıllarda tekrar bir yükseliş söz konusu olmuştur (Tablo 4). Tekir balığının işletme oranı da Karadeniz barbunyası gibi optimum işletme oranından oldukça yüksek (E=0.82) tahmin edilmiştir. Bu sonuçlara göre Karadeniz barbunyası ve tekir balıkları için aşırı avcılık ve av baskısı söz konusu iken Nil barbunyası için av baskısı oluşmaya başlamasına rağmen diğer iki türe göre durum biraz daha iyi görünmektedir.

Tablo 5. Nil barbunyası balığı üzerine yapılan çalışmalardan elde edilen parametreler

Araştırmacı	Bölge	Yöntem	Parametreler						
			L_∞	K	t_0	Z	M	F	E
Bingel vd. (1993)	Doğu Akdeniz	Yaş	25.6	0.62	-0.27	-	-	-	-
Kaya vd. (1999)	Akdeniz-Güney Ege	Yaş	26.0	0.11	-3.77	-	-	-	-
Kökçü (2004)	Akdeniz (Karataş)	Yaş	27.9	0.11	-4.04	-	-	-	-
Kökçü (2004)	Akdeniz (Karataş)	Boy	25.1	0.09	-4.71	-	-	-	-
Ismen (2005)	Akdeniz (İskenderun)	Yaş	25.2	0.19	-1.00	-	-	-	-
Ismen (2005)	Akdeniz (İskenderun)	Boy	24.3	0.22	-0,92	-	-	-	-
Özvarol vd. (2010)	Akdeniz (Antalya)	Yaş	25.6	0.14	-3.93	-	-	-	-
Mevcut Çalışma	Akdeniz (Mersin)	Boy	23.5	0.61	-1.05	2.17	0.85	1.32	0.60

Tablo 6. Tekir balığı üzerine yapılan çalışmalardan elde edilen parametreler

Araştırmacı	Bölge	Yöntem	Parametreler						
			L_∞	K	t_0	Z	M	F	E
Shanchez vd. (1983)	Akdeniz (Catalan)	Yaş	32.5	0.11	-3.65	-	-	-	-
Morales-Nin, (1986)	Akdeniz (Catalan)	Yaş	30.9	0.11	-3.85	-	-	-	-
Morales-Nin, (1992)	Akdeniz (Majorca)	Yaş	29.8	0.24	-	-	-	-	-
Djabali vd. (1993)	Akdeniz (Sicily)	Boy	27.5	0.45	-	-	0.43	-	-
	Akdeniz (Sicily)	Yaş	27.6	0.27	-	-	0.39	-	-
Machias vd. (1998)	Akdeniz (Girit)	Yaş	35.4	0.23	-1.19	-	-	-	-
Moldur (1999)	Marmara Denizi	Yaş	32.8	0.23	-2.13	-	-	-	-
İlhan vd. (2009)	Ege Denizi (İzmir)	Yaş	27.9	0.19	-1.58	-	-	-	-
Üstün (2010)	Ege Denizi (Edremit)	Yaş	25.1	0.14	-2.48	-	-	-	-
Colloca vd. (2013)	Akdeniz (Balearic)	Yaş	40.1	0.16	-1.88	-	-	-	-
Mevcut Çalışma	**Akdeniz (Mersin)**	**Boy**	**27.3**	**0.52**	**-3.65**	**4.57**	**0.80**	**3.52**	**0.82**

SONUÇ

Sonuç olarak, balıkların yaşam ortamlarındaki fiziksel ve kimyasal birçok etkene bağlı olarak populasyon ve büyüme parametreleri ile ölüm oranlarında bazı değişimler görülmesi normal bir durumdur. Bu farklılıklar türlerin beslenme faaliyetleri, sürü yapısı, bireylerin büyüklüğü, küçüklüğü, cinsiyeti, gonadların durumu, üreme ve yumurtlama zamanı, büyümesi ile türler arası rekabetten kaynaklanabilir (Bagenal ve Tesch, 1978). Ayrıca örneklerin alındığı saha, avcılık zamanı, verinin alındığı av aracının özellikleri ve materyali, verilerin alınma şekli, örnek miktarı,

kullanılan yöntemlerde araştırma sonuçlarında farklılığa neden olabilmektedir (Tiraşin, 1993). Bununla birlikte herhangi bir balık stokunda yaşayan bireylerin büyümesi ile aynı türün başka sahalarda dağılım gösteren farklı populasyonlardaki bireylerinin dağılımı, üremesi, gelişimi ve büyümeleri arasında da bazı farklılıklar meydana gelebilmektedir (Çelik ve Torcu, 2000).

Karadeniz'de barbunya türleri için üç farklı takson bulunmasına rağmen hem küçük ölçekli (uzatma ağları) hem de büyük ölçekli balıkçılıkta (dip trolü ve gırgır) yoğun olarak Mullus barbutus ponticus türünün avlandığı gözlenmektedir (Özdemir ve Erdem, 2011). Tekir olarak bilinen Mullus surmuletus 40 cm' ye kadar ulaşabilirken Mullus barbatus barbatus 30 cm' ye Mullus barbutus ponticus ise 25 cm' ye kadar büyüyebilmektedir. (Ben-Tuvai, 1990). Mullus barbutus ponticus için hesaplanan asimptotik boy ($L\infty$) yaklaşık 30 cm civarındadır (Samsun ve Erkoyuncu, 1992; Samsun ve Özdamar, 1995).

Barbunya balıkları 1 yaşında üremeye başlarlar. Mullus barbatus ponticus türü 11-12 cm boyunda ilk üremesini gerçekleştirirken (Samsun ve Erkoyuncu, 1992; Samsun ve Özdamar, 1995; Genç, 2000; Arslan ve İşmen, 2014; Erdem, 2018; İlkyaz vd., 2018). Tekir balığı 16-18 cm ilk üreme boyuna ulaşmaktadır. Balıkların üremesi Haziran ayından Eylül ayına kadar sürmekte ve Temmuz ayında maksimuma ulaşmaktadır (Ben-Tuvia, 1990).

Bu iki türün ilk üreme boyları dikkate alındığında mevcut tebliğde yasal olarak belirlenen minimum avlama boyları (13 cm ve 11 cm) ile zıtlık gösterdiği görülmektedir. Bu karışıklığın giderilmesi açısından her iki tür için 12 cm lik minimum avlama boyunun dikkate alınmasının faydalı olabileceği düşünülmekledir. Erdem (2018) Karadeniz kıyılarında yaptığı araştırmasında barbunya balığının ilk üreme boyunu 10.88 cm olarak saptamış olup barbunya ve tekir türleri için benzer asgari avlama boyunun (12 cm) uygulanmasını önermektedir.

Tüm bu kriterler, benzerlikler ve farklılıklar dikkate alındığında Türkiye denizlerinde ekonomik olarak küçük ya da büyük ölçekli balıkçılıkta avlanan barbunya türleri üzerinde bir av baskısının olduğu ortadadır. Barbunya balıklarının ilk üreme boyları dikkate alındığında türler için asgari avlanabilir boylarında düzenlemeye gidilmesi şarttır. Ayrıca sadece Ege ve Akdeniz'de değil tüm denizlerimizde dip trol ağlarında kare gözlü ağların veya kare gözlü pencere sistemlerinin uygulamaya başlanması barbunya balığı stoklarının devamlılığı ve Patterson (1992) tarafından önerilen optimum işletme oranına (E=0.5) düşürülmesinde etkili olacaktır.

Türler üzerinde oluşan bu av baskısını sadece trol ağları değil kıyı balıkçığında yoğun olarak kullanılan uzatma ağları da katkıda bulunmaktadır. Bu nedenle Türkiye kıyı balıkçılığında kullanılan uzatma ağlarında minimum ağ gözü açıklığı sınırlaması getirilmesi yerinde bir uygulama olacaktır. Bugün kıyılarımızda dip uzatma ağlarında kullanılan ağ göz açıklığı 28 mm kadar düşmüş durumdadır. Hem hedef türlerin küçük boylarının avcılığı, hem de hedef dışı birçok türün avcılığında artışlar söz konusudur.

Tarım ve Orman Bakanlığının 3/1 Numaralı Ticari Su Ürünleri Avcılığını Düzenleyen Tebliğ'de fanyalı uzatma ağlarında tor ağların minimum 36 mm göz açıklığı gündeme gelmesine karşın 2016 yılında yayınlanan 4/1 numaralı tebliğde ve 2020 yılında yayınlanan 5/1 numaralı tebliğlerde bu öneri yerini alamamıştır. Uzatma ağlarındaki ağ gözü açıklığı en kısa zamanda yeniden gündeme alınarak sade ve fanyalı ağlarda en az 36 mm göz açıklığına sahip tor ağların kullanımına müsaade edilmesi barbunya türlerinin sürdürülebilir avcılığına ve hedef diğer türlerin uygun olmayan boylarının daha az avlanmasına da büyük katkılar sağlayacaktır.

TEŞEKKÜR / AÇIKLAMALAR

Etik beyan: Çalışmada deneysel ya da diğer bilimsel amaçlarla canlı hayvan kullanılmamıştır.

Çıkar çatışması: Yazarlar aralarında bir çıkar çatışması olmadığını beyan etmektedirler.

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Kurt (Canis lupis) nervus auriculopalpebralis'i üzerinde makroanatomik bir çalışma

A macroanatomical study on the wolf (Canis lupis) nervus auriculopalpebralis

ÖZET

Bu çalışmada kurt göz kapakları ve kulağının innervasyonunu sağlayan sinirlerden birisi olan n. auriculopalpebralis'in seyri ve dallanmasının tespiti amaçlanmıştır. Bu amaçla 5 adet kurt kafatası kullanılmıştır. Materyaller öncelikle %10'luk formaldehitte tespit edilmiş ve diseksiyonları yapıldıktan sonra makroanatomik olarak incelenmiştir. Yapılan incelemede n. auriculopalpebralis'in beyinden çıkan cranial sinirlerden birisi olan n. facialis'in (7. çift beyin siniri) bir dalı olduğu tespit edildi. Nervus auriculopalpebralis'in, mandibula'nın caudal kenarı yakınında n. facialis'in dorsal kenarından tek bir dal halinde çıktığı ve daha sonra porus acusticus externus'un cranioventral'inde n. auricularis rostralis ve r. zygomaticus adlı iki dala ayrılarak sonlandığı belirlendi. Sonuç olarak n. auricularis rostralis'in, mm. auriculares rostrales ile çevre bölgenin derisini innerve ederken, r. zygomaticus'un alt ve üst göz kapağı ile m. orbicularis oculi ve m. corrugator supercilii'yi innerve ettiği gözlendi.

Anahtar Kelimeler: Göz, kulak, kurt, nervus auriculopalpebralis

ABSTRACT

In this study, one of the nerves that provide the innervation of the wolf's eyelids and ear, it was aimed to determine the course and branching of the n. auriculopalpebralis. For this purpose, 5 wolf skulls were used. The materials were firstly fixed in 10% formaldehyde and were examined macroanatomically after their dissections. In the study, it was determined that n.auriculopalpebralis is a branch of the cranial brain nerves, n.facialis (7th brain nerve). Nervus auriculopalpebralis, near the caudal edge of the mandible n. facialis emerged as a single branch from its dorsal edge and then in the cranioventral of porus acusticus externus. It was determined that it was divided into two branches called n. auricularis rostralis and r. zygomaticus. As a result of the study, n. auricularis rostrales, while innervating the skin of the surrounding area with the mm.auricularis rostrales, r. zygomaticus lower and upper eyelid, m. orbicularis oculi and m. corrugator supercilii was innervated.

Keywords: Eye, ear, wolf, nervus auriculopalpebralis

GİRİŞ

N. facialis motor, sensitif ve parasempatik liflerden oluşan bir sinirdir. İki kök halinde pons'un caudal kenarında, corpus trapezoideum'un yan kısımlarından çıkar. N. facialis, caudalinde bulunan n. vestibulocochlearis ile birlikte meatus acusticus internus'a girer. Burada n. vestibulocochlearis'ten ayrılır ve area n. facialis'ten canalis facialis'e kadar uzanır.

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Research Article

Zekeriya ÖZÜDOĞRU[1a]
Ramazan İLGÜN[1b]

[1] Department of Anatomy, Faculty of Veterinary Medicine, Aksaray University, Aksaray, Turkey

ORCİD-
[a]0000-0002-0789-3628
[b]0000-0003-0150-3008

Correspondence
Ramazan İLGÜN
rilgun1980@hotmail.com

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Sinir, kanal içinde ganglion geniculi adını alan bir sinir düğümü oluşturduktan sonra canalis facialis'in dış deliği olan foramen stylomastoideum ile kafatasını terk eder (Dursun., 2000).

N. facialis, motor lifleri ile yüz kaslarını, m. stylohyoideus ve m. digastricus'u, sensitif lifler ile dilin ön 1/3'ünün tat duyusunu, dış kulak yolu ve yumuşak damağın duyusunu alır. Parasempatik lifler ise glandula mandibularis, glandula sublingualis, glandula lacrimalis ve glandula nasalis'i innerve eder (Tecirlioğlu., 1983).

N. facialis, foramen stylomasteudeum'dan çıktıktan sonra ramus auricularis internus, n. auricularis caudalis, ramus digastricus, n. auriculopalpebralis, rami buccales, ramus marginalis mandibulae ve ramus colli adlı dalları vererek sonlanır (Nickel vd., 1984).

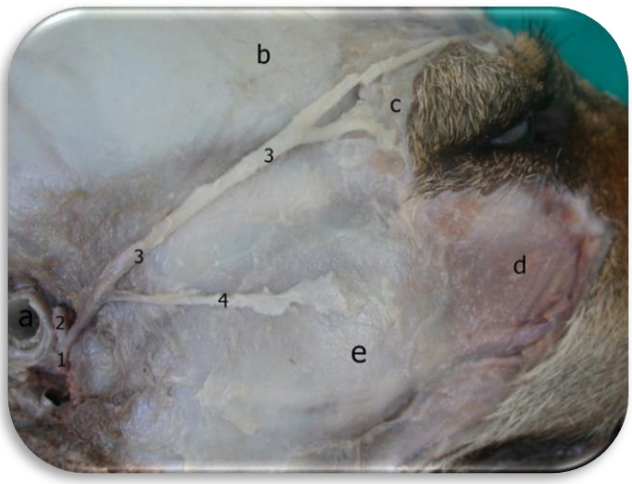
N. auriculopalpebralis, mandibula'nın caudal kenarı yakınında n. facialis'ten çıkar. Bu sinirden rami auriculares rostrales adıyla ayrılan dallar mm. auriculares rostrales ile m. paratidoauricularis'i, ramus zygomaticus dalı ise m. orbicularis oculi'yi innerve eder (McClure, 1964; Getty ve Godinho, 1975b; Teke, 1999; Özüdoğru ve Aksoy, 2005).

MATERYAL VE METOT

Çalışmada avcılar tarafından avlanan 5 adet kurt kafatası kullanılmıştır. Kafatasları 24 saat %10'luk formaldehitte tespit edildikten sonra n. auriculopalpebralis'in diseksiyonu yapıldı. Diseke edilen bölge fotoğraf makinesiyle (canon,500) fotoğraflanarak çalışmada sunuldu. İsimlendirme için kullanılan terimlerde Nomina Anatomica Veterinaria (ICVGAN, 2017) esas alındı.

BULGULAR

Nervus auriculopalpebralis'in, mandibula'nın caudal kenarı yakınında n. facialis'in dorsal kenarından tek bir dal halinde çıktığı tespit edildi. Sinir'in daha sonra porus acusticus externus'un cranioventral'inde n. auricularis rostralis ve r. zygomaticus adlı iki dala ayrılarak sonlandığı belirlendi. N. auricularis rostralis'in, porus acusticus externus'un cranialinden dorsale doğru seyrine devam ettiği ve öncelikle gl. parotis içinde daha sonra da m. temporalis üzerinde dorsocaudal yönde ilerleyerek mm. auricularis rostralis ile çevre bölgenin derisini innerve ettiği gözlendi. R. zygomaticus'un, n. auriculopalpabralis'ten orijin aldıktan sonra gl. parotis içinde m. masseter'in caudalinde craniodorsal yönde ilerlediği ve daha sonra arcus zygomaticus'u çaprazlayarak m. temporalis üzerinde seyrederek gözün lateral açısına ulaşmadan hemen önce iki dala ayrıldığı belirlendi. Dallardan birisi gözün angulus oculi'sinde m.orbicularis oculu ve alt göz kapağına dağılarak sonlandığı saptandı. Diğer dalın ise gözün dorsal kısmına seyrine devam ederek üst göz kapağını ve m.corrugator supercilii'yi innerve ettiği tespit edildi. Ramus zygomaticus'un orijininden 2-3 cm sonra yanak derisine dağılan ve m.masseter'i innerve eden r. muscularis isimli bir dal verdiği belirlendi (Resim 1).



Resim 1. Nervus auriculopalpebralis'in orijin ve dallanması 1. N. auriculopalpebralis, 2. n. auricularis rostralis, 3. r. zygomaticus, 4. r. muscularis, a. porus acusticus externus, b. m. tempotalis, c. m. orbicularis oculi, d. m. buccinator, e. m. masseter.

TARTIŞMA

Yapılan çalışmada n. auriculopalpebralis'in, mandibula'nın caudal kenarı yakınında n. facialis'in dorsal kenarından tek bir dal halinde çıktığı tespit edildi. Bu bulgumuz kedide (McClure vd., 1973; Getty ve Godinho, 1975a; Teke, 1999; Özüdoğru ve Aksoy, 2005) ve köpekte (McClure 1964; Getty ve Godinho 1975b) n. auriculopalpebralis'in r. buccalis dorsalis ile birlikte n. facialis'ten çıktığı bildirimleriyle aynıdır.

Tecirlioğlu, (1983), n. auriculopalpebralis'in, mandibula'nın caudal kenarı yakınında n. facialis'in dorsal kenarından çıktıktan sonra rami auriculares rostrales isimli çok sayıda kolu ile plexus auricularis rostralis'in oluşumuna katıldığı ve mm. auriculares rostrales ile m. parotidoauricularis'i innerve ettikten sonra sinirin devam eden kolunun m. sucutularis'e bir dal verdikten sonra, n. temporalis adını aldığı ve n. frontalis'in kolları ile plexus temporalis'i oluşturduğu bildirimiyle uyuşmamaktadır.

Dyce vd., (1996), kedi ve köpekte n. facialis'in bir dalı olan n. auriculopalpebralis'in arcus zygomaticus üzerinden geçtiğini ve gözün muayenesi sırasında göz kırpmayı (musculus orbicularis oculi) ortadan kaldırmak için bloke edildiğini bildirmişlerdir.

Godinho ve Getty, (1975a), kedide, McClure vd., (1964) köpekte n. auriculopalpebralis'in, n. auricularis rostralis ve r. zygomaticus adlı iki dala ayrılarak sonlandığını; Özüdoğru ve Aksoy, (2005), Van kedisinde r. zygomaticus'un m. orbicularis oculi ve iki göz kapağını innerve ettiğini rapor etmişlerdir.

Çalışmada, r. zygomaticus'un, n. auriculopalpabralis'ten orijin aldıktan sonra m. temporalis üzerinde seyrederek gözün lateral açısına ulaşmadan hemen önce iki dala ayrıldığı ve bu dallardan birisinin m. orbicularis oculi ve alt göz kapağını, diğer dalın ise gözün dorsal kısmına doğru seyrine devam ederek üst göz kapağını ve m. corrugator supercilii'yi innerve ettiği tespit edilmiştir.

Ramus zygomaticus'un orijininden 2-3 cm sonra yanak derisine dağılan ve m. masseter'i innerve eden r. muscularis isimli bir dal verdiği belirlenmiştir.

SONUÇ

Sonuç olarak, göz kapaklarının ve kulağın rostral kısmının innervasyonunu sağlayan n. auriculopalpabralis'in orijin ve seyrinin bilinmesi göz ve kulakta yapılacak olan operasyonlarda sinirin bloke edilmesi açısından çok önemlidir. Bu çalışma ile yabani bir hayvan olan kurt'un bu bölgedeki sinirlerinin anestezisi için gerekli bilgiler ortaya konulmaya çalışılmıştır.

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Çıkar çatışması: Yazarlar aralarında herhangi bir çıkar çatışması bulunmadığını beyan etmektedir.

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Chestnut Honey as a Complementary Medicine: Determination of Antibacterial Activity, Heavy Metal Residue and Health Risk Assessment

ABSTRACT

In this study, residue levels for heavy metal content and antimicrobial effects of 27 chestnut honey samples obtained from member beekeepers of Yalova Bee Association are examined. Honey samples are digested under high temperature and pressure in a microwave oven and Arsenic (As), Lead (Pb), Cadmium (Cd), Mercury (Hg) limits are determined using ICP-OES. Using Agar gel diffusion method, 19 of the honey samples' antibacterial activity was tested on strains of Staphylococcus aureus (ATCC 25923), Escherichia coli (ATCC 25922), E. coli O 157: H7, Bacillus cereus (ATCC 6633), Salmonella Typhimirium (NCTC 12416) and Listeria monocytogenes (ATCC 7644). As, Pb, Cd and Hg residue levels in all honey samples were below the designated limits by Turkish Food Codex Regulation. The non-carcinogenic health risk associated with heavy metal contamination in honey is found to be very low. None of the 19 honey samples show antibacterial effect on L. monocytogenes strains. At 100% honey concentration, in 18 out of 19 honey samples, antibacterial effect is observed on strains of S. Typhimirium, E. coli O 157: H7 while all 19 samples show antibacterial activity on strains of E. coli and B. cereus. The antibacterial activity on these bacteria is recorded to be maintained at 50% honey concentration. At 25% honey concentration, the antibacterial effect show a decline in most of the honey samples.

Keywords: Chestnut honey, antibacterial activity, heavy metals, health risk

INTRODUCTION

Chestnut honey produced in Turkey is rich in bioactive substances (Küçük et al., 2007). Chestnut honey forests are located across Black Sea, Marmara and Aegean regions (Sarıkaya et al., 2009). With its dark color and strong aromatic flavor chestnut honey is rich in glucose oxidase, catalase, ascorbic acid, carotenoid derivatives, organic acids, Maillard reaction products, amino acids and proteins, phenolic compounds, flavonoids and minerals such as tannin, potassium, magnesium, manganese and barium (Dağ et al., 2017). In different studies conducted on Anatolian chestnut honey, significant antioxidant compounds with high antibacterial activity are reported (Ayvaz et al., 2018; Güneş et al, 2017; Sarıkaya et al., 2019).

Honeybees are exposed to environmental pollutant as they gather plant nectar, pollen and water (De Oliveira et al. 2016). Bee products are important not only for their rich nutritional value but also together with the bees for being significant agents in monitoring environmental pollution (Yarsan et al., 2007).

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Research Article

Mesut Ertan GÜNEŞ

Department of Food Processing, Vocational School of Technical Science, Bursa Uludağ University Turkey

ORCİD-
0000-0002-9347-8307

Correspondence
Mesut Ertan GÜNEŞ
egunes@uludag.edu.tr

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Metals such as arsenic, cadmium, lead and mercury may be present in soil, water and atmosphere at certain levels. In addition, metals may contaminate food products as a result of pollution by human activities such as vehicle exhaust, industrial waste, or irresponsible farming. Individuals may be exposed to such heavy metals through the environment, consumption of contaminated products and/or water. Accumulation of metals in the body may, in time, lead to several health issues and risks (Tolgahan et al., 2018).

In this study, residue levels for heavy metal content and antimicrobial effects of 27 chestnut honey samples obtained in 2014 from member beekeepers of Yalova Bee Association are examined.

MATERIAL and METHOD

Honey samples

Locations where honey samples are collected: 27 raw honey samples are collected from 7 different spots where the bee yards of Yalova Beekeepers Association members are commonly located. The collected samples are stored in glass jars in a dark and cool environment. All 27 samples are assessed in terms of their heavy metal profiles. Although no crystallization was expected for chestnut honey, 8 of the 27 samples were crystallized, therefore antibacterial activity is tested for the 19 non-crystallized honey samples.

Antimicrobial analysis

In this study, antibacterial activity is assessed on strains of S. aureus (ATCC 25923), E. coli (ATCC 25922), E. coli O 157: H7, B.cereus (ATCC 6633), S.Typhimirium (NCTC 12416) and L.monocytogenes (ATCC 7644). Agar-well diffusion method was used to assess antibacterial activity (Perez et al.,1990). Standard strains enriched in Mueller Hinton Broth are adjusted to 0.5 McFarland and cultivated into 1 ml. Mueller Hinton agar (Oxoid, CM 0337). Samples with a concentration of 100%, 50%(v/v) and 25% (v/v) are prepared from all 19 honey samples. Parallel honey samples of 50 µl at each concentration level are added into holes with a diameter of 6 mm on the Petri dish. Plates are incubated at 37°C for 24 hours. The average of the inhibition zone diameters formed at the end of the incubation period is recorded. Zone diameters of <5.5 – 9 mm, 9 – 12 mm, 12 – 15 mm and > 15 mm indicate very low, low, average and high inhibition respectively (Küçük et al., 2007). All analyses are carried out two times as parallel processes.

Heavy metal analysis

Crystallized honey samples are kept in a water bath at 70ºC to dissolve. 10 ml HNO3 (65%, Merck) and 1 ml H2O2 (30%, Merck) are added on samples of 0.5 gr and resulting samples are burned in a microwave oven (Berghof MWS-3+ / Germany) with gradually increasing temperature (160-200 C/max. 53 min). At the end of the combustion, 5 ml of ultrapure water (18.3 MΩ ultrapure water - Human, Korea) was added to each sample. Then, the concentrations of Pb, Cd, As and Hg are determined with a calibrated Plasma Optical Emission Spectrometer (ICP-OES-Perkin Elmer Optima 2100DV). Heavy metal analyses are conducted in the Food Research laboratory of Turkish Ministry of Agriculture and Forestry. The settings of the ICP-OES device are presented in Table 1.

Potential health risk assessment of the honey samples

Health risk of honey samples collected from Yalova province is determined by Target Hazard Quotient (THQ) analysis. THQ indicates the ratio between heavy metal exposure and the reference exposure dose. The potential health risk resulting from a daily consumption of 3.3 g (FAO, 2017) of honey is calculated for a seventy-year-old adult weighing seventy kg (Magna et al., 2018).

Tablo 1. Operating Conditions of the ICP-OES (Perkin Elmer Optima 2100 DV).

Parameter	
RF power	1450 watts
Plasma gas flow	15 L/min
Auxillary gas flow	0.2 L/min
Nebulizer Flow	0.65 L/min
Sample Pump Flow	1.5 mL/min
Plasma Viewing	Dual View
Elements	Wavelenth (nm)
Pb	220.353
Cd	228.802
As	193.696
Hg	253.652

$$THQ = \left[\frac{(EFr\,.EDtot\,.IFR\,.C)}{(RfDo\,.\,BWa\,.ATn)}\right].10^{-3}$$

Formula variables are as follows; EFr, indicates the exposure frequency (365 days/year); EDtot, the exposure duration (70 year); IFR, the food ingestion rate (g day-1); C, the concentration (µg g-1); RfDo, the oral reference dose (µg g-1 day-1) (Cd: s 0.001; Pb: 0.004; As: 0.003, day-1, Hg: 0,0016 mg/kg-1/day declared by US-EPA, 2009; BWa, the adult body weight (70 kg) and ATn, is the average time for non-carcinogens which is equal to EFr multiplied by EDtot.

RESULTS

Antibacterial effects on selected Gr (-) and Gr (+) bacteria are presented in Table 2. None of the 19 honey samples show antibacterial effect on strains of L. monocytogenes.

Tablo 2. Heavy Metal Levels of Honey Samples (µg/kg), Health Risk due to intake (THQ) and tolerable daily intake limits (% PTDI)

µg/kg	Pb (ppb)	Cd (ppb)	As (ppb)	Hg (ppb)
Lowest	0.096	0.076	0.032	0.240
Highest	39.067	1.198	0.950	44.546
Average	21.301	1.183	0.516	21.490
Measurement uncertainty	±0.004	±0.001	±0.001	±0.003
THQ	$2.5x10^{-4}$	$5.56x\ 10^{-5}$	$8.1x10^{-5}$	$6.45x10^{-4}$
PTDI (%)	4.4	15.4	4.71	4.4

One hundred percent concentrations of all honey samples are recorded to have high antibacterial activity with wide zone diameters on strains of S. Typhimirium. Except for one sample, at 100% concentration level, all samples show antibacterial effect on strains of E. coli and B. cereus. Nevertheless, only 15 samples form antibacterial effect zones on S. aureus strains. The antibacterial activity of samples at 50% concentration level is found to be in line with that of 100% concentration. However, partial decreases in activity zones are recorded. When the concentration of honey samples is reduced to 25%, a decline and even loss of antibacterial activity has been detected in many samples. At this concentration level, 8 samples are observed

to show no antibacterial effect on S. Typhimirium strains while only 10 samples achieve to form activity zones on S. aureus and E. coli O 157:H7 strains. The number of samples with activity zones at 25% concentration decreases to 7 samples on E. coli (ATCC 25922) and 3 samples on B.cereus strains. At all concentration levels, the highest antibacterial activity is detected on strains of S. Typhimirium. This is followed by E. coli O 157: H7 and E. coli, S. aureus and B. cereus, respectively.

Levels of Lead (Pb), Cadmium (Cd), Arsenic (As), Mercury (Hg) in honey samples are determined by ICP-MS. Heavy metal contents of samples are recorded as x̄ 21.301348 ppb for Pb, x̄ 1.18381 ppb for Cd, x̄ 21.4909 ppb for Hg and x̄ 0.51687 ppb for As (Table 3).

When the potential health risk associated with heavy metal residue levels in Yalova chestnut honey samples are examined, exposure values are determined to be 2.5 x 10 -4 for Pb, 5.5 x 10-5 for Cd, 8.1 x 10 -5 for As and 6.45 x 10-4 for Hg. It is noted that the recorded levels are below the designated reference limits of daily consumption. (US-EPA 2009) Daily tolerable level of heavy metal intake (PTDI) determined by European Food Safety Authority (EFSA-2012) and FAO/WHO Expert Committee on Food Additives (JEFCA 2011) are 0.357; 2.1; 0.185; 0.357 mg/kg/day for Cd, As, Hg, Pb respectively. Taking these limits in consideration, a 70 kg adult's 3.3 gr of Yalova honey consumption per day corresponds to %15.4 Cd > % 4.7 As >%4.4 Pb >% 4.3 Hg of the daily tolerable intake limits per each heavy metal. (Table 3)

Tablo 3. Heavy Metal Profiles of Yalova Honey and Samples in Previous Literature (mg/kg)

Origin of Samples	Pb (ppm)	Cd (ppm)	As (ppm)	Hg (ppm)	Literature
Yalova	0.021	0.0011	0.0005	0.0214	Our values
Anatolia	0.0003	0.000038			Altunatmaz, 2018
Tokat	0.0018	0.000041			Arıkan, 2010
Elazığ	0,409	337.9	4.8		Aygün, 2020
Bingöl	Nd	nd	nd	nd	Bengü, 2020
Trakya	Bdl- 0,48	Bdl-0,01			Citak, 2012
Mid-Anatolia	1.5	0.242			Leblebici, 2008
China	0.033	0.001	0.013	0.0016	Mei-ru, 2013
Sicily	0.170	0.0153			Naccari, 2014
Karadeniz	0.04- 0.73	nd- 1.28			Nisbet, 2013
Muğla	0.005-0.016	Bdl			Silici, 2013
Comercial	1.101	0.343		0.618	Şireli,2015
Anatolian	0.008-0.106	0.9-17.9			Tuzen, 2007
Hatay	0.39	0.03			Yücel, 2013

DISCUSSION

It was first suggested in 1892 by Van Ketel et al. (1892) that honey may have antibacterial effects (Dustman, 1979). The antibacterial activity of honey was first described in 1937, by Duo and Dziao (1937) and the substance in honey enabling for bacteria inhibition was named as inhibin. It is later argued that the antibacterial efficacy may also be influenced by the floral sources from which the honey is obtained (Molan et al., 1988).

In a study focusing on honey samples from Turkey, the antimicrobial activity of chestnut, rhododendron, and multifloral honey samples is examined against the strains of Helicobacter pylori, S. aureus, B. subtilis, C. tropicalis and C. albicans and the highest antimicrobial activity is

recorded for chestnut honey (Küçük et al., 2007). Results of the studies referring to a correlation between the antibacterial activity of chestnut honey and its antioxidant capacity have shown that the types of honey with high antioxidant content, especially chestnut honey, also exhibit significantly high antibacterial efficacy (Ayvaz et al., 2018; Güneş et al., 2016; Yıldız et al., 2013).

S.aureus is the most vulnerable and sensitive bacteria against the antibacterial effect of honey. Güneş et. al. (2017) recorded antibacterial activity of chestnut honey at 100%, 50% and 25% concentrations on strains of S.aureus. In this study, strong antibacterial activity against S. aureus strains is determined for chestnut honey samples from Yalova province.

Ayvaz et al. (2018) reported that the most sensitive microorganisms to chestnut honey samples from the Black Sea region are C. albicans, S. aureus and E. coli, while the most resistant microorganism is B. subtilis. The antibacterial activity of honey samples from Yalova province on E. coli strains is in line with this study. Combarros-Fuertes et al. (2020) investigated the chestnut, avocado and polyfloral honey samples' mechanism of action on E. coli and S. aureus strains and pointed out that all honey types caused significant alterations on the cell structures of E. coli and S. aureus. The study especially highlighted the remarkable metabolic deformations caused on S. aureus by honey samples' antibacterial effect.

In their studies on chestnut honey and its antibacterial and antifungal activity on strains of E. coli ATCC (25922), Yersinia pseudotuberculosis ATCC (911), Pseudomonas aeruginosa ATCC (43288), S. aureus, ATCC (25923), Enterococcus faecalis ATCC (29212), B. cereus (709 Roma), Mycobacterium smegmatis (ATCC607), Candida albicans ATCC (60.193) and Saccharomyces cerevisiae (RSKK 251), Kolaylı et al. (2016) recorded the highest inhibition levels against S. aureus, E. faecalis, Y. pseudotuberculosis and E. coli strains.

The antimicrobial properties of honey are due to its acidity, osmolarity, H_2O_2, low water activity (aW), aromatic acids, different chemical compounds; phenolics and flavonoids content (Bogdanov et al., 2008). Chestnut honey is rich in terms of mineral content and phenolic compounds (Güneş et al., 2017; Kolaylı et al., 2016). The highest antibacterial activity for honey samples from Yalova province is observed on S.Typhimirium followed by E. coli O 157:H7 and E.coli, S.aureus and B.cereus, respectively. Studies have shown that chestnut honey accelerates the treatment of wound infections and, especially, shortens the healing process of open wounds caused by diabetes (McLoone et al., 2020). The therapeutic efficacy of Yalova chestnut honey should be considered and utilized for the same purpose.

Heavy metals such as lead, cadmium, arsenic and mercury are taken into the human body through food products, drinking water and respiration. Such metals cannot be excreted through the body's excretory system and accumulate in the soft tissues of the body. Accumulation of each metal at levels above the effective doses for children and adults may cause organ poisoning, and several other severe health issues (i.e. thyroid, neurological, autism and infertility) and even death (Özpolat, 2016).

Yalova province is surrounded by the regions where heavy industrial organizations operate. In the study, pollution levels and potential health risks are revealed by the heavy metal profiles of honey samples obtained from the region.

The average lead level of 27 honey samples analyzed in the study is found to be 0.021 ppm. This is below the levels recorded in all previous studies mentioned above. (Table 4) However, the lead levels of honey samples from Yalova are found to be above the levels recorded by Tuzen et al. (2007) for honey samples from different cities in Anatolia, Arıkan (2010) for samples

from Tokat province, Silici and et al. (2013) for samples from Muğla, Nisbet et al. (2013) for samples from Black Sea region, Altunalmaz et al. (2018) for samples from Anatolia and Bengü (2011) for samples from Bingöl province.

Tablo 4. Antimicrobial Activity of Chestnut Honey from Yalova

Con. v/v (%)	Bacteria	Honey Samples (Inhibition zone diameter mm)																		
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
100%	*B.cereus*	27	28	27	31	26	29	26	-	28	29	30	32	28	27	27	29	26	30	33
	S.aureus	20	-	15	21	18	-	-	-	14	15	17	15	13	15	15	19	18	14	14
	E.coli	29	29	28	33	32	30	-	30	32	31	35	33	30	31	34	30	31	31	32
	E.coli O157 H:7	29	33	30	-	32	28	29	26	30	30	30	32	31	30	31	31	30	32	32
	S. Typhimirium	30	32	30	38	30	28	36	41	32	34	32	36	36	40	35	32	28	38	32
50%	*B.cereus*	23	24	19	-	20	23	23	-	24	26	24	25	26	22	19	25	-	24	24
	S.aureus	12	-	13	14	14	-	-	-	11	13	14	11	-	14	-	14	15	12	11
	E.coli	25	24	26	28	20	24	-	22	27	23	29	24	24	22	27	28	29	24	27
	E.coli O157 H:7	26	23	27	-	25	27	27	22	27	25	23	28	28	27	28	23	26	29	26
	S. Typhimirium	23	18	22	26	20	19	18	21	23	20	20	18	23	24	19	22	22	20	27
25%	*B.cereus*	15	-	-	-	14	-	-	-	-	-	-	20	-	-	-	-	-	-	-
	S.aureus	10	-	10	12	11	-	-	-	-	11	11	-	-	-	-	12	11	9	-
	E.coli	22	-	-	24	-	19		20	-	19	-	19	-	-	-	-	-	19	-
	E.coli O157 H:7	22	-	24	-	-	22	-	18	23	-	16	-	-	15	-	-	19	23	-
	S. Typhimirium	18	17	19	22	18	-	-	19	22	-	17	-	-	-	-	18	18	16	-

The average cadmium level of honey samples from Yalova is recorded to be 0.0011 ppm. Considering the previous literature, a higher cadmium level is observed compared to honey samples collected from Tokat (Arıkan, 2010), Trakya (sunflower honey samples) (Çıtak, 2012), Muğla (Silici, 2013), Anatolian region Altunazmaz, 2018) and Bingöl (Bengü, 2020). Compared to the previously published studies, lower cadmium levels are observed. In a study conducted by Naccari et al.(2016) on 3 different monofloral honey samples in Sicily, higher lead and cadmium levels were recorded compared to the present stud

The average arsenic residue levels for honey samples collected from Yalova is determined to be 0,0005 ppm. This is a lower level compared to honey samples for Elazığ (Aygün, 2020).

In the study, the average mercury residue level of 30 chestnut honey samples is found to be 0.0214 ppm. The observed level is much lower compared to the residue limits recorded by Şireli et al. (2015) for commercial honey samples. The mercury residue level obtained by Ru et al. (2013) in their study on 48 monofloral honey samples is lower than that of Yalova honey. The cadmium values recorded in the same study are similar to the findings of this study while the lead and arsenic levels are higher than the values observed for chestnut honey samples from Yalova.

There is no designated maximum limit on the heavy metal contamination of honey in the Turkish Food Codex Regulation (TGK-29.12.2011). It is concluded that the target hazard quotient and health risk factors associated with lead, cadmium, arsenic and mercury residues in honey calculated for a seventy-year-old adult, weighing seventy kg with a 3.3 g of daily honey consumption, is below the reference limits set by US-EPA (US-EPA 2009). When the level of heavy metal residues in the honey samples are evaluated for consumer health and in

terms of tolerable weekly intake limits (EFSA 2012, JEFCA 2011), it is determined that a 70 kg adult's 3.3 gr of Yalova honey consumption per day corresponds to 15.4%, 4.7%, 4.4%, 4.3% of the daily tolerable intake limit for Cd, As, Pb, Hg, respectively. Adjusting for individual differences, potential health risk factors associated with a daily consumption of even 15 g of honey collected from Yalova province would be below the designated reference limits.

CONCLUSION

In conclusion, pure chestnut honey has a potential to be a complementary product in protective and therapeutic applications for human health. It has been concluded that honey samples obtained from Yalova region is a valuable source in terms of their potential use in treatments of both traditional and complementary medicine (apitherapy). period.

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Relationship between weight, volume and specific gravity of goose eggs before incubation

Research Article

Osman KARABULUT

Department of Biometrics, Faculty of Veterinary Medicine, Aksaray University, Aksaray, Turkey

ORCİD-
0000-0002-8142-2365

Correspondence
Osman KARABULUT
okarabulut02@gmail.com

ABSTRACT

In this study, the relationships between the weight, volume and specific gravity of eggs are revealed by calculating egg's volume and specific gravity depending on the weight of the egg, breadth and length, which are only three variables, with mathematical equations. Eggs to three goose genotypes, Grey China, Linda and Native geese from Aksaray region taken from six breeders were used. Eggs (n=481) were weighed and Length, Breadth measurements made with a precision of 0.01 mm. Average weights in genotypes from large to small in Linda, Native and Chinese geese were detected as; 165.9, 137.2 and 131.1 g, respectively, and the average egg volume was found as; 152.0, 126.0 and 120.3 cm^3, respectively. Specific gravity from large to small in Linda, China and Native has occurred as; 1.092, 1.091 and 1.089 g/cm^3. Egg volume was calculated with mathematical equations and results were close to real, and accordingly the calculated Specific gravity was also detected to be realistic. These results were obtained easily by only three variables, egg weight, and Length and Breadth values. This method can pave the way to obtain a lot of information about the egg with Specific gravity.

Keywords: Correlation, goose, egg, regression, specific gravity

INTRODUCTION

Breeding process of geese in an incubator involves extra procedures compared to other types of poultry. At the end of this difficult procedure followed, the desired level of incubation efficiency cannot be achieved (Golz, 1991; Tilki and İnal, 2004a; Karabulut et al., 2017). Hatching efficiency has a high variation in geese, about 50-90%, and this variation is difficult to explain (Tilki and İnal, 2004a; Ramos et al., 1989; Toth, 1991; Özbey, 1998; Arslan and Saatçi, 2003; Önk and Kırmızıbayrak, 2019).

Offspring rate is the most important reason that reduces hatching efficiency, which can be understood by the incubation of the egg. With an appropriate flock management, the fertility rate can be increased and the decline in hatchery yield can be prevented. The handicap of goose breeders is that they try to use all of the goose eggs in incubation because they are few and valuable. This causes exceeding the external quality standards in the hatching eggs, and therefore reduces the hatching efficiency (Karabulut et al., 2017; Önk and Kırmızıbayrak, 2019). Incorrect storage of eggs is an important factor that reduces hatchability. After the egg is laid, there is a decrease in its weight as it constantly loses water, and this decrease occurs rapidly if the appropriate humidity and heat environment is not provided.

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This loss of weight causes a series of reactions that cause the egg to spoil and the efficiency of the hatch is reduced (Rahn, 1977; Carey, 1994; Erensayın, 2000).

The presence of weight loss depending on the time of the egg refers to the variability of the egg over time. In other words, when talking about egg weight, weighing at a certain time should come to mind.

The average weight of goose eggs has a wide variation between approximately 123 and 185 g (Paganelli et al., 1974; Saatçi et al., 2002; Saatçi et al., 2005; Rabsztyn et al., 2010; Nedomová and Buchar, 2014; Hamadani et al., 2016; Kumbar et al., 2016; Ahmad et al., 2017). These differences may result from genetic factors such as race and age, as well as environmental factors such as care, feeding, breeding system and region differences. Arroyo (1990) used goose eggs of Africa, China, Toulouse and Embden in his study and reported that the egg weight was 170, 173, 168 and 183 g, respectively. Tilki and İnal (2004b) found that the average egg weight was 154.9 g in their study on France White geese and increased significantly after 2 years of age. Hamadani (2016) found the average egg weight as 136.65 g in Kashmir Weight geese; Razmaitė (2013) found the average egg weight as 123.40 g in the first spawning season and 186.69 g in the second season.

Due to the variability of egg weight, it alone is not enough to evaluate the egg. But it can gain a powerful meaning with specific gravity. Because, when looking at the specific gravity formula, it suggests that there is a positive correlation between weight and specific gravity. Normally the specific gravity corresponds to an approximately constant value, but as the weight decreases, the specific gravity is expected to decrease as well.

It is the safest method to calculate specific gravity according to Archimedes principle. Saatçi et al. (2002) calculated the specific gravity of domestic goose eggs as 1.113 in Kars region, Arroyo (1990) calculated it as 1.079, 1.08, 1.08 and 1.079 in Africa, China, Toulouse and Embden races respectively, Hamadani et al. (2016) detected it as 1.09/cm^3 g in Kashmir Anz geese in Kashmir Valley, India. It is seen that specific gravity values differ in studies conducted on some poultry animals other than geese. Çetingül and İnal (2009) detected the specific gravity in Nick Brown breed chickens as approximately 1.082 g/cm^3. Nemati (2020) found the specific gravity as 1.07 g/cm^3 in Japanese quails.

Volume is a variable used in the calculation of specific gravity, and the volume of the egg does not differ over time. In other words, while the specific gravity and egg weight show an alternation, the volume is constant. Calculating the volume according to Archimedes' principle is the safest. The average volume of goose eggs has a wide variation between approximately 125 and 175 cm^3 (Paganelli et al., 1974; Saatçi et al., 2002; Nedomová and Buchar, 2014; Kumbar et al., 2016). There is a positive correlation between volume and weight. Paganelli et al. (1974) calculated the volume of Embden Geese as 158.74 cm^3 depending on the egg weight.

When examining specific gravity, calculating volume or specific gravity according to Archimedes principle is both difficult and time consuming. It requires a series of difficult operations performed by immersion in water (Hamilton, 1982). Instead, it will be more accurate to use mathematical formulas that calculate the volume with a little error. Since the volume of the egg does not change, it will be appropriate to examine the weight and specific gravity of the eggs together with the volume.

Recently, photographic imaging techniques have been used frequently to obtain information about egg morphology (Lawrence et al., 2006; Sunardi, 2017; Adegbenjo et al., 2020). Length and breadth are frequently used variables in these techniques (Nedomová and Buchar, 2014; Narushin, 2005). Depending on these two

variables or egg weight, many other variables of the egg can be predicted (Çopur-Akpınar et al., 2017; Alaşahan et al., 2019; Karabulut, in press).

The aim of this study is to obtain information about the weight and specific gravity of eggs with unknown storage history by easily calculating the volume and specific gravity of eggs depending on the three variables of length, breadth and egg weight. For this purpose, mathematical equations will be used instead of Archimedes principle.

MATERIAL and METHOD

Materials

Aksaray is a province in the South east of Tuz Gölü, which has a latitude of 38° N and longitude of 34° E, and an altitude of 980 m. Although this region has a continental climate, the air temperature from January to June, which is the laying season of geese, varies between -19 - 40 ºC and relative humidity between 40% and 85% (Yayvan, 2008; 2014; Eskin, 2017; ÇŞB, 2021). Breeders brought the eggs to the hatchery in plastic containers or cardboard boxes, supplemented with straw, hay or fodder. They stated that they kept the eggs in the barn until they were brought to the hatchery. In the study, eggs of three goose genotypes; Grey China, Linda and Native geese of Aksaray region, taken from six breeders in Aksaray region were evaluated. China and Linda breeds have been found pure and most of the Native geese of Aksaray region are gray and piebald colored geese, but very few of them have blood from China goose and other breeds. The study was conducted on a total of 481 eggs and the result as follows; Egg distributions from 1st breeder to 6th breeders are 100, 6, 287, 52, 13 and 23, respectively, and the distribution of these eggs by genotypes is 113, 32 and 336 in Grey China, Linda and Native geese of Aksaray region, respectively.

To measure weight, the eggs were weighed on a scale with 1 g sensitivity. For the calculation of egg volume, the distance between the two tip points of the long axis of the egg was taken as Length and the distance at the widest part of the short axis as Breadth Measurements were carried out with a digital caliper capable of measuring 200 mm distance with a precision of 0.01 mm.

Methods

Egg specific gravity was calculated with its known formula.

$$\text{Specific gravity}=\frac{\text{Egg weight}}{\text{Egg volume}}$$

Actual measurement values were used as egg weight. Egg volume was calculated based on the ellipse shape of the egg (Preston, 1974).

Egg volume= $KvLB^2$ Here; Kv=0.507, L: Length, B: Breadth

The coefficient (Kv) for the egg volume in the formula is the coefficient corresponding to the egg of *Anser fabalis*, which weighs 142.6 g, reported by Hoyt (1979) from Schönwetter.

Statistics

Comparison of genotype and breeders for each trait was made by OneWay ANOVA, and differences were made by Duncan Test. Correlations between variables were determined with Pearson Correlation. Analyzes were made in SPSS package program (IBM, 2013).

RESULTS

The differences between the genotypes of the characteristics related to the weight, volume and specific gravity of the egg have been given in Table 1.

Table 1. Differences in Egg weight, Egg volume and Specific gravity by genotypes

Variables	Genotypes	N	$\bar{x}$	$S\bar{x}$	Min	Max	CV	p
Egg weight (g)	Native	336	137.2[b]	0.793	101	191	10.60	***
	China	113	131.1[c]	1.110	98	164	8.99	
	Linda	32	165.9[a]	2.492	141	190	8.50	
	Total	481	137.7	0.730	98	191	11.63	
Egg volume (cm^3)	Native	336	126.0[b]	0.762	94.3	175.7	11.09	***
	China	113	120.3[c]	1.054	93.1	153.4	9.32	
	Linda	32	152.0[a]	2.229	128.9	175.1	8.30	
	Total	481	126.4	0.689	93.1	175.7	11.95	
Specific gravity (g/cm^3)	Native	336	1.089	0.001	0.890	1.150	1.89	NS
	China	113	1.091	0.002	0.950	1.130	2.39	
	Linda	32	1.092	0.002	1.060	1.120	1.24	
	Total	481	1.090	0.001	0.890	1.150	1.98	

***: p<0.001, NS: No significant, n: number of eggs, $\bar{x}$: mean, $S\bar{x}$: standard error, CV: coefficient of variance, p: significance

The difference between genotypes in terms of egg weight and volume was found to be very significant (p<0.001). Among the genotypes, in terms of egg weight averages, it was observed that Linda was the highest and Chinese geese were the lowest. Egg weights averages in genotypes from large to small occurred as follows; Linda, Native and Chinese, respectively. Egg volume was calculated and the order of the averages in genotypes was found to be similar to the egg weight order. Its specific gravity was observed to be similar between genotypes (p>0.05).

The regressions where egg weight determines egg volume and specific gravity according to genotypes have been given in Figure 1.

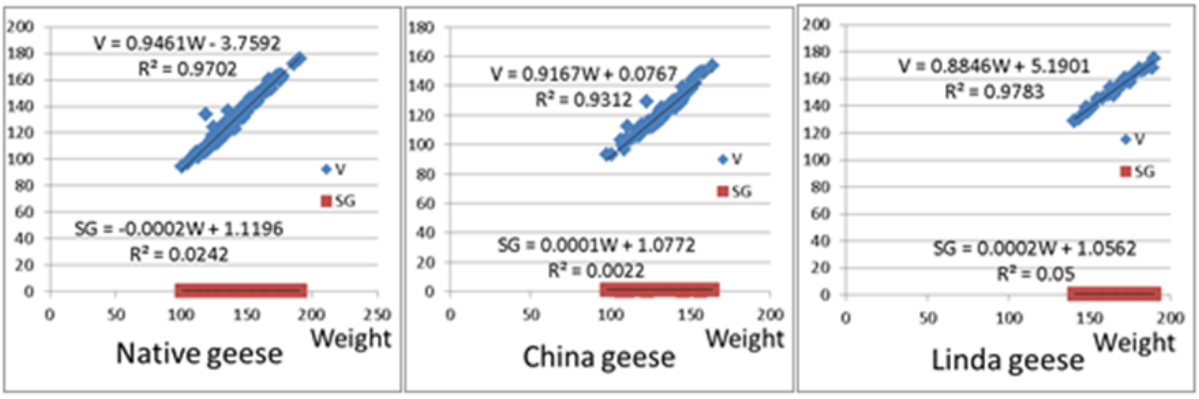

Fig 1. Regressions in which Egg weight determines Egg volume and Specific gravity according to genotypes.

In regression where egg weight determines egg volume, determination coefficients have taken values close to 1. In regression where egg weight determines the specific gravity in genotypes, determination coefficients have taken values far from 1.

Correlations between Egg weight, Egg volume and Specific gravity according to genotypes have been given in Table 2.

Table 2. Correlations between Egg weight, Egg volume and Specific gravity by genotypes.

r	Native (n=336)		China (n=113)		Linda (n=32)	
	Weight	Volume	Weight	Volume	Weight	Volume
Volume	0.985 (p<0.001)		0.965 (p<0.001)		0.989 (p<0.001)	
Specific gravity	-0.157 (p<0.01)	-0.324 (p<0.001)	0.047 (p>0.05)	-0.215 (p<0.01)	0.229 (p>0.05)	0.085 (p>0.05)

A very high positive correlation was found between egg weight and volume (p<0.001). Considering the correlations of specific gravity with egg weight and volume, significant negative correlations were found with both variables in Native geese and just volume in China goose (p<0.01, p<0.001) but not seen either in Linda (p>0.05).

The differences among the breeders of the variables related to the weight, volume and specific gravity of the egg have been given in Table 3. According to the breeders, a significant difference was found in all variables (p<0.001).

Table 3. Differences in Egg weight, Egg volume and Specific gravity according to Breeders.

Variables	Breeders	n	x̄	Sx̄	Min	Max	CV	P
Egg weight (g)	1.Breeder	100	131.8^{bc}	1.210	98.0	164.0	9.18	
	2.Breeder	6	146.2^{a}	8.284	116.0	167.0	13.88	
	3.Breeder	287	138.8^{ab}	0.965	105.0	190.0	11.78	***
	4.Breeder	52	143.3^{a}	2.603	101.0	191.0	13.10	
	5.Breeder	13	125.9^{c}	2.098	109.0	135.0	6.01	
	6.Breeder	23	140.3^{ab}	2.634	119.0	160.0	9.00	
	Total	481	137.7	0.730	98.0	191.0	11.63	
Egg volume (cm^3)	1. Breeder	100	121.2^{bc}	1.135	93.1	153.4	9.36	
	2. Breeder	6	135.1^{a}	8.658	105.2	160.7	15.70	
	3. Breeder	287	127.4^{ab}	0.903	97.2	175.1	12.01	***
	4. Breeder	52	131.4^{a}	2.461	94.3	175.7	13.51	
	5. Breeder	13	112.9^{c}	1.798	96.6	121.3	5.74	
	6. Breeder	23	130.1^{ab}	2.657	109.2	149.2	9.79	
	Total	481	126.4	0.689	93.1	175.7	11.95	
Specific gravity (g/cm^3)	1. Breeder	100	1.088^{b}	0.003	0.950	1.126	2.344	
	2. Breeder	6	1.085^{b}	0.010	1.040	1.106	2.342	
	3. Breeder	287	1.090^{b}	0.001	0.890	1.138	1.905	***
	4. Breeder	52	1.092^{b}	0.002	1.056	1.147	1.494	
	5. Breeder	13	1.115^{a}	0.004	1.089	1.133	1.390	
	6. Breeder	23	1.079^{b}	0.003	1.049	1.106	1.462	
	Total	481	1.090	0.001	0.890	1.147	1.985	

***: p<0,001, NS: No significant, CV: Coefficient of variance

In terms of egg weight average, the highest averages were in the 2nd Breeder and 4th Breeder; 146.2 and 143.3 g, the lowest averages were 131.8 and 125.9 g in 1st Breeder and 5th Breeder, and 3rd Breeder and 6th Breeder were placed in the middle with 138.8 and 140.3 g,

respectively. In terms of egg volume averages, the highest averages were 135.1 and 131.4 cm^3 in the 2nd Breeder and 4th Breeder, respectively, the lowest averages were 121.2 and 112.9 cm^3 in 1st Breeder and 5th Breeder, and with 127.4 and 130.1 cm^3, 3rd Breeder and 6th Breeder were placed in the middle. With these rates, it was found to be similar to the difference in egg weight in terms of differences. The order in specific gravities was different from the weight and volume, and there was only a difference due to the size of the specific gravity of the 5th Breeder's eggs. The regressions where egg weight determines egg volume and specific gravity have given in Figure 2.

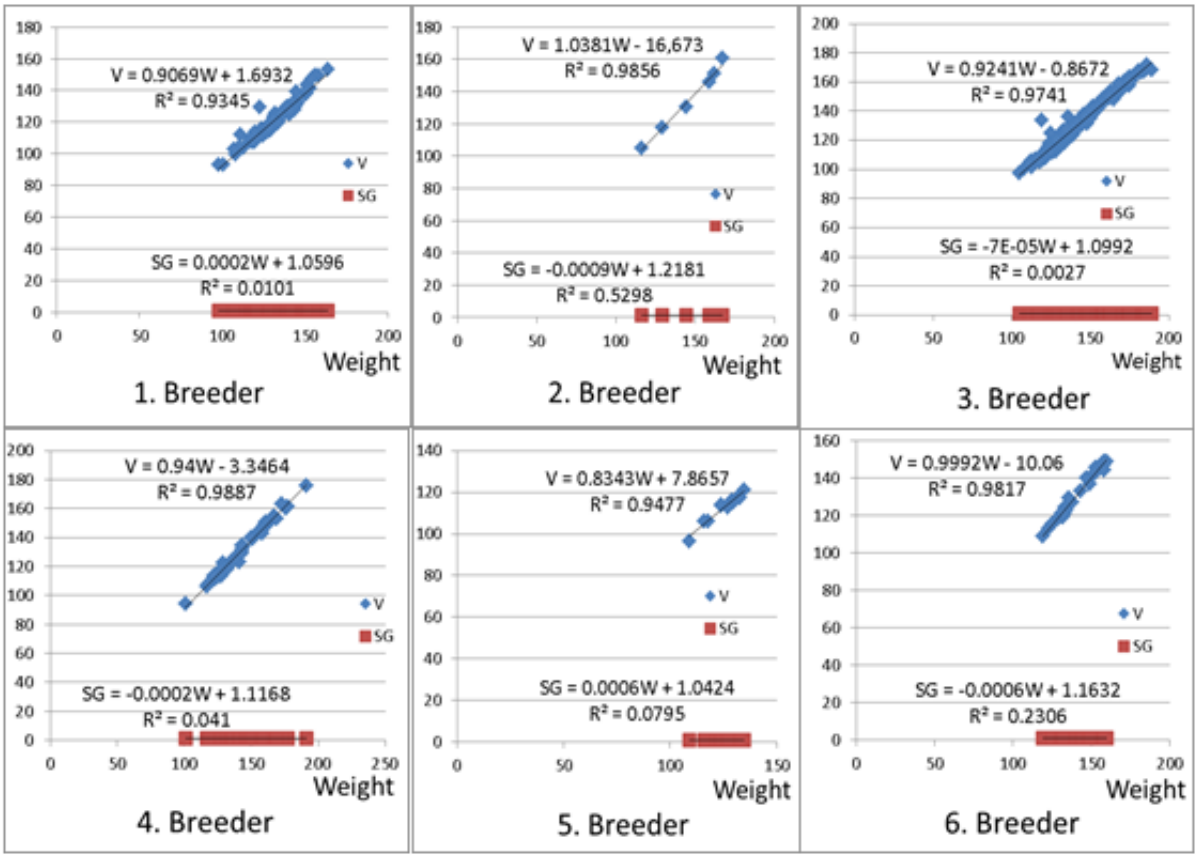

Figure 2. Regressions where Egg weight determined Egg volume and Specific gravity according to Breeders.

In the regression where egg weight determines the egg volume in Breeders, the coefficients of determination were 0.9345, 0.9856, 0.9741, 0.9887, 0.9477 and 0.9817, from 1st Breeder to 6th Breeder, respectively. Determination coefficients have values close to 1 and a similar situation in genotypes has emerged here as well. According to Breeders, the coefficients of determination in regression, where egg weight determines the specific gravity, increased from 1st Breeder to 6th Breeder, respectively, 0.0101, 0.5298, 0.0027, 0.041, 0.0795 and 0.2306. Correlations between Egg weight, Egg volume and Specific gravity according to Breeders have been given in Table 4. A very high positive correlation was found between egg weight and volume, and it was 0.967, 0.993, 0.987, 0.994, 0.974 and 0.991, respectively, from 1st Breeder to 6th Breeder ($p<0.001$). A similar situation in genotypes was seen in Breeders. A negative correlation ($r=-0.156$) was observed only in Breeder 6 between the egg weight and specific gravity variables in Breeder ($p<0.01$). This difference suggests that the 6th Breeder have kept eggs in a more humid environment during egg storage compared to other breeders.

DISCUSSION

Average egg weights, from large to small in genotypes, have emerged 165.9, 137.2 and 131.1 g in Linda, Native and Chinese geese, respectively. These averages are within the range reported in the literature. However, the average found for Chinese Geese is much lower than the 173 g reported by Arroyo (1990). Similarly, it is lower than 144.51 and 148.43 g reported by Saatçi et al. (2002) and Saatçi et al. (2005) for Native geese. These differences may be originated from genetic factors such as race and age, as well as environmental factors such as care, feeding, breeding system and region differences (Hamadani et al., 2016; Arroyo, 1990; Tilki and İnal, 2004a; Razmaitė, 2013). Storage time and shape may also have a contribution to this difference.

The ranking of average egg volume in genotypes is similar to that of egg weight and was calculated as 152.0, 126.0 and 120.3 cm^3 in Linda, Native and Chinese geese, respectively. While the average of Linda and Native geese was within the range reported in the literature, the average of Chinese geese has been found to be low (Paganelli et al., 1974; Saatçi et al., 2002; Hamadani et al., 2016; Kumbar et al., 2016). These differences can be said for egg volume, as there is a positive correlation between volume and weight. However, storage does not affect the egg volume.

Specific gravity is similar among genotypes ($p>0.05$), and it has been found as 1.092, 1.091 and 1.089 g/cm^3 in Linda, China and Native, respectively, from large to small. The averages have been found higher than those reported by Saatçi et al. (2002) for Native geese, similar to those reported by Hamadani et al. (2016) for Kashmir Anz geese and higher than those reported by Arroyo (1990) for Africa, China, Toulouse and Embden geese. It has also been detected to be higher than those reported for Japanese quail and chickens (Çetingül and İnal, 2009; Nemati, 2020). In addition to this being normal, the formula used in calculating the volume can be regarded as an indicator that it gives results close to reality. However, the storage time and how it's done may affect the specific gravity.

Those determination coefficients have taken values close to 1 in the regression between egg weight and volume is an indication that an egg volume that is close to the required (real-like) value can be calculated for each egg whose weight is known by using the regression formula. In the regression between egg weight and specific gravity in genotypes, the determination coefficients have occurred 0.0242, 0.0022 and 0.05 in Native, China and Linda, respectively. Even though the determination coefficients seem far from 1, the importance of the correlation between egg weight and specific gravity should be checked because the importance of correlation is directly related to the number of eggs.

Regarding with correlations between the variables, a high level of positive correlation have been detected between the egg weight and volume in Native, China and Linda, as 0.985, 0.965 and 0.989, respectively, which seems to confirm the coefficient of determination. The fact that genotypes have the same size order of the egg weight and egg volume averages supports the existence of this correlation. Considering the correlations of specific gravity with egg weight and volume, although the negative correlation ($r=-0.157$) with egg weight in Native geese does not seem normal, the addition of a substance with a lower specific gravity than the Specific gravity of the egg may create a negative correlation (MEB, 2011). In other words, the Specific gravity of an egg, which is under the influence of a substance with a low Specific gravity, falls but its weight, increases. The first thing that comes to mind here is that the egg has been exposed to excessive moisture, and the density of water is lower than that of the egg. That the correlation of Specific gravity with volume has emerged at a higher

level supports this view. Because the Specific gravity decreased although the egg volume remained constant, That Specific gravity has a correlation with egg volume and a positive correlation coefficient with egg weight indicates that the egg is under the influence of the high density substance. The emergence of insignificant correlations in Linda geese may indicate that they are well preserved.

Breeders were ranked as 2th, 4nd, 6th, 3th, 1th and 5th in terms of the size of average egg weights from large to small, while their weight averages were 146.2, 143.3, 140.3, 138.8, 131.8 and 125.9 g. Although these averages are within the range reported in the literature, important differences have been found among Breeders. The reasons reported for the difference in egg weight averages of genotypes can also be said for Breeders.

The order of the average egg volume in the breeders is the same as for the egg weight and it has been calculated as 135.1, 131.4, 130.1, 127.4, 121.2 and 112.9 cm^3, respectively. The ordering relationship in the genotypes was also seen in the breeder in terms of the weight and volume of the eggs averages.

In Specific gravities, the order is different from the weight and volume, and only a difference has occurred due to the height of the 5th Breeder's average Specific gravity of 1.115 g/cm^3. This average is close to the 1.113 g/cm^3 average reported only by Saatçi et al. (2002) among the reviewed literature. Having the lowest value in terms of coefficient of variance, it has been detected that the most homogeneous eggs in terms of specific gravity are in the 5th breeder. Although the eggs have been preserved under the same conditions, regression and correlation should be considered in order to understand the height in Specific gravity.

When the regressions in which egg weight determines egg volume and specific gravity in breeders have been examined, the coefficients of determination in egg weight and egg volume have taken values close to 1, and egg weight and specific gravity have taken values far from 1. The importance of the correlation coefficients should be checked in order to make a definite decision about the determination coefficients.

When the correlations between the variables in breeders have been examined, the correlation coefficients in egg weight and egg volume have been positive and close to 1 as in the genotypes. Therefore, the determinations about the correlations in genotypes are also valid for Breeders. When the correlations of specific gravity with egg weight and volume have been examined, it has been found that it is insignificant in 1st Breeder and 2nd Breeder ($p>0.05$). It can be said that the eggs of these two Breeders have not been affected by any substance.

Correlations have been found significant and negative between Specific gravity and volume in 3rd Breeder, 4th Breeder and 5th Breeder ($p<0.001$, $p<0.01$). The negative correlation coefficient between Specific gravity and egg weight in 3rd Breeder and 4th Breeder suggests that it has been under the influence of a substance lower than egg Specific gravity. If this substance is caused by water depending on the ambient humidity, it should be evaluated together with the ambient temperature because as the temperature increases, the volume of the substances expands and the specific gravity decreases (Erensayın, 2000; MEB, 2011). In other words, this suggests that moist eggs have been weighed in a place with high ambient temperature. For this reason, it can be said that there is an insignificant increase in egg weight but a significant decrease in specific mass. In 5th Breeder, the positive correlation coefficient of Specific gravity and egg weight may indicate the inclusion of a substance with a higher specific gravity than that of the egg or the output of a substance with a low specific gravity. The first thing that comes to mind here is the possibility that the egg has lost water. In the 6th Breeder, the correlation coefficients have been found to be

significant and negative. Most likely the egg has moistened.

CONCLUSION

In the study, egg volume was calculated with mathematical equations and results were obtained close to reality. Accordingly, the calculated Specific gravity has also been found to be realistic. These results have been obtained easily by knowing only three variables, egg weight and Length and Breadth values. These results will be easier to obtain with photographic imaging techniques. It is thought that this method can pave the way for obtaining a lot of information about the relation between egg and specific gravity. For this, it is necessary to measure and determine the changes that occur as a result of the controlled storage of eggs obtained from controlled flocks and examine these data together with the changes in specific gravity, weight and volume.

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Ethical approval: An ethical approval is not needed according Turkish regulations as only samples were taken for calculations.

Conflict of interest: Authors declare that they have no competing interests.

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Isolation and identification of yeasts in white cheese

Research Article

Sevda URÇAR GELEN[1a]
Ziya CEYLAN[2b]

[1]Department of Food Hygiene and Technology, Veterinary Medicine, Atatürk University 25240 Erzurum, Turkey
[2]Department of Food Hygiene and Technology, Veterinary Medicine, Atatürk University 25240 Erzurum, Turkey

ORCİD-
[a]0000-0002-1852-3614
[b]0000-0001-8644-5905

Correspondence
Sevda URÇAR GELEN
surcar@atauni.edu.tr

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ABSTRACT

In this study, firstly yeast was isolated from 50 white cheese samples produced by traditional methods from raw milk. The yeast species were isolated from 50 traditional white cheese samples and identified with the VITEK2 Compact system. There were obtained from white cheese samples 90 isolates 35.6% *Candida sake*, 13.3% *Candida zeylanoides*, 8.9% *Candida famata* and 8.9% *Candida kefyr* and the remaining 33.3% *Candida sphaerica*, *Candida colliculasa*, *Candida boidinii*, *Candida lusitaniae*, *Candida parapsilosis*, *Candida sphaerica*, *Cryptococcus laurentii*, *Candida krusei*, and *Saccharomyces cerevisiae*. Yeasts are known as a saprophyte found in many foods, but that some of them positively contribute to the fermentation. More over some yeasts are could be used of starter culture during the manufacture of certain dairy product for maturation by supporting the functions. some yeasts species were isolated in this study, could be used as support culture.

Keywords: *Candida* spp., cheese, yeast

INTRODUCTION

Yeasts, which play important roles in the production and deterioration of many dairy products, are an important member of microflora (Jakobsen & Narvhus, 1996; Cosentino et al., 2001). Yeasts contribute to the aroma formation in dairy products with the aroma compounds and other metabolites they have (Gadaga et al., 2001; Fröhlich-Wyder et al., 2019). Cheese is the most frequently isolated yeast product among dairy products. The reason for this may be because dairy enterprises are suitable for yeast development. In addition, it is also considered that cheese brine is an important source for yeast contamination (Kesenkaş & Akbulut, 2006; Haastrup et al., 2018).

The role of yeasts in cheese varies depending on the type of cheese produced. Some yeast types cause deterioration in cheese like discoloration, fruity flavor, and adhesive structure formation; some other yeast types contribute to the development of tissue and flavor compounds by taking part in the maturation process (Ferreira & Viljoen, 2003; Atanassova et al., 2016). It is considered that yeasts can be used as auxiliary starter cultures during production stage because of their positive contributions to cheese. *Kluyveromyces marxianus*, *Debaryomyces hansenii*, *Saccharomyces cerevisiae*, *Yarrowia lipolytica*, *Trichosporon cutaneum* (*Trichosporon beigelii*), *Rhodotorula mucilaginosa*, *Torulaspora delbrueckii* are the most common yeast species (Jakobsen & Narvhus, 1996).

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Cosentino et al. isolated *Debaryomyces hansenii*, *Geotrichum candidum*, *Kluyveromyces lactis*, *Kluyveromyces marxianus*, *Pichia*, *Candida*, *Dekkera*, *Yarrowia* and *Rhodotorula* from different cheese varieties (Cosentino et al., 2001). The most frequently isolated species of fresh cheese samples were *Candida zeylanoides*, *Debaryomyces hansenii*, *Kluyveromyces marxianus*, *Yarrowia lipolytica* (Lopandic et al., 2006).

Fadda et al. conducted a study on feta cheese samples, and isolated *Kluyveromyces lactis*, *Debaryomyces hansenii*, *Dekkera anomala*, *Dekkera bruxellensis*, *Geotrichum candidum*, *Kluyveromyces marxianus*, *Rhodotorula rubra*, *Candida tropicalis*, and *Candida sake* (Fadda et al., 2001). In a study that was conducted in feta cheese by Kaminarides and Laskos, it was determined that *Saccharomyces cerevisiae*, *Candida famata*, *Torulaspora delbrueckii*, and *Pichia membranaefaciens* were dominant in brine juice (Kaminarides & Laskos, 1992).

In a study conducted in Egypt, *Issatchenkia orientalis*, *Candida albicans*, *Clavispora lusitaniae* (*Candida lusitaniae*), *Kodamaea ohmeri* (*Pichia ohmeri*), *Kluyveromyces marxianus* and *Candida catenulata* were isolated from cheese (El-Sharoud, Belloch, Peris, & Querol, 2009). In a study in Denmark, *Debaryomyces hansenii*, *Kluyveromyces lactis*, *Torulaspora delbrueckii*, and *Yarrowia lipolytica* were isolated from cheese (Westall & Filtenborg, 1998). Cardozo et al., it was determined that *Candida*, *Myxozyma*, and *Debaryomyces* species were dominant in soft cheeses (Cardozo, Fusco, & Carrasco, 2017). Karasu Yalcin et al. were identified *Candida*, *Geotrichum* and *Trichosporon* yeast species from traditional Mihalic cheeses (Karasu Yalcin et al., 2017). In a study in Portuguese, *Debaryomyces hansenii*, *Kluyveromyces marxianus*, *Candida* spp. and *Pichia* spp. were isolated from Serpa cheeses (Dos Santos, Benito, de Guía Córdoba, Alvarenga, & de Herrera, 2017). On the other study was found *S. cerevisiae*, *Wickerhamomyces anomalus*, *Pichia kudriavzevii*, *Kluyveromyces lactis*, *Geotrichum candidum*, *Debaryomyces hansenii*, *Candida tropicalis*, *Cryptococcus neoformans*, *Rhodotorula glabrata* and *Trichosporon cutaneum* (Helmy et al., 2019). Ozmen Togay et al. were isolated *Torulaspora delbrueck*ii and *Candida zeylanoides* from white cheeses (Ozmen Togay et al., 2020).

Yeast types vary in the environment depending on the cheese types, production techniques, and production locations. Yeasts can have both positive and negative effects on cheese with their proteolytic and lipolytic activities. While they contribute to the maturation of some cheeses, they also cause some to deteriorate. For this reason, it is important to determine the yeast profile in cheese produced with traditional methods. In this study, the purpose was to determine the yeast flora of white cheese, which is a soft cheese consumed extensively in Turkey.

MATERIAL and METHOD

The white cheese samples used in the study were produced in local businesses with traditional methods from raw milk. Yeasts were isolated from 50 samples of White Cheese. For isolation twenty five grams of the samples was homogenized with 225 ml of Ringer solution. Samples were serially diluted in the physiological solution and plated on a RBC (Rose Bengal Chloramphenicol) Agar (Merck). The colony forming units (cfu) were determined after incubation for 5 days at 25±1°C. From the primary cultures different colonies with identical morphological appearance were selected for further purification. The selected colonies were inoculated on a RBCA medium and after incubation at 25±1°C for 5 days. Strains for identification were selected based on the morphological and physiological characterisation. The suspension prepared with

pure yeast cultures was loaded into yeast cards. Finally, all the yeast isolates were subjected to identification using the Vitek2 Compact system (bioMérieux). The test panels (ID-YST) contained 46 fluorimetric tests that included carbon source utilization, enzymatic activities and resistance (Pincus, 2006).

Statistical analyses

SPSS for Windows 21.0 was used. The physicochemical data were analysed by a one-way analysis of variance (ANOVA) was used for the microbiological data.

RESULTS

The 90 isolates that were obtained from white cheese samples were *Candida sake* (32%) at a rate of 35.6%, *Candida zeylanoides* (12) at a rate of 13.3%, *candida famata* (8) at a rate of 8.9%; *Candida kefyr* (8) at a rate of 8.9%; and the rest 33.3% consisted of *Candida sphaerica* (4), *Candida colliculasa* (2), *Candida boidinii* (2), *Candida lusitaniae* (4), *Candida parapsilosis* (2), *Candida sphaerica* (4) *Cryptococcus laurentii* (4), *Candida krusei* (4) and *Saccharomyces cerevisiae* (4) yeasts. The yeast types, which were derived from white cheese samples, are given in Table 1.

Table 1: The yeast types, which were derived from white cheese samples

Yeast species	Number of yeast	Percentage of total	Number of samples with yeast	Percentage of Sample with yeast
Candida sake	32	35.6	18	36
Candida zeylanoides	12	13.3	4	8
Candida sphaerica	8	8.9	4	8
Candida famata	8	8.9	4	8
Candida kefyr	8	8.9	2	4
Candida krusei	4	4.4	4	8
Candida lusitaniae	4	4.4	3	6
Cryptococcus laurentii	4	4.4	2	4
Saccharomyces cerevisiae	4	4.4	3	6
Candida boidinii	2	2.2	2	4
Candida colliculasa	2	2.2	2	4
Candida parapsilosis	2	2.2	2	4
Total	90	100	50	100

DISCUSSION

The yeasts that can develop in dairy products have positive effects on ripening with their physiological and biochemical properties like lactose fermentation or assimilation, high lipolytic and proteolytic activity, lactic acid and citric acid, the ability of developing at low temperatures and tolerating high salt concentrations (Fleet, 1990; Fröhlich-Wyder et al., 2019). Yeasts vary depending on the cheese type they are isolated from. The white cheese used in this study had similarities to feta cheese.

Candida sake, which was isolated from many white cheese samples, was similarly isolated from Danish feta cheese, although not dominant in Sardinian feta cheese, this yeast species was also isolated (Westall & Filtenborg, 1998; Fadda et al., 2001). Although *Candida zeylanoides*, which was isolated in this study, is one of the most isolated yeast species in studies on both soft and hard cheese varieties, its lactose and lactate are negative (Seiler &

Busse, 1990; Pereira-Dias et al., 2000; Callon et al., 2006; Lopandic et al., 2006; Karasu-Yalcin et al., 2017; Dos Santos et al., 2017; Ozmen Togay et al., 2020)

Candida famata species, which were reported to contribute to aroma formation in cheese, have previously been isolated at similar rates in studies conducted on different cheese varieties (Besancon et al., 1992; Pereira-Dias et al., 2000; Romano et al., 2001; Atanassova et al., 2016; Karasu-Yalcin et al., 2017; Dos Santos et al., 2017; Helmy et al., 2019; Ozmen Togay et al., 2020). *Candida famata* (perfect form is *Debaryomyces hansenii*) may be used as a starter culture to help in cheese production as it can assimilate lactose, lactic and citric acids, produce lipoidase and proteases and develop in high salt concentrations and low pH values (Fatichenti et al., 1983; Welthagen & Viljoen, 1999; Van Den Tempel & Jakobsen, 2000). *Candida kefyr* (perfect form is *Kluyveromyces marxianus*) was a yeast species that was often isolated, even if it is not dominant flora, in studies conducted in different cheese varieties, as it was the case in this study (Welthagen & Viljoen, 1997; Welthagen & Viljoen, 1998; Romano et al., 2001; Callon et al., 2006; Atanassova et al., 2016; Dos Santos et al., 2017; Ozmen Togay et al., 2020). *Candida kefyr*, which can ferment lactose, is usually available in milk and dairy products. It encourages reproduction in cheese where other yeasts are low especially because they can ferment lactose (Padilla et al., 2014).

Saccharomyces cerevisiae (Callon et al., 2006; Atanassova et al., 2016; Helmy et al., 2019; Ozmen Togay et al., 2020), *Candida colliculasa* (Romano et al., 2001), *Candida sphaerica* (Besancon et al., 1992), *Candida lusitaniae* (Facchin et al., 2013) *Candida krusei* (Facchin et al., 2013; Karasu-Yalcin et al., 2017) and *Candida parapsilosis* (Seiler & Busse, 1990; Callon et al., 2006; Facchin et al., 2013; Dos Santos et al., 2017; Ozmen Togay et al., 2020) were found in different cheese varieties as in previous studies but were not dominant.

CONCLUSION

As a result, we determined in our study that *Candida* spp. constituted the predominant yeast flora in white cheeses. Yeasts have effects on both ripening and deterioration in cheese. For this reason, firstly the yeast profile in cheese must be determined, and then it should be determined which yeast species can be used as auxiliary starters in different cheese types. In this way, both the ripening time will be shortened, and the products will be more aromatic.

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Comparison of impact of synchronization protocols applied to ewes on pregnancy rate in Turkey with bayesian meta-analysis

ABSTRACT

The present study carried a meta-analysis of the pregnancy rates of different synchronization protocols implemented in ewes in Turkey. A common pregnancy rate was estimated by coalescing pregnancy rates, and the heterogeneity between the studies was explored. All studies were carried out independently. This article compiled 28 studies that determined the pregnancy rate for 2,437 ewes in the synchronization studies between the years 1995–2020. The overall effect size of the meta-analysis was found to be 0.66 (95% confidence interval; 0.58–0.74) ($p<0.001$). This meta-analysis indicates that fertility parameters were improved with the highest effect size in the synchronization protocol used in ewes during the breeding season by combining various doses of PMSG at the end of intravaginal sponge application as a source of progesterone. In terms of subgroups, 40 mg FGA + 300 IU PMSG (95% confidence interval; 0.50–0.61), 40 mg FGA + 500 IU PMSG (95% confidence interval; 0.44–0.52), and 40 mg FGA + 700 IU PMSG (95% confidence interval; 0.41–0.61) protocol effect size was estimated to be higher than other protocols during the season. Thus, it is possible to control the reproductive performance in enterprises with estrus synchronization protocols and mating of ewes. However, it is important to validate which of these methods are optimum in terms of economy and efficiency in enterprise conditions. Considering the effects of synchronization protocols on pregnancy, it is predicted that Bayes meta-analysis will guide enterprises as a decision support system in achieving optimum pregnancy rates.

Keywords: Ewes, meta-analysis, pregnancy rate, synchronization, Turkey

INTRODUCTION

In ewes breeding, lamb yield is more important than milk production. The most critical factor affecting the profitability in ewes breeding enterprises is the number of lambs born and the conversion of these lambs into profit (Arıkan, 2021). The main goal in ewes breeding is to increase lamb production and, accordingly, profitability. This is directly proportional to the reduction of lambing interval and the increase of fertility (Doğruer et al., 2015). The seasonal estrus of ewes and goats, especially in ovine breeding enterprises, makes reproductive management vital. The anoestrus period of ewes and goats, which are seasonal polyestrous animals, is considered as out of season period. During the anoestrus period, the enterprises are deprived of brood and milk production. This period of increasing costs, coupled with decreased production, adversely affects the profitability of enterprises. Different synchronization methods are exploited to channelize the anoestrus period in a reproductive manner in ovine breeding enterprises. A significant contribution to enterprises, in terms of reducing costs and increasing profitability, could be obtained by applying these methods.

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Research Article

Burak MAT[1a]
Murat POLAT[2b]
Zeynep ÖZEL[3c]
Mert DEMİRSÖZ[3d]

[1]Department of Animal Health Economics and Management, Faculty of Veterinary Medicine, Selçuk University, Konya, Turkey
[2]Department of Animal Health Economics and Management, Faculty of Veterinary Medicine, Kastamonu University, Kastamonu, Turkey
[3]Department of Biostatistics, Faculty of Veterinary Medicine, Selçuk University, Konya, Turkey

ORCİD-
[a]0000-0002-0455-8736
[b]0000-0002-2659-3495
[c]0000-0002-1077-1250
[d]0000-0002-4800-2529

Correspondence
Burak MAT
burak_mat2004@yahoo.com

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With synchronization in the enterprises, conception in a short time throughout the herd, reducing the number of excesses per pregnancy, births at the desired time, more efficient use of labor, feed consumption, and enterprise capacity are enabled. Furthermore, synchronization facilitates planning when the prices of animal products produced and marketed by the enterprise are the highest (Whitley & Jackson, 2004).

The synchronization protocols in ewes employ hormones such as melatonin, gonadotropin-releasing hormone (GnRH), PGF2α and their analogs, estrogens, human chorionic gonadotropin (hCG), pregnant mare serum gonadotropin (eCG/PMSG), progestogens, and their combinations (Aköz et al., 2015; Özyurtlu et al., 2010;).

Many studies have been conducted on the effect of various synchronization applications on the pregnancy rate in ewes. However, the wide distribution of pregnancy rates from different studies indicates the need for a more precise conclusion. One of the most effective methods to draw such a conclusion is meta-analysis. Meta-analysis is a method of compiling the results of many independent studies conducted on a specific subject and reinterpreting them with the aid of suitable statistical analysis of the findings (Lipsey & Wilson, 2001). Meta-analysis improves the precision and power of the parameter estimates and, in turn, the statistical significance by increasing the sample size. For this reason, meta-analysis, in ewes (Palacin et al., 2011) and cattle (Borchardt et al., 2017; Borchardt et al., 2018), is a frequently used tool in the literature to ascertain the effectiveness of different synchronization methods.

In this study, the effect of different synchronization protocols pregnancy rates applied to ewes seasonal and out of season in Turkey considering Bayesian meta-analysis of studies examined with high pregnancy rates was determined. It was then calculated which protocol was lower cost. This method for ewe enterprises; As a decision support system, it is aimed to guide in catching the high pregnancy rate and low protocol cost point.

MATERIAL and METHOD

The present study extracted pregnancy rates from 28 independent studies. These studies determined the pregnancy rates using different synchronization methods in Turkey between the years 1995–2020. Based on the literature review, 371 studies were enlisted within the scope of the analysis. Among these studies, the abstracts of 343 articles that remained after excluding duplicate articles were read in accordance with research strategies, exclusion, and inclusion criteria. Thereafter, 294 articles were excluded, and 49 studies were included for the present meta-analysis. According to the research literature search strategy, 21 studies that failed to provide the necessary statistical data were excluded, and 28 studies were analyzed in terms of content and transferred to the coding form. The literature search method is outlined in the flow chart in Figure 1.

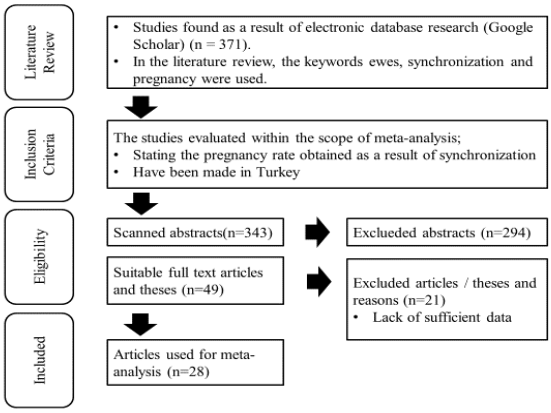

Figure 1. Flowchart about the literature search methodology employed in the meta-analysis

A total of 2,437 ewes from different studies were included in the meta-analysis. The information on the pregnancy rates obtained by the synchronisation method in ewes in Turkey and studies included in the meta-analysis is detailed in Table 1.

The linearity of the study effect sizes and standard errors of the studies included in the meta-analysis were determined by Egger's Linear Regression test. A funnel plot was employed to identify publication bias, and the Trim and Fill method of Duval and Tweedie (2000) were applied to eliminate the bias, and the common exposure value was re-calculated (Duval & Tweedie, 2000). A random-effect model (DerSimonian-Laird method) was used to estimate the variance between studies as well as within-study variance (Sutton et al., 2001).

Table 1. Characteristics of the studies conducted about synchronization in ewes in Turkey between the years 1995–2020, incorporated in the meta-analysis

Studies, Year	Total Number of Ewes	Number of Pregnant Ewes	Pregnancy Rate (%)
Duymaz (2020)	120	103	85.83
Karakaya (2019)	50	32	64.00
Kaya (2019)	70	16	22.86
Özbilek (2019)	238	166	69.75
Akbaş (2016)	75	52	69.33
Doğanay (2011)	90	53	58.89
Solak (2009)	78	12	15.38
Ocak (2007)	89	72	80.00
Topcu (2004)	88	54	61.36
Özyurtlu (2010b)	62	32	51.61
Uçar (2002)	59	46	77.97
Ataman (2009)	75	49	65.33
Kulaksız (2011)	29	24	82.76
Baştan and Kuplülü (1995)	50	33	66.00
Emrelli (2003)	30	16	53.33
Kaya (2003)	80	69	86.25
Algan (2014)	217	92	42.40
Koyuncu (2001)	95	92	96.84
Timurkan and Yıldız (2005)	130	118	90.77
Köse (2016)	124	95	76.61
Öztürkler (2003)	40	34	85.00
Doğan and Nur (2006)	69	36	52.17
Aköz (2006)	88	84	95.45
Kuru (2017a)	148	61	41.22
Kaçar (2008)	29	12	41.38
Kaya (2013)	60	26	43.33
Doğruer (2015)	76	50	65.79
Esen and Bozkurt (2001)	78	71	91.03

Cochrane's Q statistics with degrees of freedom was used to evaluate the heterogeneity of the effect sizes of the studies (k-1). Further, to determine the level of heterogeneity I^2 statistics and to determine the true variance between studies τ^2 statistics were used. I^2 value was assessed by using three categories (low if below 25%, medium between 25–50%, high heterogeneity above 50%) (Patsopoulous et al., 2008).

A priori distributions were classified into two groups as "informative" and "non-informative". In the case of the non-informative prior distribution, the data containing the sample information were weighted more in the posterior distribution, while the data containing the sample information were less weighted in the informative prior distribution (Bolstad, 2007). As the values of parameters, a and b, of the beta distribution changed, the informative and non-informative prior distributions were formed. If a and b are given a value of 1, respectively (equivalent to Uniform (0, 1) distribution), it gives a non-informative a priori

distribution so that θ takes any value in the interval (0,1) with equal probability. In this case, the values of a and b are considered 0.5; it is called low informative a priori distribution because θ indicates that the probability of taking extreme values is higher as compared to the probability of taking middle values. Finally, the values of a and b being greater than and equal to 1 and 0.5 provides stronger information about θ. In this case, it emphasizes that θ takes the middle values in the interval (0,1) with a higher probability and that the probability of realization and non-realization is equal.

As a result of the comparison, it was affirmed that the Bayesian meta-analysis obtained by using different a priori distributions gives a narrower estimation range than the classical method. One of the most important advantages of the Bayesian approach over the classical method is that it can compute for all uncertain sources of change. The simulation algorithms developed in recent years have reduced the hurdle of calculating the complex integral operations required for the Bayesian approach.

RESULTS

The list of synchronization studies in ewes that were meta-analyzed in this article, the name of the researchers, the year of publication of the study, the number of synchronized ewes, the number of pregnant ewes, and the pregnancy rate (%) are compiled in Table 1. The heterogeneity test results in the study are summarized in Table 2.

Table 2. Publication bias summary statistics

Fail-Safe N Analysis (File Drawer Analysis)		Rank Correlation Test for Funnel Plot Asymmetry		Regression Test for Funnel Plot Asymmetry		Heterogeneity Statistics						
Fail-safe N	p	Kendall's tau	p	Z	p	Tau	Tau²	I²	H²	df	Q	p
2.328.000	<.001	0.587	<.001	-8.996	<.001	0.381	0.1452 (SE = 0.0425)	48.18%	54.940	27.000	465.999	<.001

Note Fail-safe N Calculation Using the Rosenberg Approach

When Table 2 was evaluated based on the heterogeneity test, the meta-analysis of the studies included in the study was found to be heterogeneous since the p-value was less than 0.05, and the Q value was greater than the value corresponding to the df value. Since the statistical value of I^2 was used to determine the level of heterogeneity, which was calculated to be 48.18, there was a moderate bias in our study, and the random effects model was opted.

The funnel plot obtained in the study to determine the publication bias is illustrated in Figure 2.

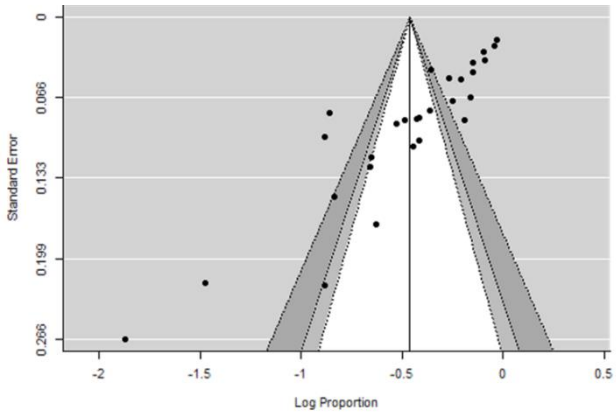

Figure 2. The funnel (Funnel Plot) chart of studies included in the meta-analysis of synchronization studies in ewes published between the years 1995–2020

It is quite evident from the funnel scatter plot in Figure 2 that most of the pregnancy rates of 28 studies included in the study were located near the vertical line designating the combined effect size and at the top of the graph.

The homogeneous distribution value, the average effect size, and confidence intervals of the random effect model are outlined in Table 3.

Table 3. Statistical Values of Random Effect Model

Random-Effects Model (k = 28)						
	Estimate	**se**	**Z**	**p**	**Lower Bound**	**Upper Bound**
Intercept	0.658	0.0407	16.02	<.001	0.571	0.744

Note Tau² Estimator: Empirical Bayes

The effect size value (Estimate) was 0.658, the lower limit of the 95% confidence interval was 0.571, and the upper limit was 0.744 ($p<0.01$). Thus, it can be inferred that there was a significant effect of synchronization operation performed in ewes in Turkey of pregnancy rates in the period we determined (1995–2020). It was also observed that hormone applications to increase pregnancy rates significantly augmented this value statistically. The fit statistics and information criteria for the model are summarized in Table-4.

Table 4. Model Fit Statistics and Information Criteria

	Log likelihood	**Deviance**	**AIC**	**BIC**	**AICc**
Maximum-Likelihood	3.754	113.037	3.508	0.844	-3.028
Restricted Maximum-Likelihood	3.138	-6.275	-2.275	0.317	-1.775

Considering the fit of the model with the information criteria in Table 4, it is predicted that the model is moderately compatible and can provide guidance for further studies.

The forest graph obtained as a result of the meta-analysis applied is shown in Figure 3.

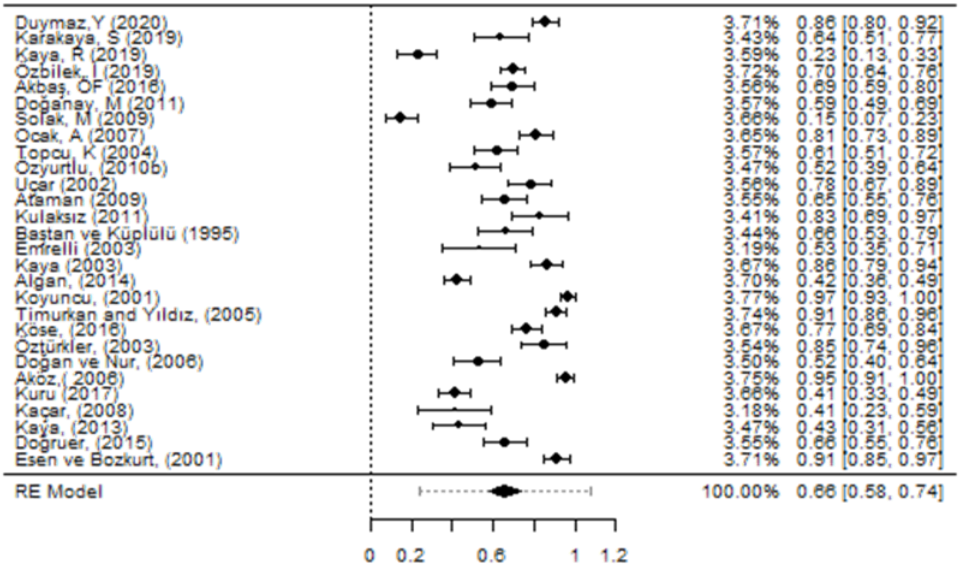

Figure 3. Forest plot of the synchronization studies effective on pregnancy rate in ewes

Figure 3 summarizes the effect sizes and relative weights of each study with the findings of the forest plot. Relative weight is calculated as the percentage of the working weight and it is observed that the study of Aköz et al. (2006) and Koyuncu et al. (2001) revealed the highest weight and effect size. In the forest plot, on the left, the squares represent the effect size of each study, the dimensions of the squares designate the study sizes, and the bars extending to the right and left symbolize the lower and upper limit of the 95% confidence interval of each study's effect size. The diamond on the x-axis in the graph reflects the overall effect size and it is documented that the overall effect size is 0.66 (95% confidence interval; 0.58–0.74) ($p<0.001$). The squares representing the studies delineate summary information about the proximity or distance to the diamond, which portrays the general effect.

Table 5. Statistical values of the fixed effect model in subgroups in the study of Aköz et al. (2006)

Fixed-Effects Model (k = 6)						
	Estimate	**se**	**Z**	**p**	**Lower Bound**	**Upper Bound**
Intercept	0.431	0.0205	21.0	<.001	0.391	0.471

When Table 5 is examined, the effect size value (Estimate) was determined as 0.431, the lower limit of the 95% confidence interval was 0.391, and the upper limit was 0.471 ($p<0.001$). The forest chart created for subgroups is illustrated in Figure 4.

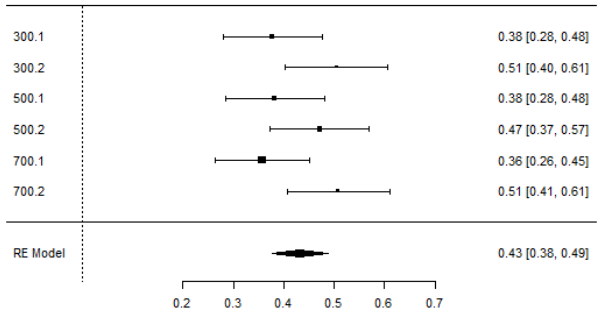

Figure 4. The forest graph obtained from the meta-analysis applied to the subgroups of the study of Aköz et al. (2006)

Scrutinizing Figure 4 in terms of subgroups in the study of Aköz et al. revealed that the protocol where the highest effect size was obtained was 40 mg FGA+300 IU PMSG (95% confidence interval; 0.50–0.61) and 40 mg FGA+700IU PMSG (95% confidence interval; 0.41–0.61).

When Table 6 is investigated; the effect size value (Estimate) was 0.484, the lower limit of the 95% confidence interval was 0.443, and the upper limit was 0.524 ($p<0.001$). The forest chart created for subgroups is presented in Figure 5.

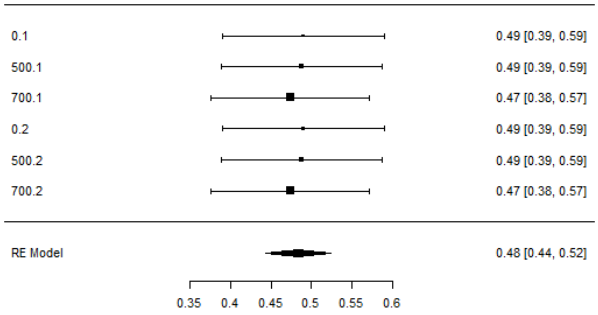

Figure 5. The forest plot acquired from the meta-analysis applied to its subgroups in the study of Koyuncu et al. (2001)

Figure 5, when evaluated in terms of subgroups in the study conducted by Koyuncu et al. (2001), claimed that the protocol with the highest effect rate was 40 mg FGA+500 IU PMSG (95% confidence interval; 0.39–0.59).

DISCUSSION

Although meta-analysis is a method that combines and reviews independent and comparable studies, it summarizes the effect sizes obtained from each study with a single statistic. By eliminating inconsistencies in individual studies, stronger and more precise estimates can be made for the effect size of the population with the analysis. These estimates

also found their relevance in the field of veterinary medicine and became widespread in field applications.

Table 6. Statistical values of the random effects model in subgroups in the study of Koyuncu et al. (2001)

Random-Effects Model (k = 6)						
	Estimate	**se**	**Z**	**p**	**Lower Bound**	**Upper Bound**
Intercept	0.484	0.0207	23.4	<.001	0.443	0.524

As far as the effect of synchronization methods on fertility parameters is concerned, meta-analysis has found its use as a valid method in recent studies. The consequences of melatonin implants on pregnancy rates and the number of lambs born in ewes were deciphered by meta-analysis (Palcini et al., 2011). Two different synchronization protocols in dairy cattle, effect on the rate of conception (Borchardt et al., 2017; Borchardt et al., 2018), the effect on pregnancy rate per insemination (Bisinotto et al., 2015), the effect on pregnancy rate (Rabiee et al., 2005) have been elucidated by meta-analysis method. Nonetheless, since these studies are generally limited to paired comparisons, it was beyond the scope of this article to evaluate the advantages of the protocols with a holistic approach.

As with primary research, meta-analysis studies begin with determining the purpose and developing relevant research questions. The data obtained from the studies are synthesized in common size, and the relationships between the characteristics of the studies and the calculated common size are examined. For this common size, the effect size is generally preferred. The most important reason why the effect size, which is a measure of practical significance, is preferred over the p-value, which gives an idea of whether the results are statistically significant. This value is independent of the sample size. In the study, the effect size (Estimate) was determined as 0.658 (0.571-0.744) p <0.01. We can say that the exposures made in Turkey or the methods applied between the periods we have determined (1995-2020) have a significant effect. We can say that interventional or non-invasive methods on pregnancy rates increase the birth rate statistically significantly.

The application of the intravaginal sponge progestagen for the aggregation of heat in ewes breeding attracts all ewes to the luteal stage but accelerates the entry of PMSG into the follicular stage of ewes after the elimination of the sponges. Other studies have reported that PMSG administration reduces the duration of oestrus but has an equal effect on fertility parameters (Zonturlu et al., 2011).

In this study, it was aimed to achieve a common result with the Bayesian meta-analysis by combining the outcomes of the studies comparing the synchronization protocols with the pregnancy rates in ewes and with the control groups.

In this meta-analysis, it was found that two studies had the highest effect size during the breeding season (Aköz et al., 2006; Koyuncu et al., 2001) with the synchronization protocol used in ewes; the fertility parameters were ameliorated by combining with various doses of PMSG at the end of intravaginal sponge application as a source of progesterone. These findings validated the effectiveness of progestagen and PMSG application used for synchronization of pregnancy in ewes, among other protocols. The effect size was 0.66.

In the evaluation made in terms of subgroups in the study conducted by Aköz et al. (2006), the effect size of the 40 mg FGA + 300 IU PMSG (5.99 US$) and 40 mg FGA + 700 IU PMSG (6.05 US$) protocol was calculated higher as compared to the other protocols. It would be more rational to opt for the method whereby costs, ease of application, and success

rates are optimum in the selection of the protocol to be applied in large ewes herds. According to Koyuncu et al. (2001) reported that the effect size of the 40 mg FGA + 500 IU PMSG (6.02 US$) protocol was higher than the other groups. Considering the two studies with high effect sizes as a result of meta-analysis in our study, it can be concluded that 40 mg FGA + PMSG (300–700 IU) applications are more effective on pregnancy rate compared to other synchronization protocols.

CONCLUSION

Estrus synchronization in ewes is employed to control pregnancy in the enterprises. Planning the pregnancy under enterprise conditions ensures optimum yield by propagating the lamb or milk yield to the whole year in line with the purpose of the enterprise. It is possible to regulate the reproductive performance in enterprises with estrus synchronization protocols and mating of ewes. However, it is essential to hunt for the optimal method in terms of economy and efficiency in enterprise conditions. Combining this study with the results of studies on the effects of synchronization protocols on pregnancy, it is predicted that Bayes meta-analysis will guide enterprises as a decision support system in achieving the optimum.

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Ethical approval: In the study, no experiments were made on any living thing or any personal information was used.

Conflict of interest: There is no conflict of interest.

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The effects of solutions of maca (*Lepidium meyenii*) powder as a food/feed supplement on the viability of murine macrophage cells by digital image analysis

ABSTRACT

Maca (*Lepidium meyenii*) is a tuber root plant and belong to Brassicaceae family and recently used as a supplement in human and animal nutrition. In this study, it was aimed to investigate the cytotoxicity of two solutions (aqueous and ethanol) of maca root powder by digital image analysis. Maca powder was mixed in ultra distilled water and ethanol (1:2 w/v) for 24 h at 4 °C. The mixtures were centrifuged and the supernatants were ten-fold diluted for cytotoxic analysis of Raw 264.7 murine macrophage cells. After seeded the cell cultures in microplates, ten-fold dilutions (from 10^{-1} to 10^{-7}) of both maca solutions were added as six replicates for 24 h. While the aqueous maca solution increased the number of dead cells at 10^{-1} (50 mg mL^{-1}), the ethanolic solution statistically increased the number of dead cells at 10^{-1}, 10^{-2} and 10^{-3} (50, 5 and 0.5 mg mL^{-1}) ($P<0.01$). In conclusion, the alcoholic preparation of maca powder caused a higher cytotoxic effect on the murine macrophage cells than the aqueous preparation due to the solvents and the dilution factor in this study.

Keywords: *Lepidium meyenii*, Food/Feed Supplement, Cytotoxicity

INTRODUCTION

Maca (*Lepidium meyenii*) is a tuber root plant and belong to Brassicaceae family such as broccoli, cabbage and turnip. On both human and animal nutrition, many researches were published about the effects of maca (Korkmaz et al., 2016; Bilal et al., 2016; Gonzales et al., 2020), its extracts and bioactive compounds such as polysaccharides, macamide, macaene, phenols and glucosinolates (Korkmaz, 2018; Gonzales et al., 2020). So, there are many products in various forms of maca as food and feed supplements for the consumptions of human and animals. Therefore, it was reported that the maca extracts had antioxidant, free-radical scavenging, cell viability and cytotoxic effects in vitro studies. These effects vary due to the extraction methods and the most used in the studies are aqueous and alcoholic extractions methods (Rodríguez-Huamán et al., 2017; Ye et al., 2018).

Besides to the methods of biochemical, colorimetric and spectrophotometric analysis, the methods of digital image analysis were recently developed to quantify the cell viability and the cytotoxicity for both *in vivo* and *in vitro* studies (Collins, 2007; Sandhya, 2011). The advantages of these methods were to be rapid, economic and pollution-free (Grishagin, 2015; Freitag et al., 2020).

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Research Article

Serol KORKMAZ[1a]
Ayşe PARMAKSIZ[2b]
Ahmet SAİT[2c]
İrem OMURTAG KORKMAZ[3d]

[1]Institute of Health Sciences, Marmara University, Istanbul Turkey
[2]Pendik Veterinary Control Institute, Istanbul, Turkey
[3]Institute of Health Sciences, Marmara University, Istanbul Turkey

ORCİD-
[a]0000-0001-8970-6883
[b]0000-0003-1242-7987
[c]0000-0001-7658-8793
[d]0000-0001-7918-6212

Correspondence
Serol KORKMAZ
serolkorkmaz@yahoo.com

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In this study, the aim was to investigate the effect of aqueous and alcoholic solutions of maca powder on the cell viability using image analysis.

MATERIAL and METHOD

Materials

Ultra-pure water was from PURELAB flex 3, Elga, United Kingdom. Ethanol (%99.8, CAS 64-17-5) and trypan blue solution (0.4%, sterile-filtered) was purchased from Sigma-Aldrich, Germany. DMEM (500mL, Dulbecco's Modified Eagle Medium (w/o L-glutamine, w/o sodium pyruvate), L-alanyl-L-glutamine (200 mM), penicillin (10,000 units mL^{-1})-streptomycin (10 mg mL^{-1})-amphotericin B (0.025 mg mL^{-1}) solution, foetal bovine serum (FBS, European grade), trypsin-EDTA solution (w/o phenol red) and Dulbecco's Phosphate Buffered Saline (PBS w/o calcium magnesium) were obtained from Biological Industries, USA. For cell culture, the 75 cm^2-flasks were from EasYFlask, Thermo Scientific and 96-well microplates were obtained from CellStar, Greiner Bio-One, Germany. The syringe filters (sterile, 0.22 µm) was purchased from Merck Millipore, Germany. Sterile centrifuge tubes were from ISOLAB, Germany.

Plant material and preparation

Organic maca (*Lepidium meyenii* Walp.) root powder was cultivated in Junin and imported from Peru. Maca powder samples were added to ultra-pure water (1:2, w/v) (MW) or ethanol (1:2, w/v) (ME) and mixed well. Then, they were kept at 4 °C overnight. After centrifuged at 3500 rpm for 15 min, both supernatants were filtered using the sterile syringe filters (0.22 µm) and stored in the sterile tubes at 4 °C for further analysis.

Cell culture

Raw 264.7 murine macrophage cells were from American Type Culture Collection (ATCC), Manassas, VA, USA). Serum heat-inactivation was performed in a water bath at 56 ◦C for 30 minutes before use. All solutions were heated to 37 °C before the process of cell cultivation. The cells were cultured in DMEM supplemented with 10% inactivated foetal bovine serum, L-alanyl-L-glutamine and 1% penicillin-streptomycin at 37 °C. It was maintained in cell culture flasks (75 cm^2) in the incubator with the condition of 37 °C and 5% CO_2 for 24 h to confluence. After the incubation, all waste medium was discarded and the cell monolayer was disaggregated with trypsin-EDTA in the incubator for 3 min. The suspension was centrifuged with a refrigerated centrifuge at 1200 rpm/15 min. the supernatant was discarded and the pellet was suspended in fresh medium. Then, viable and dead cells were counted by the method of trypan blue (0.4%) staining with a haemocytometer as in the section of Cell Staining and Image Analysis. For the preparation of 96-well microplates, 100 µL of the stock viable cell suspension (3 x 10^5 cell mL^{-1}) was seeded in each well (3 x 10^4 cell) and kept in the incubator for 24 h to confluence at least of 90%.

Cytotoxicity assay

Both of maca solutions (MW and ME) was 10-fold serially diluted with DMEM supplemented 10% FBS and antibiotics from 10^{-1} to 10^{-7} (from 50 to 0.00005 mg maca mL^{-1}). Then, 100 µL of each dilution was added to six-replicated wells of the 96-well microplate seed with the cell culture. DMEM solution was only added to the control wells. The microplates were incubated at 37 °C and 5% CO2 for 24 h. The inert microscopy (Olympus ix71, Tokyo, Japan) was used to observe and image the morphological changes of the cells for the cytotoxicity assay.

Cell staining and image analysis

The supernatants were discarded from the microplates and the wells were washed with PBS. 40 µL of trypan blue solution (0.4%) was added to each well and kept for 5 min Then, all wells were washed with PBS (Aung et al., 2019). Blue color dyed cells was considered as dead and were photographed at 20x (1300 x 1300 pixels,

96 DPI) for the monolayer area imagination and the measurement of cell mortality with ImageJ 1.53 software (National Institutes of Health, Bethesda, Maryland, USA) (Figure 1.).

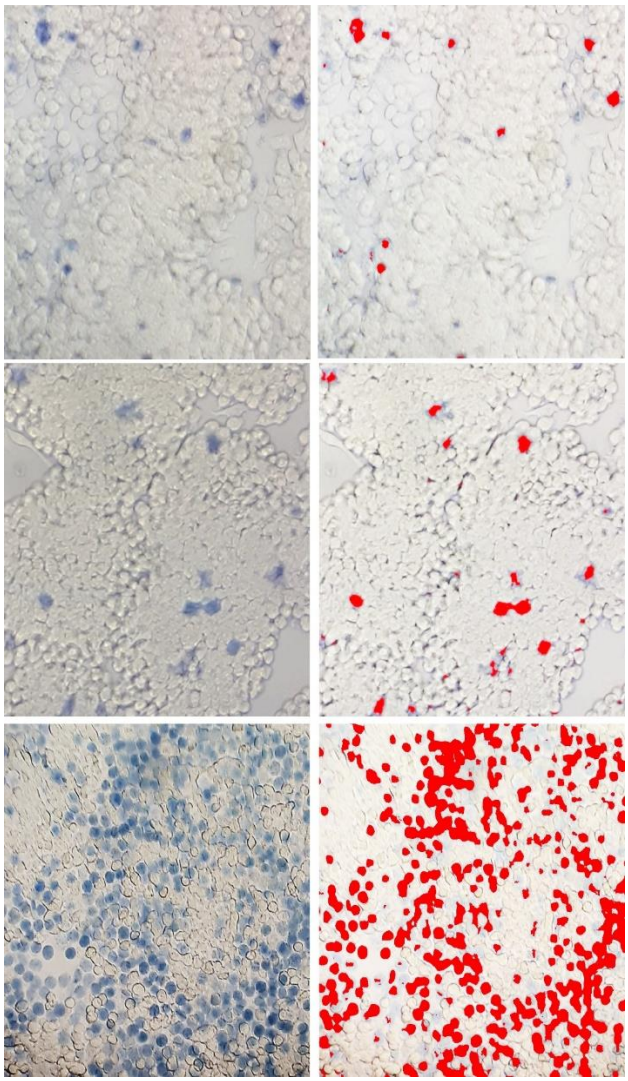

Figure 1. The dead cell counting (red coloured on right) and image analysis by ImageJ software.

Statistical analysis

The means of data and the standard deviations (SD) were calculated for each group using SPSS 21 software (SPSS Inc., IL, USA). The effects on cell viability were analysed by one-way analysis of variance (ANOVA). Differences among data means were compared using the Tukey post hoc test at a $P < 0.05$ level of significance.

RESULTS

The mean area of dead cells was determined as %0.76 of the total area in control wells and the mean dead cells were counted as 75.71 (±18.73) cells by the image analysis (Table 2.). And, no cytotoxic sign was observed in all six-replicate wells (0/6) of control (Table 1). For the aqueous solution of maca, the cytotoxic effect and the mean dead area of 2.45% was determined in the three of six-replicate wells (3/6) treated with the log-1 dilution (50 mg mL^{-1}) (Table 1. and 2.). But no cytotoxic effect was observed for the lower dilutions (from 10^{-2} to 10^{-7}) of the aqueous solution of maca (Table 1.). For the ethanol solution of maca, the mean dead areas of 29.17%, 25.66% and 22.51% were measured in the cells treated with the 10^{-1}, 10^{-2} and 10^{-3} dilutions (50, 5 and 0.5 mg mL^{-1}), respectively (Table 1. and 2.). These cytotoxic effects were observed in all (6/6), three (3/6) and one (1/6) of six-replicate wells for 10^{-1}, 10^{-2} and 10^{-3} dilutions respectively. However, there was no observed cytotoxic effect for the lower dilutions than the log-3 of the ethanol solution of maca (Table 1.).

When the results of cytotoxic dilutions of both solutions and the control were statistically compared with each other, the number of dead cells was significantly higher in the dilutions (331.5, 328.83 and 301.27 in the 10^{-1}, 10^{-2} and 10^{-3} respectively) of the ethanol solution than the aqueous solution and the cell control ($P<0,01$). Likewise, the dead cell count of the 10^{-1} (50 mg mL^{-1}) dilution of aqueous maca solution was statistically higher than the cell control ($P<0.01$)

Table 1. The cell mortality results of six-replicated wells on 96-well microplate.

	MW		ME	
Dilution (Log) (mg mL^{-1})	**Dead Area (%)**	**CPE of Wells**	**Death Area (%)**	**CPE of Wells**
10^{-1} (50 mg mL^{-1})	2.45	3/6	29.17	6/6
10^{-2} (5 mg mL^{-1})	0.95	0/6	25.66	3/6
10^{-3} (0.5 mg mL^{-1})	0.91	0/6	22.51	1/6
10^{-4} (0.05 mg mL^{-1})	0.71	0/6	0.97	0/6
-5 (0.005 mg mL^{-1})	0.56	0/6	0.91	0/6
-6 (0.0005 mg mL^{-1})	0.60	0/6	0.76	0/6
-7 (0.00005 mg mL^{-1})	0.71	0/6	0.81	0/6
Control	0.76	0/6	0.76	0/6

Table 2. The comparison of effects of the dilutions (10^{-1}-10^{-3}) on the cell viability by the image analysis of cell culture

Items	**Control**	**MW**	**ME**	**MW**	**ME**	**MW**	**ME**
		10^{-1} (50 mg **mL^{-1}**)		10^{-2} (5 mg **mL^{-1}**)		10^{-3} (0.5 mg **mL^{-1}**)	
Dead cell number (n)	75.71a	189b	331.5c	112.64a	328.83c	112.29a	301.27c
% of dead area (pixel^2)	0.76	2.45	29.17	0.95	25.66	0.91	22.51
SD	18.73	8.41	13.18	6.53	15.44	9.30	12.87
p value		<0.01		<0.01		<0.01	

DISCUSSION

Many compounds isolated from the plants, also maca, have been reported to possess potential cytotoxic activity (Bai et al., 2015; Zhou et al., 2017; 2018). Alkaloids and flavonoid, isolated from the aqueous acetone and ethanol extracts of maca powder, was reported as a cytotoxic compound on five human cancer cell lines at higher 40 µM (Bai et al., 2015; Zhou et al., 2017; 2018). Also, flavonoids in maca negatively affected the RAW 264.7 macrophages when compared the control cells (Bai et al., 2015). In the study of Del Valle Mendoza et al. (2014) the methanol extract of maca showed low cytotoxicity on MDCK cells, but the aqueous extract of maca powder did not show cytotoxic effect. Likewise, Wang et al. (2016) reported the immunomodulatory and antioxidant effects of aqueous polysaccharides from maca powder on RAW 264.7 macrophages without cytotoxic effect when compared the control. Ye et al. (2018) showed that the strong solvent (dichloromethane and ethyl acetate) extracts had serious cytotoxic effects. In the different solvent extracts of maca leaves, the aqueous extract showed more antioxidant capacity than chloroform, dichloromethane and chloroform-ethanolic extracts (Rodríguez-Huamán et al., 2017). In addition to the solvents, it was shown that the cytotoxic effects of maca extracts differed due to the kinds of cell cultures (Xia et al., 2018; Fu et al., 2021). In this study, the ethanolic dilutions of maca powder up to log 10^{-3} (50, 5 and 0.5 mg mL^{-1}) caused more cell mortality than the aqueous dilutions ($P<0.01$), similar to the results of Wang et al. (2016), Del Valle Mendoza et al. (2014) and Rodríguez-Huamán et al. (2017). However, the aqueous dilutions also increased the number of dead cells at only log 10^{-1} dilution (50 mg mL^{-1}) when compared with the control.

CONCLUSION

Many researchers reported that maca extracted by strong solvents such as alcohols adversely affected the cell viability, despite their rich bioactive compound content. Similarly, the alcoholic preparation of maca powder caused the

higher cytotoxic effect on the murine macrophage cells than the aqueous preparation in this study due to the dilution factor of the toxic solvent.

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Ethical approval: In the study, no experiments were made on any living thing or any personal information was used.

Conflict of interest: There is no conflict of interest.

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The antimicrobial efficiency of green seaweeds from the Mediterranean coast against some pathogenic bacteria

ABSTRACT

During the past several years, microbial resistance to common antibiotics has continually increased, and this growing resistance threatens the effective treatment of bacterial infections. Thus, there is increased research on novel Antimicrobial agents like seaweeds. In this study, crude extracts of three seaweeds (Ulva clathrate, Ulva lactuca and Ulva compressa) were obtained with a Soxhlet extraction apparatus. Evaluation of antimicrobial efficiency was carried out using well diffusion method and microdilution method (MIC) at different concentrations (100-0.195mg/ml) for Staphylococcus aureus and Escherichia coli. The green seaweed extracts produced inhibition zones ranging from 7 to 12.5 mm. Methanol extracts produced the strongest inhibitory activity against the tested bacterial species. Overall, this study provides data on the potential use of algal extracts for the development of antimicrobial agents to treat infectious diseases.

Keywords: Antimicrobial, green seaweeds, well diffusion method, microdilution method (MIC).

INTRODUCTION

Diseases affecting livestock can have a significant effect on animal productivity and production and human health (Das et al., 2019). The increasing spread of antibiotic resistance created serious problems for treatment of bacterial infections and continue to be a major challenge for public health worldwide (Elmanama et al., 2019). Infectious poultry shows an assortment of manifestations such as diarrhea, paralysis, and respiratory issues. It ought to be accentuated at the start that the counteraction of infection in a poultry rush through sound administration is vital (Das et al., 2019). This is because albeit some irresistible illnesses can be dealt with, for some it is an exercise in futility since cash and infectious fowls ought to be discarded immediately. Explicit medications are normally suggested for resistant microorgansims that might be partitioned into three classes: bacterial, protozoan, and fungal infections, while some popular illnesses can be forestalled by immunization (Logue et al., 2020). Damage to the intestinal quality due to pathogenic microbes may cause an issue in feed proficiency and diminished pace of gain to raise the absolute creation costs. Nevertheless, further extreme enteric harmed by bacterial diseases will cause plain sickness and high mortality in poultry rush, and food creatures can likewise communicate microbes to other creature species straightforwardly or by implication through natural dispersal (Laptev et al., 2019).

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Research Article

Adil AKSOY[1a]
Mahmoud EL HİNDİ[2b]

[1]Eskil Vocation of High School, Laboratory Veterinary Science, Aksaray University, Aksaray, Turkey
[2]Department of Biotechnology, Faculty of Science, Islamic University of Gaza, Gaza, Palestine

ORCİD-
[a]0000-0002-1521-3100
[b]0000-0002-9815-6526

Correspondence
Adil AKSOY
adilaksoy@aksaray.edu.tr

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Escherichia coli infections are one of the significant issues that cause an incredible danger to the productivity of poultry ranchers worldwide. The intestinal tract *E. coli* has become a normal inhabitant of poultry under the influence of predisposing factors such as inadequate and faulty ventilation, overcrowding, hunger, thirst, extremes of temperatures and low vitality, high mortality during rearing, reduced weight gain, and condemnation of birds at the time of slaughter (Elmanama et al., 2019; Khanal et al., 2019). Broiler chickens that are frequently infected with *E.coli* have reported depression, loss of appetite, tendency to huddle respiratory distress, reduction of weight gain, dropped wing, closed eyes, cyanosis, and labored breathing (El Seedy et al., 2019). *Staphylococcus aureus* (*S. aureus*) is a non-motile, non-spore producer, Gram-positive coccus causing a wide assortment of suppurative infections in people and animals. In people, it is perhaps the most widely recognized reason for bacteremia, skin, and delicate tissue infection (Madzgalla et al., 2016; Thomer et al., 2016). While in animals, it is perhaps the most common causative specialists of clinical and subclinical mastitis in dairy ranches, causing roughly 33% of cases in steers, and bringing about huge financial misfortunes (Gussmann et al., 2019). It is a huge reason for extreme nosocomial diseases and a universally significant wellbeing concern (Benrabia et al., 2020). The chance of human pollution of poultry corpses by slaughterhouse representatives can't be precluded (Amoako et al., 2019). The natural products with biological activities from living organisms are very important for discovering new Antimicrobial agents. Such interest was expanded over the most recent very long time because of the development of pathogenic microorganisms that are impervious to significant classes of antimicrobials. Algal constituents a modest wellspring of crude materials for the extraction of different mixes of organic exercises. In addition, among all macroalgae, the green growth has a place with the variety Ulva are the least researched for antimicrobial activities when contrasted with the red (Rhodophyta) and earthy colored green growth (Phaeophyta). This work was performed to evaluate the efficacy of algae extracts *U. clathrate, U. Lactuca and U. compressa* against *E. coli* and *S. aureus.*

MATERIAL and METHOD

Algal source: The three marine Ulva species (*U. clathrate*, *U. Lactuca* and *U. compressa*) were that corresponded roughly to the months of June, July and August in 2016, and July and August in 2017, from uncovered rough destinations close to the edge of the shore of the Mediterranean Sea (https://goo.gl/maps/r3y4h UrfR12jkuz96). The ordered ID of species was finished by utilizing standard writing and ordered keys. The gathered green growth was moved to the research facility, where they were washed completely with tap water to eliminate any related flotsam and jetsam like sand and shells, and afterward, the drying-of examples were left in the shadow of the room temperature for fourteen days.

Preparation of algae sample: The drying process is followed by samples' cutting process into little pieces, ground to a fine powder, and put away in firmly shut plastic cups in dull at room temperature until usage.

Preparation of algae extracts: 30 grams of finely ground test powder extracted in a Soxhlet extractor by using 300 ml of with methanol, chloroform, and hexane for 8 hours. The resulting extracts were evaporated using oven temperature 37 °C for 3 days. Then all extracts were dissolved in DMSO. One gram of each extract was dissolved in 5 ml of DMSO. The standard concentration of extracts was 200 mg / ml. Then extracts were sterilized using 0.22 µm membrane filters and all samples were maintained at -4°C until used (Pina-Pérez et al., 2017).

Test Microorganisms: For the antibacterial assessment, cultures of Gram-positive bacteria *S. aureus* and Gram-negative *E. coli* were utilized as test microorganisms. All microorganisms provided by the Department of Biology and Biotechnology, Faculty of Science, Islamic University of Gaza, Palestine.

The antibiotic sensitivity assay: Antibiotic sensitivity test was performed by disc diffusion method. By using sterile forceps, the selected of antibiotics (Table 1.) were put on the surface of plate. The plates were incubated at 37^0C for 24 h. Then the zones of inhibition were measured in millimeter by using ruler. (Kirbag et al, 2009; Mabrouk, 2012). Sensitivity assay was labeled sensitive, center, or protected, considering the CLSI standards. A total of 15 antimicrobials were used in this examination as exhibited in (Table 1.)

Table 1. List of antibiotic potency.

Antibiotics	Symbol	Antibiotics potency	Manufactured by
Cefotaxime	CXM	30 mg	Himedia, Indian & Bioanalyse, Turkey
Cefuroxime	CTX	30 mg	Himedia, Indian & Bioanalyse, Turkey
Cefaclor	CEC, CF	30 mg	Himedia, Indian & Bioanalyse, Turkey
Cefalexin	CL, CN	30 mg	Himedia, Indian & Bioanalyse, Turkey
Ofloxacin	OFX	5 mg	Himedia, Indian & Bioanalyse, Turkey
Ciprofloxacin	CIP	5 mg	Himedia, Indian & Bioanalyse, Turkey
Norfloxacin	NOR	10 mg	Himedia, Indian & Bioanalyse, Turkey
Nalidixic acid	NA	30 mg	Himedia, Indian & Bioanalyse, Turkey
Amikacin	AK	30 µg	Himedia, Indian & Bioanalyse, Turkey
Gentamicin	GMN	10 mg	Himedia, Indian & Bioanalyse, Turkey
Ampicillin	AM	10 mg	Himedia, Indian & Bioanalyse, Turkey
Oxacillin	OX	1 mg	Himedia, Indian & Bioanalyse, Turkey
Amoxiclav	AMC	30 mg	Himedia, Indian & Bioanalyse, Turkey
Rifampicin	RIF	5 mg	Himedia, Indian & Bioanalyse, Turkey
Penicillin G	P	10 mg	Himedia, Indian & Bioanalyse, Turkey
Tetracycline	TE	30 mg	Himedia, Indian & Bioanalyse, Turkey

Well-diffusion test: Antibacterial activity was examined utilizing the agar well-diffusion test. Muller Hinton Agar Medium (MHA) were disinfected via autoclaving at 121 ºC and 15 lbs. pressure for 20 minutes. Autoclaved mediums were inoculated with bacterial strain under aseptic conditions and wells (diameter=6mm) were filled with 50 µl of the test samples and incubated at 37 °C for 24 hours. After incubation, all plates were noticed for a clear zone around the well. The diameter of the growth inhibition zones was measured in millimeters. (El-Hindi et al., 2017; Sharma, 2011).

Algal Extracts Least Inhibitory Concentration (MIC): The antibacterial activity of extracts against tested bacteria was also evaluated by the broth macrodilution method (96- well plates). Algal Extracts were diluted a number of times through a sterile diluent and the obtained concentration ranges were from (100 to 0.1953) mg/ml. 10 µl of bacterial suspension (10^6 CFU/ml) was added to each well except a positive control. Extracts with media was used as a positive control and inoculum with media was used as a negative control. The test plates were incubated at 37°C for 24 h. After 18 h 50 µl of a 0.01% solution of 2, 3, 5 triphenyl tetrazolium chloride (TTC) as indicator was added to the wells and the plate was incubated for another hour. The colorless tetrazolium salt is reduced to red colored

product by active bacteria, the inhibition of growth can be detected when the solution in the wells remains clear after incubation with TTC (Adwan, and Mhanna, 2008; Gupta and Sharma, 2006; Souza et al., 2005).

RESULTS

Antibiotics assays Measurement: The results in (Table 2.) showed the antimicrobial activity of some Antibiotics including Ciprofloxacin (CIP), Ampicillin (AM), Cefotaxime (CTX), Nalidixic destructive (NA), Norfloxacin (NOR), Cefuroxime (CXM), Cefaclor (CF or CEC), Ofloxacin (OFX), Cefalexin (CL or CN), Tetracycline (TE), Rifampicin (RIF), Amoxyclav (AMC), Gentamycin (GMN), Penicillin (P) and Oxacillin (OX), while Amikacin (AK) by disc diffusion method against tested microorganisms (Table 2.).

Table 2. Impact of antibiotic reference standard on pathogenic microorganisms (hindrance zone communicated by mm).

Antibiotics / Bacteria	CFF	AM	CTX	NA	NOR	CXM	CF	OFX	CL	TE	RIF	AMC	GMN	P	OX	AK
E. coli	0	0	0	0	0	0	0	0	0	9	8	0	11	*	*	20
S. aureus	0	0	0	*	0	0	0	0	0	*	*	0	*	7	0	22

*. Not tested, mm. millimeter.

Well-diffusion test: The results in (Table 3.) showed the antimicrobial activity by well diffusion method against tested microorganisms. Methanol and chloroform extracts of *U. clathrate*, *U. lactuca* and *U. compressa* showed antibacterial action toward *S. aureus* and *E. coli*. In each case, the hexane concentrations of the three Ulva species didn't show any antibacterial action against the tested bacteria. The methanol and chloroform extracts of *U. clathrate* showed antibacterial action against all tested microorganisms and the methanol extracts showed better antibacterial action than the chloroform extracts. The information additionally indicated that are Gram-negative and Gram-positive bacteria were influenced by algal extracts. Notwithstanding, *U. clathrate*, *U. lactuca* and *U. compres* were active towards Gram-negative bacteria (*E. coli*) than Gram-positive bacteria (*S .aureus*).

Table 3. The inhibition zone in millimeter (mm) of methanol extracts of *U. clathrate*, *U. lactuca*, and *U. compressa* against *E. coli* and *S. aureus*.

Bacteria	Algae	Experiment 1 (mm)	Experiment 2 (mm)	Standard Deviation ± Mean (mm)
E. coli	*U. clathrate*	15	12	2.1±13.5
	U. lactuca	12	13	0.7±12.5
	U. compressa	13	11	1.4±12
S. aureus	*U. clathrata*	12	12	0±12
	U. lactuca	-	-	-
	U. compressa	-	-	-

Methanol extract: The methanol extract of *U. clathrate* showed 13.5 mm zone of inhibition against *E. coli* and 12 mm against *S. aureus*. The Methanol extract from *U. lactuca* showed 12.5 zone of inhibition mm against *E. coli*. The least activity action (12 mm) was seen with a methanol extract of *U. compressa* and *U. clathrate* towards *E. coli* and *S. aureus* independently. No effect of the methanol extracts from *U. lactuca* and *U. compressa* against *S. aureus*.

Chloroform extract: The chloroform extract of *U. compressa* showed of 12.5 mm zone of inhibition against *E. coli* and 10.5 mm against *S. aureus*. The chloroform extract of *U. clathrate* showed 12 mm zone of inhibition against *S. aureus*, and 10.5 mm against *E. coli*. The chloroform extract of *U. lactuca* showed 11 mm zone of inhibition against *E. coli* (Table 4.).

Table 4. The zone of inhibition in millimeter (mm) of chloroform extracts of *U. clathrate*, *U. lactuca*, and *U. compressa* against *E. coli* and *S. aureus*.

Bacteria	Algae	Experiment 1 (mm)	Experiment 2 (mm)	Standard Deviation ± Mean (mm)
E. coli	*U. clathrate*	11	10	0.7±10.5
	U. lactuca	10	12	1.4±11
	U. compressa	12	13	0.7±12.5
S. aureus	*U. clathrata*	12	12	0±12
	U. lactuca	-	-	-
	U. compressa	11	10	0.7±10.5

Minimum Inhibitory Concentrations (MICs): MIC values of all tested fruit extracts against tested microorganisms are summarized in (Tables 5.-7.).

Methanol extract: As observed in (Table 5.), the growth of *E. coli* and *S. aureus* was inhibited by the methanolic extract of *U. clathrate* at concentrations of 3.125 and 12.5 mg/ml separately. For the methanolic extract of *U. lactuca*, the growth of both *E. coli* and *S. aureus* was repressed at 12.5 mg/ml. Methanolic extract from *U. compressa* showed antibacterial action against the two tested bacteria, and the strong activity showed against *E. coli* (MIC= 6.25 mg/ml), and against *S. aureus* (MIC=12.5 mg/ml).

Table 5. The MICs (mg/ml) of methanol extract of green algae against the tested bacteria

Algae	Bacteria	
	E. coli	***S. aureus***
U. clathrata	3.125	12.5
U. lactuca	12.5	12.5
U. compressa	6.25	12.5

Table 6. The MICs (mg/ml) of chloroform extract of green algae against the tested bacteria

Algae	Bacteria	
	E. coli	***S. aureus***
U. clathrata	25	25
U. lactuca	12.5	25
U. compressa	12.5	100

Chloroform extract: As observed in (Table 6.), the MIC of the chloroform extract of *U. clathrate* for *E. coli* and for *S. aureus* was 25 mg/ml. The MIC of chloroform extract of *U. lactuca* against *E. coli* was 12.5 mg/ml and was 25 mg/ml for *S. aureus*. The growth of *E. coli* was inhibited by the chloroform extracts 12.5 mg/ml, and 100 mg/ml for *S. aureus*.

Table 7. The MICs (mg/ml) of hexane extract of green algae against the tested bacteria.

Algae	Bacteria	
	E. coli	***S. aureus***
U. clathrata	25	25
U. lactuca	25	25
U. compressa	12.5	25

Hexane extract: As observed in (Table 6.), the MIC of the chloroform extract of *U. clathrate* for *E. coli* and *S. aureus* was 25 mg/ml. The MIC of chloroform extract of *U. lactuca* against *E. coli* was 12.5 mg/ml and was 25 mg/ml for *S. aureus*. The growth of *E. coli* was inhibited by the chloroform extract of *U. compressa* at 12.5 mg/ml, and 100 mg/ml for *S. aureus*.

DISCUSSION

The seaweeds (*U. clathrate, U. lactuca and U. compressa*) were separated by three solvents and assessed for their antibacterial activity against the two clinically pathogenic bacterial species of *S. aureus* and *E. coli*. The action of each algal concentration was assessed by two methods: well-diffusion method and lowest minimum inhibitory concentration (MIC). This study shows clearly that the green, marine seaweeds contain antibacterial compounds. They can be considered as a promising source for bioactive compounds including antibacterial agents. The outcomes here are in concurrence with the other report performed by Elnabris (Elnabris et al., 2013), which introduced the antimicrobial properties of the methanolic concentrations of marine ocean growth *U. lactuca, U. compressa* (Chlorophyta), Padina Pavonica (Phaeophyta), and Jania Rubens (Rhodophyta). The natural activity of Green Seaweeds was ascribed to the presence of Sulfated Polysaccharide (SP) of uncommon substance arrangement and construction in the cell mass of these green Seaweeds called Ulvan. Recently, Ulvan has been broadly assessed for advancing other medications and practical food sources (Wijesekara et al., 2011). Studies proposed that sulfated polysaccharides can likewise display useful bioactivity mixes such as anticoagulant, antiviral, cancer prevention agent, calming (Costa et al., 2010), and antiproliferative (Mohamed et al., 2012). The outcomes demonstrate that the methanolic concentrations of all Seaweeds showed preferable antibacterial activity over chloroform and hexane extracts. Antimicrobial activity relies upon both algal species and the effectiveness of the extraction. A couple of assessments concerning the suitability of extraction procedures include that the methanol extraction yields higher antibacterial activity than various solvents such as hexane and chloroform (Sastry and Rao, 1994; Seenivasan et. al, 2010) and likewise propose that methanol is the best for separating the successful antibacterial materials from the green algae species. Other studies reported that chloroform is superior to methanol (Omar et al., 2012; Rajasulochana et al., 2009) and found that the petrol ether, diethyl, and ethyl acetic acid derivation were better than methanol for extracting the bioactive compounds from green algae. The algal extracts of *U. clathrat*e have higher antibacterial activities against the tested bacteria than extracts of *U. lactuca* and *U. compressa* which may indicate that *U. clathrat*e has good antibacterial activity-related compounds. Methanolic separates *U. clathrate* exhibited more noteworthy cell reinforcement potential and the most elevated phenolic substance and flavonoid content in correlation with those of different types of green kelp from Northern Coasts of the Persian Gulf (Farasat et al., 2014). Sulfated polysaccharides Ulvan from *U. clathrate* was found to have antiviral action

and repress viral connection/section and cell-cell combination in NDV contamination (Aguilar-Briseño et al., 2015). Furthermore, the new examination has another huge outcome which was the moderate antibacterial movement of the concentrations of *U. lactuca* when tried against the *E. coli* and *S. aureus* development. No antimicrobial action was additionally announced by (Perez et al., 1990) when the natural concentrations of similar green seaweeds were assessed. Variety in effects of these investigations might be because of species variety, the time (season) and area of test assortment, and the amount of test utilized (Sivakumar, and Vignesh, 2014). In the present study, the well-diffusion and MIC method using 2,3,5,-triphenyl tetrazolium chloride (TTC) as an indicator was used as biological assays to evaluate the antibacterial activities of seaweeds because of their technical simplicity, effectiveness, sensitivity, rapidity, and relatively low cost of these methods. Additionally, if the color intensity of the red formazan formed in the microdilution method is measured using a spectrophotometer at 480 nm, the test could also provide a quantitative determination of the antibacterial activity of antibacterial agents (Moussa et al., 2013). Seaweeds were evaluated and exhibited higher antimicrobial activity towards Gram-negative bacteria than the Gram-positive bacterial species. *E. coli* was noted to have larger inhibition diameters and nearly have fewer MICs than *S. aureus*. Similarly, Seenivasan et al., (2010) reported a more inhibitive effect of Acetone, Methanol, and Ethanol extracts of three marine green algae species (*Chaetomorpha aerea*, *Enteromorpha intestinalis*, *Ulva fasciata*) on the growth of Gram-negative bacteria such as *E. coli*, than Gram-positive bacteria such as *S. aureus*. This is intriguing since a few reports concerning the antimicrobial action of kelp indicated opposite outcomes (Ibtissam et al., 2009; Taskin et al., 2010). A few researchers propose that the Gram-negative bacteria have an external layer act as a boundary to numerous natural substances, including antimicrobials (Tortora et al., 2001; Tshikalange et al., 2005). The perceptions of the current investigation show that the connections between the measurements of the restraint zones and the MIC esteems differed and may rely upon the sort of dissolvable utilized and the bioactive compounds included (Cox et al, 2010). Hexane separates didn't show any antimicrobial action against the tried microbes in the well-diffusion method. This outcome could be likely due to the non-polar nature of the compound extracted by hexane which couldn't diffuse well in the agar polar climate. Comparatively opposed to the well-diffusion method, all algae seaweed showed antimicrobial action against microorganisms in the microdilution technique.

CONCLUSION

In conclusion, the results demonstrated that the crude extract of green seaweeds under investigation exhibited antimicrobial activities against the tested bacteria species. This proved that the marine macroalgae are potential sources of biologically active compounds which are effective in resisting bacterial growth and could be investigated for Antimicrobial agents invention for many drug-resistance microorganisms. Marine algae are also gaining growing prominence in the pharmaceutical industries around the world. Further biochemical analyses, however, are necessary to isolate, characterize, and determine the bioactivity compounds. Overall, the present study provides data to show the potential utilization of green growth extracts for the advancement against microbial specialists for treating irresistible illnesses.

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Versatile analysis of some biochemical and hematological parameters of sheep

ABSTRACT

Biochemical, whole blood hematological and macro-mineral values are critical health and disease status parameters, which depend on animals' conditions, age, gender, breed, region, husbandry, geographical differences, seasonal changes, rearing location and diet. In this study, all parameters were measured in blood samples obtained from healthy Akkaraman sheep, composed of lambs and adults of both sexes. Analyses of the parameters were carried out with commercial assay kits. When comparing the values of the biochemical variables, a significant difference ($P<0.05$) between the four groups was observed in the concentrations of the following variables evaluated: glucose, total bilirubin, urea, and creatinin. A significant difference ($P<0.05$) between the four groups was found in the concentrations of the following variables: evaluated red blood cell, mean red blood cell volume, mean red blood cell hemoglobin, platelets and mean platelet volume. When comparing the values of the variables, a significant difference ($P<0.05$) between the four groups was observed in the concentrations of iron. The values of calcium and phosphorus were not found statistically significant. With a summary of the current literature, it could not be found a detailed study on advanced biochemical, mineral and hematological reference values for Akkaraman breeds in Aksaray region. Thus, our goal is to identify and present the values for the total biochemical and hematological parameters of Akkaraman sheep raised in the Aksaray region of Turkey. It is expected that these results may be used as reference values for Akkaraman sheep in this region.

Keywords: Age, akkaraman sheep, biochemical parameters, gender, hematological parameters, mineral assay

INTRODUCTION

The analysis of blood content contributes to detailed check up the organisms. So, blood as a vital tissue, plays a unique role in the metabolic, physiological, nutritional and also pathological status of a living organism. Blood biochemistry is the primary diagnostic tool in determining the health status of and in investigating diseases in human and animal (Braun et al., 2010 and Onasanya et al., 2015). Factors such as nutrition, stress, temperature-climate, seasonal differences, disease, muscle activity, age, sex and race affect the physiological values of parameters biochemically, and these conditions are important in clinical biochemistry (Gunduz, 2000). The determination of specific reference intervals is important for both clinicians and academicians so they can accurately interpret biochemical values in different animal species and breeds (Kaneko et al., 2008; Meyer and Harvey, 2004). The primary use of clinical biochemistry in human and animal are following up on an individual's health status, diagnosing disease and monitoring treatment (Braun et al., 2010).

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Research Article

Neşe Hayat AKSOY[1a]
Tahir KARAŞAHIN[2b]
Şükrü DURSUN[3c]
Neffel Kürşat AKBULUT[4d]
Ali Evren HAYDARDEDEOĞLU[5e]
Ramazan İLGUN[6f]
Olga BÜYÜKLEBLEBİCİ[7g]

[1]Department of Biochemistry, Faculty of Veterinary Medicine, Aksaray University, Aksaray, Turkey
[2]Aksaray University, Faculty of Veterinary Medicine, Department of Physiology, Aksaray, Turkey
[3]Aksaray University, Faculty of Veterinary Medicine, Department of Reproduction and Gynecology, Aksaray, Turkey
[4]Bahri Dagdas International Agricultural Research Institute, Konya, Turkey
[5]Aksaray University, Faculty of Veterinary Medicine, Department of Internal Medicine, Aksaray, Turkey
[6]Aksaray University, Faculty of Veterinary Medicine, Department of Anatomy, Aksaray, Turkey
[7]Mersin University, Technical Sciences Vocational School, Mersin, Turkey

ORCİD-
[a]0000-0001-9039-555X
[b]0000-0003-2358-0389
[c]0000-0002-2453-3464
[d]0000-0002-2829-4712
[e]0000-0002-8473-0072
[f]0000-0003-0150-3008
[g]0000-0002-6702-5769

Correspondence
Neşe Hayat AKSOY
nhaksoy@gmail.com

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Biochemical profiles provide reliable information on the health status of animals and reflect the animal's responsiveness to its internal and external environment (Onasanya et al., 2015). Because there are so many different ovine breeds and production systems differ so much among regions and countries, reference values for sheep definitely show variations. On the basis of data from the "Clinical and Laboratory Standards Institute (CLSI)", it is recommended that reference intervals be produced or validated by each laboratory with its own techniques in a similar as possible population of animals (Braun et al., 2010; CLSI, 2010).

Nowadays the hematological analyzes are easy, quick and dependable keys of clinical monitoring (Braun et al., 2010; Coles, 1986; Onasanya et al., 2015; Polizopoulou, 2010; Rahman et al., 2018). Hematology is a science that deals with whole blood physiology, metabolism, and biochemistry. It investigates the physiological and pathological states of cells and proteins found in blood (Bush 1991; Coles, 1986; Polizopoulou, 2010). Also, interpretation of hematological blood results which crucial for clinicians and academicians mainly depend on the determination of specific reference intervals (Kaneko et al., 2008; Meyer and Harvey, 2004; Simpraga et al., 2013; Vojta et al., 2011). Hematological diagnostic methods and processes have become a necessary and fundamental part of the minimum base of information for determining health status and the diagnostic observation of ovine medical problems. The widespread use of advanced hematological instruments, kits and materials that can process small ruminant blood samples has made blood assays important diagnostic methods in managing sheep health (Polizopoulou, 2010). For healthy ovine as with other mammals, nutritional status, age, sex and breed of the sheep as well as the season and environmental conditions should be considered when determining hematological reference values and ranges, as in biochemical parameters. The value of blood sample diagnosis lies in its ability to identify the effects of diseases on blood cells and platelets. Blood composition is continuously variable. Rapid modifications could occur as a response to multiple physiological events initiated by physiological or pathological stress. In living organisms, especially in mammals, identification of hematological values with specific reference intervals based on breed-specific differences is vital in disease prognosis and treatment processes, in both academic and clinical prospects (Polizopoulou, 2010).

Most of the elements play important roles in maintaining health, and normal metabolic balance and growth in human and animals (Carlson, 2008; Rucker et al., 2008). Minerals are involved in all biochemical mechanisms, as structural elements and as regulators of almost all metabolic course (Kaneko, et al., 2008). In living organisms, macrominerals fulfill many vital functions in biochemical metabolic processes. Minerals act as catalytic co-factors for normal enzyme functions and essential for constituents. Minerals are important also in the synthesis of many hormones that are required for normal functions of basic biochemical and hematological processes in the body. Arrangement of cell replication and differentiation are other regulatory impacts of minerals. Structurally, minerals can form macro-components of organs and tissues, such as calcium and phosphorus (P) (bones and teeth, tendons). Physiologically, minerals play significant roles in body fluids and tissues, as electrolytes (Na, K, Ca, Mg, etc) concerned with the maintenance of osmotic pressure, acid–base balance, membrane permeability and transmission of nerve impulses (Kaneko, et al., 2008; Suttle, 2010; Yatoo et al., 2013). Phosphorus plays a role in numerous biological processes, including energy metabolism and bone mineralization. P, participates in the structure of DNA and RNA and also takes

mainly part in various biochemical pathways such as glycolysis and beta oxidation of fats. As a component of signal transduction, phosphate is used in cyclic AMP and products of deoxyribonucleoside diphosphates (Raina, 2012). The majority of intracellular P is found either as inorganic phosphate esters, phospholipids in cell membranes, or as phosphorylated intermediate molecules involved in a wide variety of biochemical processes, including energy production (ATP), storage and transfer (Favus, 2006; Suttle, 2010). Serum iron levels have both biochemical and hematological importance. The daily requirements of iron vary according to the age, gender, nutritional and physiological status of the individual. Iron is an essential element in the body, but its effect on the body is like a double-edged knife (Harvey 2008a, 2008b; Suttle 2010). Iron ion is critical for Fe-containing proteins, oxygen transport and storage, respiration, DNA synthesis, Krebs cycle, and various enzymatic reactions. However, the same physical properties that allow iron to function as a cofactor in controlled redox biochemistry also make iron potentially toxic to cells, as it catalyzes the formation of the reactive oxygen species (Harvey 2008a).

The identification and use of specific ranges for breeds and species of animals are required to establish reference sites specific to regions where animals are raised and to their racial values (Altintas and Fidanci, 1993; Braun et al., 2010; Meyer and Harvey, 2004). Since these parameters are vital for animal health and may vary depending on different variables, regional reference values for animals are clinically valuable. The goal is to investigate the reference values for clinically healthy Akkaraman sheep breed raised in Aksaray and the nearby environment, by measuring some parameters and considering age and sex-related differences.

MATERIAL and METHOD

Current study protocol involved to take blood from animals following by ethical rules. Animals were separated into four groups based on gender and age. For biochemical and mineral assays, 15 mL of blood was taken from the vena jugularis, in anticoagulant-free serum tubes. The hemolysis-free serum samples were separated from clotted blood samples by centrifugation and stored at -25 °C till analysis (Coles, 1986). Biochemical variables determined in each sample were total protein (TP), glucose (Glc), albumin (Alb), globulin (Glb), triglycerides (TG), total cholesterol (Tot. Chol.), high-density cholesterol (HDL), low-density cholesterol (LDL), total bilirubin (Tot. Bil.), urea, and creatinine (Crea). Analyses of the parameters were carried out with commercial assay kits (Assel, Italy) and a biochemical analyzer (Humalyzer 3000 semi-analyzer, Germany), according to the method of administration of each parameter described in the commercial kit procedure. Analyses of the serum calcium (Ca), phosphorus (P) and iron (Fe) levels were carried out with commercial assay kit (Assel, Italy) and biochemical analyzer (Humalyzer 3000 semi-analyzer, Germany). For hematological assays, the blood samples were taken from the vena jugularis into EDTA-containing hemogram tubes and commercial test kits (Mindray V-28, China) with the auto-hematology analyzer Mindray, BC-2800-Vet (China) were used.

White blood cell-WBC (10^9/L), hemoglobin-HGB (g/dL), hematocrit-HCT (%), red blood cell-RBC (10^{12}/L), mean red blood cell volume-MCV (fL), mean cell hemoglobin-MCH (pg), mean cell hemoglobin concentration-MCHC (g/dL), red cell distribution width-RDW (%), platelet-PLT (10^9/L), mean platelet volume-MPV (fL), platelet dispersion width-PDW and platelet relative volume-PCT (%) variables were measured.

The descriptive statistics for the properties studied were mean, standard deviation, standard error, minimum values and maximum values. Variations among the groups were analyzed by Student t-test. "One-way ANOVA" was fulfilled to crosscheck the group averages in terms of continuous variables. A Duncan multiple comparison test was used to identify the different groups following the analysis of variance. The data are given as the means ± standard error ($\bar{X}$ ±SE). Statistical significance was accepted as $P<0.05$ level.

RESULTS

In this study, various biochemical values were measured according to gender and age, statistical analyses of these values were performed and the results were examined (Table 1).

Table 1. Biochemical findings for four groups of sheep

Parameter (Unit)	Ewes	Female Lambs	Rams	Male Lambs	*P*
Glucose (mg/dl)	68,91±2,07^{c}	77,04±5,17b^{c}	92,86±5,95^{a}	82,51±2,24^{ab}	0.001
Total Protein (g/dl)	11,27±0,43	10,85±0,39	11,39±0,40	11,76±0,31	>0.05
Albumin (g/dl)	5,46±0,28	5,82±0,31	6,18±0,24	6,16±0,39	>0.05
Globulin (g/dl)	5,32±0,31	4,73±0,31	4,94±0,30	5,21±0,31	>0.05
Triglycerides (µg/dl)	138,71±7,40	129,83±3,43	123,45±5,47	123,91±6,30	>0.05
Total Cholesterol (µg/dl)	76,57±2,97	72,03±5,45	72,31±3,10	67,55±4,91	>0.05
HDL Cholesterol (mg/dl)	45,00±1,62	42,86±3,28	42,28±2,85	43,50±4,03	>0.05
LDL Cholesterol (mg/dl)	29,21±2,75	28,23±2,73	28,89±2,01	23,00±1,71	>0.05
Total Bilirubin (mg/dl)	0,61±0,06^{a}	0,42±0.05^{b}	0,65±0,07^{a}	0,65±0.05^{a}	0.020
Urea (mg/dl)	20,07±1,88^{b}	15,25±1,38^{b}	32,01±2,16^{a}	19,62±1,40^{b}	0.000
Creatinine (mg/dl)	0,89±0,087^{b}	0,71±0,038^{b}	1,24±0,08^{a}	0,91±0,07^{b}	0.000

When serum Glc values of healthy groups of different ages and genders were investigated, a higher amount of Glc was detected in males than in females; and the difference was statistically significant ($P<0.05$). Total protein values were the highest in male lambs and the lowest in female lambs, and the amount of protein was higher in ewes than in female lambs; however, neither of these findings were statistically significant ($P>0.05$). It was also observed that the total protein ratios are not age-dependent but sex-dependent, with the protein concentration in males being higher than in females. When the Alb values were examined, it was found that the rams had the highest values and female lambs had the lowest, but these were not statistically significant ($P>0.05$). There were no statistically significant ($P>0.05$) differences between the four groups in the amount of globulin, which was higher in ewes than in female lambs and higher in male lambs than rams. In the present study, regardless of age, measured triglyceride levels were found to be higher in females than in male Akkaraman sheep. The levels of triglycerides, which were also higher in ewes than in female lambs, were slightly different in rams than in male lambs. The triglyceride values of the four groups were not statistically significant ($P>0.05$). Total cholesterol values were observed to be lowest in male lambs and highest in ewes. The cholesterol levels in adults was relatively higher than lambs. Though cholesterol levels were higher in ewes than in rams and lower in male lambs than in female lambs, these results were not statistically significant ($P>0.05$). The ewes had the highest HDL cholesterol compared to the other three groups, but the results were not significant. LDL cholesterol was found to be higher in the adult groups (ewes and rams) than in the lamb groups. The highest LDL cholesterol was in the

ewes, but the difference was not statistically significant. There was no age-related difference in total bilirubin values in males. Looking at gender-dependent differences, the total bilirubin levels were higher in males than in females. In female group, total bilirubin values were higher in ewes than in female lambs and this difference was statistically significant (P<0.05). The higher urea values were found in rams and ewes as compared to lambs (P>0.05). The urea values were found to be statistically significantly and this status applies to all groups. Urea values of rams are higher at statistical significance level compared to all other groups. There is no difference between the other groups. When creatinine was assessed, it was found to be the highest in rams and these results were statistically significant (P<0.05) (Table 1).

With presented study, it could be declared that whole blood hematological parameters in Akkaraman sheep breed (Aksaray region) can be vary on age and gender. The comparative graphics of the total parameters were demonstrated in Figure 1 and the median and mean values and the statistical analysis of the hematological variables of this study are represented in Table 2.

Table 2. Hematological findings of Akkaraman sheep

Parameter (Unit)	Ewes	Female Lambs	Rams	Male Lambs	*P*
WBC (10^9/L)	7,86±0,98	9,08±0,43	9,75±0,55	9,52±1,09	> 0.05
RBC (10^{12}/L)	7,95±0,53^b	9,77±0,34^a	9,96±0,30^a	9,63±0,83^a	0.046
HGB (g/dL)	9,38±0,56	9,33±0,25	9,63±0,36	9,35±0,81	> 0.05
HCT (%)	28,0±1,62	28,95±0,75	29,96±1,27	28,68±2,4	>0.05
MCV (fL)	33,74±0,46^a	29,88±0,67^b	30,83±1,24^b	30,0±0,53^b	0.004
MCH (pg)	11,075±0,20^a	9,55±0,19^b	10,075±0,36^b	9,57±0,17^b	0.000
MCHC (g/dL)	32,05±0,35	32,20±0,41	31,95±0,52	31,52±0,48	>0.05
RDW (%)	22,68±0,42	23,30±0,49	23,70±0,52	24,41±0,51	>0.05
PLT (10^9/L)	261,75±15,31^b	412,75±45,28^a	392,17±46,81^a	401,75±41,18^a	0.031
MPV (fL)	3,96±0,15^a	3,8±0,06^{ab}	3,62±0,05^b	3,58±0,078^b	0.025
PDW (fL)	15,60±0,17	15,27±0,96	15,08±0,21	15,13±0,12	>0.05
PCT (%)	0,10±0,005	0,15±0,016	0,13±0,017	0,21±0,07	>0.05

It was determined that the WBC count was higher in rams and male lambs than in ewes and female lambs. The lowest value was found in ewes; however, the results were not statistically significant. According to the measurements of RBC counts, the highest values belonged to the rams whereas the ewes had the lowest amount of red blood cells. These results were statistically significant (P< 0.05).

The HGB values of Akkaraman sheep were found to be higher in adults than in lambs (both sex). So though the findings were not statistically significant (P> 0.05), there was difference in HGB values depending on age (higher in adults than in lambs), not on sex. HCT values were found to be very close between all groups: no difference was found and these values were found to be statistically insignificant (P> 0.05). When MCV and MCH were examined, it was found that the values were higher in ewes than in rams and lambs of both sexes. Both parameters (MCV P <0.005 and MCH P <0.001) were found to be statistically significant when compared to other 3 groups. The mean MCHC concentrations in female group had higher values than males, but again the data was not found to be statistically significant. The highest RDW value belonged to male lambs whereas the lowest values belonged to ewes. In general, females had lower levels of RDW than males, but the data was statistically insignificant. The highest PLT value was found

in female lambs and the lowest value belonged to ewes. This difference was significant ($P<$ 0.05). MPV was higher in females than males. There was no difference in MPV between the young and adult males. The differences between ewes and rams, and ewes and male lambs were statistically significant ($P<$ 0.05). PDW values were also higher in females than in males, but the difference was not statistically significant ($P>$ 0.05). When relative volumes of PCT were measured and examined statistically, values were highest in lambs and lowest in ewes, but the differences were not statistically significant ($P>$ 0.05). The hematological data obtained in this study will contribute to form of the reference values based on the age and sex of Akkaraman sheep and will also provide valuable clinical information (Figure. 1.).

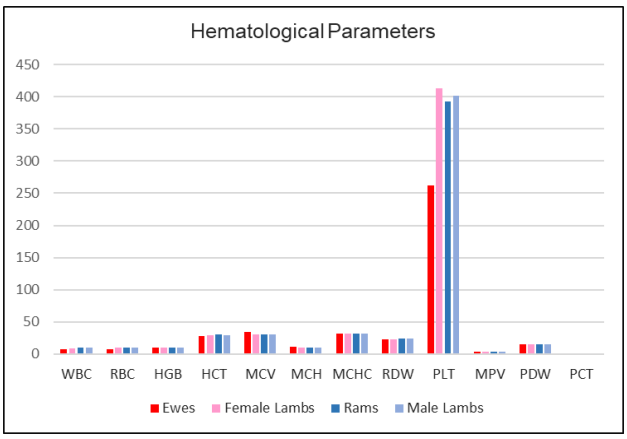

Figure 1. Levels of hematological parameters in four groups of sheep

The values of the iron levels for all groups found statistically significant differences ($P<0.05$) (Table 3).

Table 3. Macromineral levels of Akkaraman sheep

Parameter (*SI* Unit)	Ewes	Female Lambs	Rams	Male Lambs	*P*
P (mmol/L)	2,19±0,13	2,40±0,11	2,00±0,14	2,40±0,12	>0.05
Ca (mmol/L)	3,8±0,22	3,53±0,23	3,20±0,17	3,37±0,719	>0.05
Ca / P Ratio	1,74	1,47	1,60	1,40	
Fe (*μ* mol/L)	22,46±1,11 ^{ab}	19,52±1,35^{b}	24,25±1,40^{a}	24,90±1,50^{a}	0,028

Differences between groups in the values of Ca and P were not statistically significant ($P>0.05$). There was no significant correlation between ewes and rams and the values were statistically insignificant. The Ca levels did not show any significant differences between all four groups. High Ca values detected in ewes were not statistically significant compared to the other groups.

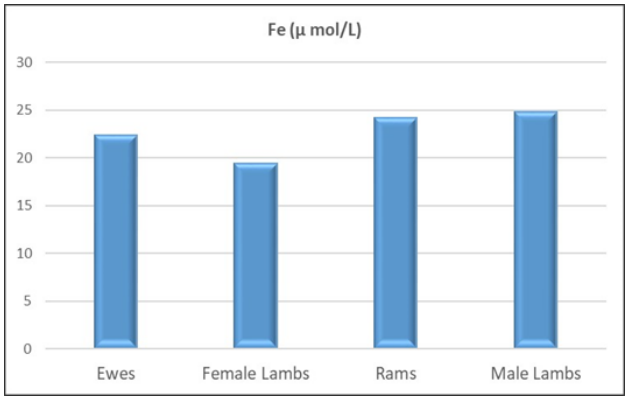

Figure 2. Levels of Fe in four groups of Akkaraman sheep

The highest Fe level was in male lambs. It's found that, ewes had higher Fe values compared to female lambs, which had the lowest iron levels measured ($P<0.05$) (Figure 2 and 3).

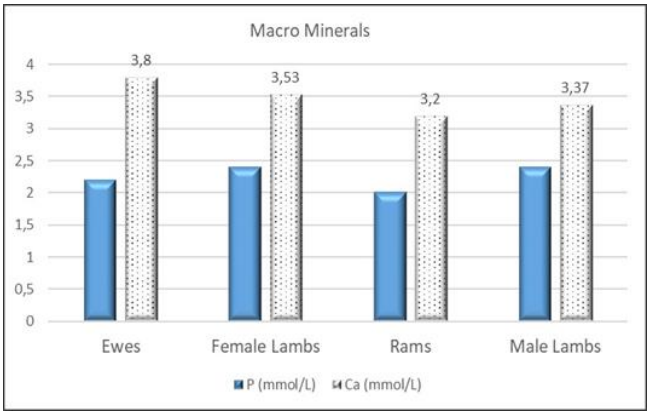

Figure. 3. Serum Ca and P Levels in Akkaraman sheep

DISCUSSION

Blood analyzes are the indispensable distinctive pieces in identifying the health status and following up the treatment in investigating diseases in living beings. At this point, determining the changes in the hematological and biochemical values and mineral levels of the Akkaraman sheep breeds will be very important in terms of the diagnosis and interpretation of disease in these animals as well as the choice of treatment and the follow-up it. Thus, our goal is to identify and present the values for the some important and vital hematological, biochemical parameters and mineral levels of Akkaraman sheep raised in the Aksaray region of Turkey. It is also a major aim of the study to compare the data obtained in the current study with previously identified reference intervals for other sheep breeds.

As mentioned earlier, the unique biochemical blood profile can be influenced by age, gender, nutrition, breed, species, metabolic periods, and environmental and seasonal conditions, which are reflected in its biochemical structure (Cruz et al., 2017). Braun and co-workers recommended that, because there are many different breeds and breeding systems in sheep, each laboratory specify their own reference values and ranges. As a result, there must be different reference ranges (Braun et al., 2010). They and other scientists suggested that seasonal impact is often difficult to discern from a variety of misleading factors, such as food supply and the reproductive condition of female animals (Braun et al., 2010; Yokus et al., 2004, and 2006; Yokus and Cakir, 2006). A study that appraised the physiological alterations caused and influenced by age and gender on biochemical variables in male and female Dorper sheep at different ages. They concluded that age has a significant influence on the values of most biochemical parameters of Dorper sheep aged 15 to 121 days, but that there is no effect based on sex (Cruz et al., 2017). In clinical biochemistry, monitoring of fasting blood Glc levels in every biochemical assay is crucial for tracking health-disease states in all metabolic pathways, especially vital tissues such as the liver. Similar to our study, which we found the variability of glucose levels depending on age and gender in all four groups statistically significant, Durak et al. (2015) demonstrated that the serum Glc levels of the Zom sheep, were significantly lower in the female group than those of male group (P<0.05). Similarly, the researchers declared that for Glc, they found significant differences between groups of different gender and age (Durak et al., 2015). In the current study, while the Glc results were found to be within normal limits for ewes and rams (Kaneko, 2008), they were higher than in the studies performed in different geographical regions, such as Iraq (Fartosi et al., 2010) and Pakistan (Kiran et al., 2012). It was declared that in a study on Akkaraman sheep raised in the Ankara region of Turkey (Altinsaat, 2001), the amount of Glc in healthy ewes and rams was lower, than our adult male and female sheep's glucose results. The possible differences in Glc levels could be attributed to age, gender, physiological and endocrinological changes, nutrition, the individual metabolic activity of animals and geographical differences (Braun et al., 2010; Burtis et al., 2012; Carlos et al., 2015; Kaneko, 2008). Besides these, in three different studies, performed in tropical regions, the biochemical variables glucose and total cholesterol were investigated. Compared with the current study, Glc and Hb values were lower and cholesterol levels were higher. Considering that these studies were conducted in tropical regions, it is understandable that their results are similar to each other and differ from the results of our study. It is certain that gender, geographical distribution, ecological and geological differences, nutritional properties and health conditions can influence hemoglobin levels (Bhat et al., 2014; Kiran et al., 2012; Pradhan, 2016). These values which were obtained in

studies conducted with different sheep breeds; female Karakachan sheep (Stevanović et al., 2015), Dalmatian pramenka sheep (without sex and age differences, total mean) (Vojta et al., 2011), Tsigai sheep (total mean values) (Antunović et al., 2009) and female Lika pramenka sheep (Vugrovečki et al., 2017) had lower total protein, Alb and globulin values than our results. The total protein and its components measured in ewes in the current study were higher than that in the studies conducted in Adıyaman province, Turkey (Kurt et al., 2008) and in Ankara, Turkey (Altinsaat, 2001). In another study the age of the sheep affected the blood chol., trig., urea, crea., prot., and glob. values as well as the Alb concentrations; however, the difference between the different age groups was not significant (Carlos et al., 2015). When the values of determined by Carlos et al., (2015) are compared with our results; in ewes and rams, Glc, tot. prot., alb and glob levels were lower, whereas tot. chol., trig. and urea values were higher. These all differences may due to physiological variations between the breeds as well as geographical distinctions between the different regions. Furthermore, these unsimilarities in the results probably stem from also the different management conditions, climates and nutrition levels of the animals (Vugrovečki et al., 2017). The level of cholesterol, a health indicator for many issues, especially cardiovascular diseases, is the sine qua non of the body (Bruss, 2008; Nelson, and Cox, 2006). Literature information is insufficient with region-specific prior studies of Akkaraman sheep with triglyceride, total cholesterol, HDL, and LDL cholesterol parameters. In studies conducted in different geographical regions, the overall mean values of cholesterol were higher than in our study whereas the overall triglyceride mean values were lower (Fartosi et al., 2010; Kiran et al., 2012). In the study conducted in seven different districts of Adıyaman province, Turkey, the overall mean values of lipid parameters were close to the current study regardless of gender difference (Kurt et al., 2008). In the present study, total bilirubin, urea and creatinine levels, which are closely related to renal and hepatic health, the circulatory system and fluid-electrolyte balance, were also investigated in Kurt's research (2008), while the overall mean values of the urea levels were approximately the same, and creatinine and total bilirubin levels were lower, Altinsaat et al (2001), declared lower levels of the overall mean values of urea, creatinine and bilirubin. Because creatinine is formed by the degradation of phosphocreatine for energy release in skeletal muscle, the serum creatinine is an important marker proportional to the muscle mass (Kreutzer and Turk, 2008; Meyer and Harvey 2004). In the current study, creatinine was the highest in rams and generally higher in males than in females, and these differences were statistically significant ($P<0.05$). In the current study, bilirubin and urea values were highest in rams. So, Akkaraman adults had higher values of urea, crea and bil than in lambs.

Examinations that complement physiological findings and biochemical analyses are extremely important for veterinarians. Descriptive and determinant biochemical investigations carried out in animals raised in different countries, or in different regions of the same country, as well as clinical studies, also provide useful information (Altintas and Fidancı, 1993; Kaneko et al., 2008). These mentioned parameters are used in diagnosing diseases, revealing nutritional disorders, and following up on treatments. They also help to pioneer and assist in future research on these animals. In this completed study, we observe that some values can change with age, some can differ by gender and some parameters can be influenced by both. After all, when all parameters are taken into consideration, differences in region, breed, sex, age, season and nutritional sources affect biochemical values and can cause changes. The

identification and monitoring of these parameters reflecting the metabolic profile show that animals' homeostatic mechanisms maintain blood composition at physiological limits under different conditions (breeds, species, regions, feed-nutrition regimes and age and sex characteristics).

Identifying the biochemical and hematological values of the Akkaraman sheep breeds is crucial for the monitoring general health status, diagnosis of diseases and also choosing of the treatment and to follow this cure. In a study conducted in Bangladesh, obtained that hematological values found significantly different between adult and lamb and between male and female indigenous sheep, respectively health, similarly with our results (Rahman et al., 2018). In 2014, it's determined that the hemogram values obtained, which have great regional and geographical differences, nonetheless have approximate values (with the exception of WBC). The mean WBC values obtained in the current study were high when compared to those in some other (Njidda et al., 2014) and close to normal values in others (Kaneko, 2008), considering both gender and age differences (Celebi and Uzun, 2000). Compared to some other studies, in our research the hemoglobin values were higher in both genders and at different age ranges (Kiran et al., 2012). In present study, RBC values in rams were higher than those obtained by Njidda et al., whereas the values in the females were close (2014). In a hematological study performed in healthy Akkaraman Kangal lambs in the Sivas region of Turkey, the RBC was within the limits of the hemogram values (for lambs) obtained in the present study, the WBC was lower, and the HGB, HCT, MCV and PLT were higher than this study's (Kockaya and Ozsensoy, 2016). The MCV, MCH, MCHC values found in this study were close to those of some other studies carried out in Turkey. The current HCT values were lower in different age and gender groups as compared to the results obtained from Tuj and Morkaraman sheep (Celebi and Uzun, 2000). In our study, the HCT values of four groups were close to each other and similar to the literature (Kaneko, 2008). Firstly, Vojta and co-workers (2011) and later Simpraga and colleagues (Simpraga et al., 2013) and lastly in 2017 (Vugrovečki et al., 2017) presented a model study for reference intervals of organically raised dalmatian pramenka sheep by the robust method. In 2013, it's specified that, these sheep have special hematological and biochemical reference ranges which depend on the conditions, breeding, and food supply, environmental and seasonal influences. According to their findings, it seemed some similarity of values with our results. Although not statistically significant some of our findings (WBC, RBC, HGB, HCT), also differ between upper and lower limit of this study (Simpraga et al., 2013). In the present study, differences in RBC and PLT were found to be significant only in sheep compared to other groups ($P<0.05$). Significant differences in MPV, MCV and MCH were found to be especially in adults statistically significant ($P < 0.05$). Often, blood values are effecting by many factors, genetic and non-genetic. As already mentioned, all these diversities in hematologic parameters may be due to maturity of sheep, metabolism and hormonal differences of sexes. And also depend on the feed, stress, hormonal influence, and environmental status (Vugrovečki et al., 2017). As Etim et al., pointed out, we also believe that it's so vital to build basic indicators for blood variables on the basis of many factors and also perform further researches to define all influences of these factors on these (2014).

Many of the minerals are cofactors of vital catalytic proteins in all metabolic pathways. The most of trace minerals should also be determined in ruminant animals to assign if deficiencies, imbalances and toxicities are present (Balamurugan, et al., 2017; López-Alonso, 2012; McDowell and Arthington,

2005). Since marginal mineral deficiencies can affect growth, development, reproduction, and production and are not frequently diagnosed, animals are considered to be equally as important as mineral deficiencies in which they show clinical signs that can be detected and treated (Suttle, 2010). In our research, the Ca and P values obtained are slightly higher, and Fe values were found lower, than the general reference values. In the study where iron, calcium and phosphorus levels were examined in Sivas-Akkaraman Kangal lambs without discriminating sex, it was observed that in the healthy control group had lower levels compared to our study (Kockaya and Ozsensoy, 2016). Calcium and phosphorus values of our groups were higher and iron levels were observed lower, when compared to the study conducted in Akkaraman sheep raised in the districts of Adıyaman (Kurt et al., 2008). In different studies comparing some biochemical parameters of sheep, reported that calcium and phosphorous values were lower than our study and there were no statistical differences (Angelov et al., 2013; Gürsu and Aygün, 2014; Stevanović et al., 2015; Stojković et al., 2014). As mineral deficiencies may influence all metabolic mechanisms include growth, development and reproduction, they are evenly important as mineral deficiencies in the most living beings display clinical indicators that can be defined and cured (Suttle, 2010). According to these comparisons, it is clear how effective the diet, the type and breed of the animal, regional differences, seasonal differences and lactation period and pregnancy (health status), and also the gender differences are on blood mineral levels (Rucker et al., 2008; Suttle, 2010).

CONCLUSION

It is especially important to know the values of the blood parameters of indigenous breeds, which form a critical component of the economy of our country. Biochemical values obtained in breed studies and regional studies are of great importance among the studies complementing, supporting and strengthening clinical findings. Detection and follow-up of health status provides important information on diagnosis, early diagnosis, etiology, pathogenesis of disease, disease detection and control of the selection and usefulness of applied treatment methods to veterinarians working in the field. It is significant to determine the reference ranges of domestic and region specific breeds' blood parameters, which form a dominant and precious component of the economy of our country. According to the findings presented in the literature, there are variations in the hemato-biochemical reference values for Akkaraman sheep breed, (in Aksaray region) compared to other countries and regions and other sheep species. In sight of all these evaluations, detect and determine the blood values differences would further emphasize the need to establish appropriate health baseline values (physiological, hemato-biochemical, metabolic, pathological, etc.) for livestock in Turkey in establishing the physiological status of farm animals. It is therefore important to perform serum hemato-bio-chemistry and mineral profiles in livestock in order to detect and prevent imbalances that can lead to reduced production and reproductive disturbances and as a result, economic losses.

At this point, the identification, calculation and use of biochemical reference intervals for species variations in breed and region will be the most useful. In this regard, we believe that this study will aid and help to propagate future research. In further, it is planned to make researches with more detailed variables in order to create reference intervals in the hematological and biochemical blood values of Akkaraman sheep.

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Determination of the average intraocular pressure values, optimum anesthesia dose and phenotypic characteristics in Oscar fish (Astronotus ocellatus)

Research Article

Tuba Özge YAŞAR[1a]
Mehmet YARDIMCI[2b]
Çetin YAĞCILAR[3c]

[1]Department of Surgery, Faculty of Veterinary Medicine, Tekirdağ Namık Kemal University, Tekirdağ, Turkey
[2]Department of Biostatistics, Faculty of Veterinary Medicine, Tekirdağ Namık Kemal University, Tekirdağ, Turkey
[3]Department of Aquaculture, Faculty of Veterinary Medicine, Tekirdağ Namık Kemal University, Tekirdağ, Turkey

ORCİD-
[a]0000-0003-2778-5779
[b]0000-0001-5650-437X
[c]0000-0002-4683-820X

Correspondence
Tuba Özge YAŞAR
dr.tozgeyasar@gmail.com

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ABSTRACT

This research was carried out to determine the intraocular pressure values, optimum anesthesia dose and phenotypic characteristics in Oscar fish (Astronotus ocellatus). A total of 11 adult, male Oscar fish were used in the study. Optimum anesthesia dose, pre-and post-anesthesia intraocular pressure values and phenotypic measurements (total length, fork length, standard length, dorsal fin base length, head length, body length, eye diameter, body depth, pectoral length, pelvic fin bottom length, anal fin bottom length) were determined and reference values specific to this fish species were obtained by performing a statistical analysis. When exposed to 3 cc/L of the concentrations, fish achieved a deep state of anesthesia (induction time 1.31 min; recovery time 6.42 min). The most balanced anesthesia without risking vital functions was achieved using Clove oil with a dose of 2.5 cc/L. Compared to the other dimensions, total length, fork length and standard length were the most consistent body measurements with high positive correlations. No statistically significant difference ($p > 0.05$) was seen in both eyes between the intraocular pressure values before (5.36-5.86) and after (5.59-5.36) anesthesia. It is recommended that the ideal concentration of clove oil was 2.5 cc/L to reduce stress and injury damage during handling procedures. Additionally, intraocular pressure values, body measurements and Clove oil optimum anesthesia dose obtained in this study can be used as reference values for Oscar fish raised in aquarium conditions.

Keywords: Anesthesia, clove oil, intraocular pressure, Oscar fish, phenotype

INTRODUCTION

In terms of the aquarium fish farming sector, precise anesthesia dosages that can be taken as reference are needed for transportation, operative interventions and experimental studies. Regarding the Oscar fish as a species with economic importance, there is no reference source with data on adult body phenotypic measurements, normal intraocular pressure values and optimal anesthesia dosage. On the other hand, since there is a limited number of studies aiming to establish physiological reference values for various parameters of Oscar fish, especially intraocular pressure, the need for such studies in the field has not yet been met (Keeney et al., 2019; Link et al., 2004) because it is not commonly preferred in aquaculture due to its slow growth rate and troublesome maintenance (Pinheiro et al., 2018). Nevertheless, studies that will provide reference data in the field of aquarium fish farming, which is a dynamic sector, are welcomed by both breeders and researchers.

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In recent years, different types of anesthetics have been used in aquaculture and scientific research to eliminate the negative effects on the health and growth of fish and to reduce the stress responses, minimize physical injuries, taking photographs, processing artificial reproduction and making several measurements (Roubach et al., 2005; Küçük et al., 2016; Chatigny, 2017; Fernandes et al., 2017). On the other hand, intraocular pressure (IOP) is required to maintain the eye into a shape allowing it to function and it is sustained by the balance between the production of aqueous humor by the ciliary body and the resistance to its outflow from the eye in vertebrates (Zouache et al., 2016).

Oscar fish are mostly known as omnivorous but they seem to prefer a carnivorous diet. They mainly feed on aquatic and terrestrial insects, small fish and invertebrates, fruits, benthic algae and plants (Yılmaz & Arslan, 2013). The Astronotus ocellatus species was chosen in this study due to lack of work on it. It is difficult to find breeders who have large numbers of these fish in their aquariums.

It was aimed to determine the optimum anesthesia dose, analyze the pre and post-intraocular pressure changes and determine some phenotypic features to provide scientific information about Oscar fish. In this respect, it is hoped that the data obtained from this research will fill an important gap to some extent and will shed light on future studies for both industry and experimental studies.

MATERIAL and METHOD

Fish material and experimental conditions

A total of 11 adult, male Oscar fish reared in the 100 cm x 35 cm x 45 cm size aquarium were used as the study material and the experiments were performed in duplicate under the similar experimental conditions in a two-week interval after the end of the first applications.

Feeding was made on the base of 5% of live weight with commercial feed. Bottom cleaning of the aquariums was done twice at 3-day intervals and water change up was performed up to 30% each time. All experiments were conducted at 27.2°C water temperature with a standard 16-h light: 8-h dark photoperiod. As routine measurements pH, salinity, Total Dissolved Solids (TDS), conductivity, aquarium temperature measurements were recorded with a waterproof ExStik® II pH/conductivity meter, EC500, "Extech" brand device, ambient temperature and humidity with a "Thermo HYGRO" device (Table 1).

Table 1. Ambient conditions during the trials

Ambient temperature (°C)	29.1
Humidity (%)	40.0
Salinity (mg/L)	333.0
Aquarium water temperature (°C)	27.2
Total Dissolved Solids (TDS)	518.0
pH	8.2

Anesthesia

Clove oil used in the research was supplied from "Kimbiotek Chemical Substances Joint Stock Company, Turkey". For anesthesia 1.5, 2, 2.5, and 3 cc/L clove oil dosages were tried to determine the optimal dosage in Oscar fish (Figure 1). Each dose was tested at 3-day intervals on the same animals. The solutions were prepared by dissolving clove oil in 95% ethanol (1:10 ratio) as described by Anderson et al [1997] to facilitate mixing.

Figure 1. Experimental setup: A simple recirculating system maintains anesthesia by delivering anesthetic water from a reservoir to the gills and recycles the effluent back to the fish through the use of a submersible pump.

The fish was anesthetized and positioned with the lateral side up during measurements (Figure 2). The induction and recovery times of the fish were monitored with a digital stopwatch, and the stimulation reaction was determined by contact with a plastic pipette.

Figure 2. Fish position on a small fish anesthetic unit at the time of anesthesia

Intraocular pressure (Ocular tonometry) measurements

After determining the optimum anesthesia dosage, the fish were individually placed in containers for preparation of the measurements. Before and after the anesthesia, bilateral intraocular pressures of the fish were measured with a digital tonometer, TonoVet (Icare, Finland) using the needle of the device brought into contact with the outer surface of the fish eye (Figure 3).

Figure 3. Intraocular pressure measurements using a digital tonometer

Bodyweight and other phenotypic measurements

Fish were weighed with a digital weighing scale precise to 0.1 g. Other phenotypic measurements (total length, fork length, standard length, dorsal fin base length, head length, body depth, eye diameter, pectoral fin length, pelvic fin bottom length, anal fin bottom length) were taken on a flat platform while the fish were under anesthesia. Metric measurements were taken using a ruler on a straight line over the curves of the body (Figure 4).

Figure 4. Phenotypic measurements of fish on the anesthetic unit

Morphological measurements were taken using the procedure below (Figure 5)

Total length: The distance measured from the anterior part of the head to the end of the most posterior part of the caudal fin.

Fork length: The distance measured from the anterior tip of the jaw to the endpoint of the caudal fin.

Standard length: The distance measured from the anterior part of the head to the end of the vertebral column/caudal peduncle.

Dorsal tail bottom length: The distance measured between the anterior and posterior base of the fin at the base of the dorsal tail.

Head length: The distance measured from the tip of the mouth to the most distant point on the opercular membrane.

Body depth: The vertical line at the widest part of the body.

Eye diameter: Maximum diameter of the eye.

Pectoral fin length: The distance measured between the anterior and posterior lines of the pectoral fin.

Pelvic fin bottom length: The distance measured between the anterior and posterior base of the pelvic fin.

Anal fin bottom length: The distance measured between the anterior and posterior base of the anal fin.

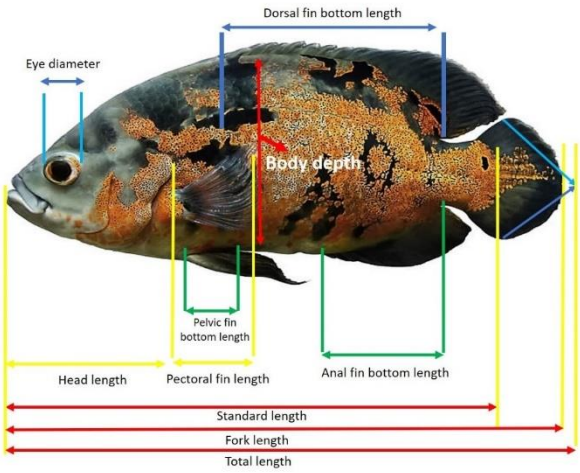

Figure 5. Morphological measurements of Oscar fish

At the end of all measurements, each fish was returned to the aquariums for recovery with clean water allocated for it.

Statistical analysis

IBM SPSS Statistics 25.0 package program was used for statistical analysis. ANOVA with the Tukey test was applied to compare the induction and recovery times ($p < 0,05$). Intraocular pressures were compared by Paired Samples t-Test ($p < 0.05$) while descriptive statistics were used to demonstrate the phenotypic measurements.

RESULTS

Behavioral characteristics observed in fish during the anesthesia induction were loss of balance, decreased respiratory rate, decreased reactions to external stimuli, immobility at the bottom of the water, stopping of gill and mouth movement, respectively. Observations during recovery from anesthesia were light movements in swimming, gills and mouth, increased response to external stimuli, improvement of balance, normal movement and regaining the swimming balance, respectively.

When applying different doses to determine the optimal dosage, 3 cc/L clove oil was noticed to cause deep anesthesia in the fish which gave the impression to be detrimental. Those fish were immediately taken to clean water and sober up before they died. It was decided that a dose of 3 cc/L would not be appropriate, since the anesthetic agent to be used was aimed to create anesthesia at the level of surgical anesthesia. On the other hand, the most balanced anesthesia without risking vital functions was achieved with a dose of 2.5 cc/L whereas 1.5 and 2 cc/L were found inadequate in terms of timing and efficacy (Table 2).

Table 2. Intergroup analysis of the effect of different doses of Clove oil on induction and recovery from anesthesia in the Oscar fish

Process	n	Dosage (cc/L)	$\bar{x} \pm S_{\bar{x}}$	p
Anesthesia induction (min)	11	1.5	14.63 ± 0.4^{a}	0.000
	11	2.0	6.95 ± 0.2^{b}	
	11	2.5	3.35 ± 0.1^{c}	
	11	3	1.31 ± 0.1^{d}	
Recovery from anesthesia (min)	11	1.5	0.40 ± 0.1^{a}	0.000
	11	2.0	3.21 ± 0.1^{b}	
	11	2.5	3.55 ± 0.2^{b}	
	11	3	6.42 ± 0.2^{c}	

$p < 0.05$, p: ANOVA. Means within a column with the different letter are significantly different

Those fish that had long induction time emerged from anesthesia early while the ones with a short induction time were delayed from anesthesia.

As seen from the measurement results, the body size and live weight of Oscar fish were much higher compared to many other aquarium fish (Table 3).

Table 3. Descriptive values for the phenotypic characteristics of Oscar fish

	n	x̄ ± SD	Min	Max
Live weight (g)	11	471.21 ± 67.80	368.1	593.0
Total length (cm)	11	27.47 ± 0.88	26.0	29.5
Fork length (cm)	11	27.35 ± 0.94	25.5	29.0
Standard length (cm)	11	22.88 ± 1.21	21.3	25.4
Dorsal tail bottom length (cm)	11	11.94 ± 2.14	7.4	14.7
Head length (cm)	11	8.12 ± 0.70	7.0	9.2
Body depth (cm)	11	11.05 ± 0.67	9.7	12.2
Eye diameter (cm)	11	1.43 ± 0.11	1.2	1.6
Pectoral fin length (cm)	11	6.68 ± 0.83	6.0	9.9
Pelvic fin bottom length (cm)	11	1.80 ± 0.48	1.2	3.0
Anal fin bottom length (cm)	11	5.89 ± 0.79	4.5	7.5

No statistically significant difference was found between intraocular pressure values in both eyes during the pre-and post-anesthesia periods ($p > 0.05$) (Table 4).

Table 4. Intraocular pressure measurements before and after anesthesia

	n	Pre-anesthesia (x̄ ± Sx̄)	Post-anesthesia (x̄ ± Sx̄)	p
Right eye IOP	11	5.36 ± 0.24	5.59 ± 0.26	0.107
Left eye IOP	11	5.86 ± 0.29	5.36 ± 0.19	0.625

$p < 0.05$; p: Paired samples t-test

Regarding the relationships between the body measurements, high positive correlations were determined between the main three dimensions. They were total length, fork length and standard length. They were all positively correlated with each other and with head length, body depth, and anal fin bottom length while negative correlations were found between body weight and the total length, fork length, standard length, head length, body length, and anal fin bottom length (Table 5).

DISCUSSION

The results of the current study showed that clove oil could be used effectively at a dosage of 2.5 cc/L in Oscar fish without any harmful effects. Depending on this dose with balanced anesthesia without risking vital functions, the mean induction time was 3.4 min with a range from 3.1 to 3.7 min and mean recovery time was 3.7 min with a range from 2.3 to 5.1 min. At other doses, induction occurred too late without obvious symptoms or too early in a deep anesthesia situation. In accordance with our results, researchers refer to clove oil as an attractive anesthetic for fish due to its efficacy, low price and safety (Fujimoto et al 2018, Silva-Souza et al 2015). It also refers to a natural substance that has no side-effects on fish and does not represent any ecological or hygienic risks (Hamackova et al 2006). Another advantage of Clove oil is that it can easily be found in pharmacies, spice shops or markets.

Regarding the phenotypic properties, Oscar fish is not among the ubiquitous species and attracts attention with their large body structures. It is difficult to keep and feed them compared to most of the other aquarium fish species. Since it is not a widely grown fish

species, it is not easy to come across the literature regarding most of the phenotypic measurements of this species. Considering the available reports of studies on body measurements of Oscar fish, several researchers reported the mean length for adults varying between 19-33 cm and weight between 0.4 kg and 1.5 kg generally referring to the wild types (Castro-Castellón et al 2020, De Boeck et al 213, Paes et al 2011, Rodrigues et al 2017, Trindade and Queiroz 2012, Yılmaz and Arslan 2013). The average total length of adult Oscar fish reared under laboratory conditions was found as 27.5 cm with a range from 26.0 to 29.5 and average live weight as 471.2 g with a range from 368.1 g to 593.0 g in the current study. The standard length which was determined as 23 cm (21-25 cm) in this study was similar to those reported by some researchers in the range of 16-28 cm for Oscar fish (Trindade and Queiroz 2012, Yılmaz and Arslan 2013). Any discussion could not be made on other phenotypic measurements due to the lack of accessible literature.

Table 5. Correlations between morphometric features of Oscar fish

		Total length	Fork length	Standard length	Dorsal fin bottom length	Head length	Body depth	Eye diameter	Pectoral length	Pelvic fin bottom length	Anal fin bottom length
Live weight	r	-.732*	-.767**	-.661**	.270	-.693**	-.671**	-.517*	.172	-.505*	-.628**
	p	.024	.003	.010	.466	.017	.036	.131	,214	.099	.047
Total length	r	1	.907**	.829**	.035	.736**	.529*	.415	-,272	.490*	.604**
	p		.000	.000	.830	.005	.031	.094	,336	.124	.017
Fork length	r		1	.923**	-.072	.780**	.717**	.585**	-,250	.511	.676**
	p			.000	.794	.005	.008	.039	,417	.107	.021
Standard length	r			1	-.104	.731**	.678**	.518*	-,202	.492*	.645**
	p				.615	.003	.016	.099	.821	.165	.011
Dorsal fin bottom length	r				1	.069	-.196	.164	-.031	.413	-.228
	p					.799	.473	.573	.934	.189	.507
Head length	r					1	.439*	.377	-.264	.235	.646**
	p						.212	.267	.422	.415	.022
Body depth	r						1	.791*	.006	.480	.668**
	p							.015	.996**	.148	.039
Eye diameter	r							1	.214	.530*	.311
	p								.533	.094	.381
Pectoral length	r								1	-.112	-.150
	p									.567	.404
Pelvic fin bottom length	r									1	.205
	p										.612

*p < 0.05; **p < 0.01

Intraocular pressure values provide important clues for the health of fish in routine clinical examinations. Therefore, a complete ocular evaluation should be included in every routine physical examination (Williams and Whitaker 1997). For example, elevated intraocular pressure (IOP) is recognized as the main risk factor in Glaucoma (Adornetto et al

2020). The changes observed in intraocular pressure values due to the effect of anesthesia are among the indicators of the sensitivity of animals towards anesthetic substances. The fact that there was no statistically significant difference between the intraocular pressure values before (5.36-5.86) and after (5.59-5.36) anesthesia in both eyes in Oscar fish in the present study could be considered as an indicator of how resistant these fish were against side effects of anesthesia. Parallel to the findings of this study, the normal range for intraocular pressure in fish is reported between 2.24 and 13.63 (Lynch et al 2007, Waser and Heisler 2005, Zouache et al 2016) depending on the corneal thickness and other biomechanical properties of the eye (Bennett et al 2018).

The high positive correlations between body measurements indicate that these fish have a consistent body structure. On the other hand, negative correlations between body weight and body measurements indicate variability in body weight values. This situation can be explained by nutritional skills depending on the social hierarchical order and individual physiological differences. Among the body measurements, total length, fork length and standard length were the most consistent ones. They were all positively correlated with each other and with head length, body depth, and anal fin bottom length. Therefore, this study suggests that considering a standard body shape total length, fork length and standard length should be used rather than the other measurements.

It is seen that not only pet animals but also aquatic creatures are becoming popular by people day by day. In this context, the diagnosis and treatment of diseases of such organisms has an important place in veterinary medicine. The fact that the clove oil used in this study did not cause a serious change in intraocular pressure is extremely valuable data, especially for ophthalmological operations and intraocular procedures.

The fact that there was no significant change in the intraocular pressure value showed that these fish were not metabolically affected at the optimum anesthetic dose.

CONCLUSION

Oscar fish was resistant to side-effects of anesthesia having a wide tolerance since the metabolic activity was not affected under the 2.5 cc/L dose. Therefore, Clove oil can safely be used for surgical anesthesia purposes. Additionally, intraocular pressure values, body measurements and Clove oil optimum anesthesia dose obtained in this study can be used as reference values for Oscar fish raised in aquarium conditions.

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Total phenolic content, antiradical, antimicrobial and antibiofilm properties of grape and apple vinegar

ABSTRACT

Vinegar is a natural product- produced from alcoholic fermentation- that has shown strong antimicrobial activity. The aim of this study was to determine the total phenolic content and antiradical activity of the commercial grape (GV) and apple vinegar (AV) as well as to evaluate their antibiofilm and antimicrobial activities against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. GV showed higher total acidity and total phenolic content, and lower antiradical activity (DPPH activity) compared to AV. The populations of *S. aureus* and *P. aeruginosa* were significantly reduced by neat GV and AV samples. The antibacterial activity of GV was superior to AV ($p<0.05$). While AV and GV samples at 50% concentration did not form a visible zone of inhibition against *S. aureus*, they showed an inhibitory effect against *P. aeruginosa* (16.25 mm for GV and 16.5 for AV). The vinegar applied at the lowest concentration (25%) did not show any antibacterial effect on either bacteria. Solutions containing 25% to 6.25% vinegar samples prevented almost 100% biofilm formation in both bacteria. Taken together, commercial GV and AV significantly reduced the viability of *S. aureus* and *P. aeruginosa*, thereby decreasing biofilm formation.

Keywords: Antimicrobial activity, Antibiofilm activity, Antiradical activity, Food pathogens, Vinegar

INTRODUCTION

Microorganisms are able to grow on food matrixes, food industry equipment, surfaces and biofilm which is an extracellular matrix formed by many different bacteria, including *Bacillus* spp., *Listeria monocytogenes*, *Staphylococcus* spp., *Escherichia coli* and *Pseudomonas aeruginosa* in different environments (Giaouris et al., 2015). The presence of more than one bacterial species in a biofilm facilitates their attachment to surfaces (Galie et al., 2018). The extracellular matrix, which consists mainly of polysaccharides, is responsible for the strong endurance of these complexes (Flemming et al., 2016). Several pathogens, a major cause of foodborne diseases, related with bacterial biofilms on food matrixes or factory equipment may lead to intoxications or infections in humans. The formation of biofilm and spread of biofilm-related infections in food cause significant health risk for human and great economic problems in food industry (Camargo et al., 2017). *Staphylococcus aureus*, a non-spore-forming, non-motile spherical bacterium, *P. aeruginosa*, a heterotrophic, motile, rod-shaped bacterium, are commensal bacteria with carriage rate in humans and wide distribution in environment and can form biofilms on surfaces along the food production chain. Therefore, consumption of food contaminated with these bacteria may pose a threat to human health (Xu et al., 2019; Pometto and Demirci, 2015).

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Research Article

Hatice Ahu KAHRAMAN[1a]
Hidayet TUTUN[2b]
Muhammet Mükerrem KAYA[2c]
Soner TUTUN[1d]
Melike Sultan USLUER[2e]
Jerina RUGJI[1f]
Özen YURDAKUL[1g]

[1]Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University, Burdur, Turkey

[2]Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University, Burdur, Turkey

ORCİD-
[a]0000-0001-6600-239X
[b]0000-0001-9512-8637
[c]0000-0002-7781-5342
[d]0000-0002-6208-476X
[e]0000-0002-9391-2839
[f]0000-0001-7930-6704
[g]0000-0001-7680-015X

Correspondence
Hatice Ahu KAHRAMAN
h.ahuerdem@mehmetakif.edu.tr

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Disinfection is defined as the treatment of surfaces to control foodborne pathogenic bacteria, using physical and chemical methods. Disinfection represents one of the most crucial processing steps affecting the quality and safety of a food product (Deng et al., 2019). Various chemicals are used to disinfect the surfaces and equipment and their use is the most efficient way of disinfection. Meanwhile, due to their toxic effects on environment and health, there are doubts about the use of these synthetic chemicals as disinfectants, especially in the food industry. However, the development of sanitizers that are not harmful to non-target organisms, animals and human, is necessary for use in the food industry (Ölmez and Kretzschmar, 2009).

Sanitization based on the application of organic acids includes one of the most important interventions in the food industry to control microbiological safety and quality (Loretz et al., 2010). Organic acids are an inexpensive and effective alternative to synthetic disinfectants that reduce both the population and the prevalence of pathogenic bacteria (Loretz et al., 2011a, 2011b). Vinegar, due to the presence of significant amounts of organic acids and other natural substances with antibacterial effect, has been proven to have some disinfectant properties (Chang and Fang, 2007; Sengum and Karapinar, 2004; Wu et al., 2000).

Vinegar is a natural fermented product containing various nutrients and bioactive compounds such as acetic acid, gallic acid, catechin, epicatechin, chlorogenic acid, caffeic acid, p-coumaric acid, and ferulic acid which have a wide variety of therapeutic properties including antioxidant, antibacterial, antiobesity, antihypertensive, and cholesterol-lowering (Budak et al., 2014; Kahraman et al., 2021). Its antibacterial activity is attributed to the presence of organic acids, polyphenols, and melanoidins. Polyphenols and melanoidins, which are produced from raw materials and fermentation processes, also contribute to the antioxidant properties in vinegars (Chen et al., 2016). It has been reported that both grain and fruit vinegar can improve antioxidant capacities and reduce oxidative damage in *in vitro* and *in vivo* experiments (Chou et al., 2015; Verzelloni and Tagliazucchi 2007; Coelho et al., 2017). Vinegar is produced by carbohydrates-rich foods such as grape, apple and other fruit juices. Rice, malt and beer can be also used as raw materials for producing vinegar. The materials used in the production of vinegar change the vinegar content, so their therapeutic effects (Budak et al., 2014; Samad et al., 2016). The aim of the present study was to assess the total phenolic content and antiradical activity of the commercial grape and apple vinegar as well as to evaluate their antibiofilm and antimicrobial activities against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

MATERIAL and METHOD

Sample preparation

The apple (AV) and grape (GV) vinegar samples used in the current study were commercially supplied from a local market. Samples were stored at +4 °C until use.

Physicochemical properties

The pH values of the vinegar samples were determined by a digital pH meter (704 pH Meter, Metrohm) at 25ºC ± 2ºC. Total acidity quantification of the vinegar samples was performed by titration method described by Kahraman et al. (2021).

Total phenolic compounds

In this study, the total phenolic content of vinegar samples was determined by using the Folin-Ciocalteu method based on the procedure of Pawar and Dasgupta (2018), and gallic acid (Sigma, USA) was used as a standard. The results were expressed as milligrams of gallic acid equivalents (GAE, mg gallic acid/g). Gallic acid was dissolved and diluted in ethanol

(Merck, Germany) for two-fold serial dilutions ranging between 3.12 and 200 µg/mL. After mixing 200 µL of filtered samples or standard solutions with 400 µL of distilled water in tubes, 200 µL of 10 % Folin–Ciocalteu's (F–C) phenolic reagent diluted in distilled water was added to the tubes. After 5 min incubation, 200 µL of 1 M sodium carbonate solution was added to the tubes. The mixtures (300 µL), after 30 min incubation in the dark at room temperature, were added into a 96-well plate. Ethanol was used as blank. Absorbance measurements were performed at 750 nm using a microplate spectrophotometer (Multiskan Go, Thermo Scientific).

Radical scavenging activity

Antioxidant activity (%) of each sample was assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay. The DPPH solution at the concentration of 200 µM was prepared in methanol. 50 µL of two concentrations (10% and Neat) from each vinegar sample (prepared in distilled water) and 150 µL of the DPPH solution were added to the well of the 96-well plate. After 30 min incubation in the dark at room temperature, the absorbance was measured at 517 nm by using a microplate reader (Multiskan Go, Thermo Scientific). Absolute methanol was used as blank. The percentage of the radical scavenging activity (RSA) was calculated based on the following equation:

$$\text{DPPH scavenging activity (\%)} = \frac{(\text{Ac} - \text{As})}{\text{Ac}} \times 100$$

Ac: Absorbance of control [DPPH + Methanol without sample]

As: Absorbance of sample [DPPH + Sample (vinegar)]

Bacterial strains and preparation of inoculum

Staphylococcus aureus (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853) strains were obtained from the Laboratory of Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University stock culture collection. The bacterial strains were grown in Tryptic Soy Agar (BK047HA, BİOKAR) and incubated for 18-24 h at 37 °C. Each bacterial cell was transferred into 0.9% sterile saline buffer and adjusted to 0.5 McFarland scale (approximately 1.5 x 10^8 CFU/mL).

Agar well diffusion

The antibacterial activity of vinegar samples was determined by agar well diffusion method (Collins et al., 1995). The two-fold serial dilutions of samples (Neat, 50%, 25%, 12.5%, 6.75%, 3.12% and 1.56%) were prepared in sterile distilled water. Each strain of bacteria was adjusted to a 0.5 McFarland standard in 0.9% sterile saline buffer solution. Bacterial suspensions were streaked on Mueller Hinton Agar (BK048HA, BİOKAR) using sterile cotton swabs. Wells (4 mm height and 6 mm in diameter) were made using a sterile borer and filled with the different concentration of vinegar (100 µL/well). Enrofloxacin (64 µg/mL) and sterile distilled water were used as positive and negative controls, respectively. The plates were incubated at 37°C for 24 h. Following incubation, the diameter of inhibition zone was measured with digital caliper.

Biofilm formation

The effects of apple and grape vinegar on biofilm formation was determined as previously described by Čabarkapa et al. (2019) and Sudagıdan & Yemenicioğlu (2012) with slight modifications. Briefly, three wells of a sterile flat-bottomed 96-well polystyrene microtiter plates (TPP 92096, Switzerland) were filled with 145 µL TSB+1% sucrose and 55 µL vinegar dilutions prepared in 0.9% sterile saline solution (Neat, 50%, 25%, 12.5%, 6.75% and 3.12%). Afterwards, 20 µL of each bacterial suspension (adjusted to 0.5 McFarland standard) was inoculated into each microplate well. The final volume of each well was 220

µL. The following controls were used for each microplate; positive control: TSB+1% sucrose (145 µL), 0.9% sterile saline solution (55 µL) and bacterial suspension (20 µL); negative control I: TSB+1% sucrose (220 µL); negative control II: TSB+1% sucrose (145 µL) the appropriate vinegar concentration in sterile distilled water (55 µL) and 0.9% sterile saline solution (20 µL). The plates were incubated at 37°C for 24 h. After the incubation period, the nonadherent bacteria were removed and the microplate wells were gently washed three times with 250 µL sterile distilled water. The formed biofilm on the bottom of wells was fixed with 200 µL of methanol, then incubated for 15 min at room temperature. The wells were emptied and allowed to dry at 55 °C for 1 h, and stained with 200 µL of crystal violet (CV; 0.5%) for 10 min. The excess dye was washed under tap water. Glacial acetic acid (250 µL, 33%, v/v) was added into the wells to extract the absorbed CV from bacterial cells, and the absorbance of the eluted solution was measured at 600 nm using the microplate reader (Epoch, BioTek, USA). The effect of vinegar samples on biofilm formation was calculated based on the following equation (Čabarkapa et al. 2019):

$$\text{Reduction (\%)} = [1 - \frac{(A1 - A2)}{(\text{Apc} - \text{Anc})}] \times 100$$

A1: Absorbance of test wells,

A2: Absorbance of wells with negative control II,

Apc: Absorbance of positive control,

Anc: Absorbance of wells with negative control I (broth only).

Statistical analyses

All experiments were replicated three times. Homogeneity and normality test were applied on data. The biofilm reduction results of the vinegars were evaluated with one-way ANOVA followed by Duncan post hoc multiple comparison test and data of inhibition zone were analyzed with students t-test using statistical software SPSS 21.0. Results were expressed as the mean±standard deviation (SD) and values of $P<0.05$ were considered significant.

RESULTS

Physico-chemical properties of vinegar samples are given in Table 1. The pH values of AV and GV were 3.03±0.16 and 2.94±0.09 respectively. The total acidity for GV (4.08±0.10 g/100 ml) was superior to AV (3.99±0.21 g/100 ml) (Table 1). Total phenolic content was 58.68 ± 2.06 mg gallic acid/g in GV and 96.11 ± 1.14 mg gallic acid/g in AV. Apple vinegar exhibited higher DPPH radical scavenging activity than GV. DPPH activity (%) of AV and GV was 90.39 ± 0.004 and 88.34 ± 0.002, respectively.

Table 1. Physico-chemical properties of vinegar samples.

	Grape vinegar	Apple vinegar
pH	2.94±0.09	3.03±0.16
Total acidity (g/100 ml)	4.08±0.10	3.99±0.21
Total phenolic (ug Gallic acid/mg)	96.11±1.14	58.68±2.06
DPPH 10%	22.88±0.01	17.01±0.009
DPPH 100%	88.34±0.002	90.39±0.004

Antimicrobial activities of apple and grape vinegar against selected pathogens are given in Table 2. Agar well diffusion method showed that both AV and GV samples had antibacterial activity against *S. aureus* and *P. aeruginosa* (Figure 1). The antibacterial activity of GV was superior to AV ($p<0.05$; Table 2). The populations of *S. aureus* and *P. aeruginosa* were significantly reduced by neat GV and AV samples. While AV and GV samples at 50% concentration did not form a visible zone of inhibition against *S. aureus*, they showed an

inhibitory effect against *P. aeruginosa* (16.25 mm for GV and 16.50 for AV). The vinegar applied at the lowest concentration (25%) did not show any antibacterial effect on either bacteria. Overall, both neat vinegar samples showed strong inhibition against the bacteria tested (Table 2).

Table 2. Antimicrobial activity of apple and grape vinegar against *S. aureus* and *P. aeruginosa* strains using agar well diffusion method

	Zone of inhibition (mm)			
	***S. aureus* ATCC 25923**		***P. aeruginosa* ATCC 27853**	
Concentration (%)	**GV**	**AV**	**GV**	**AV**
Neat	22.75±0.21[a]	21.75±0.07[b]	22.0±0.98	21.55±0.63
50	ND	ND	16.25±0.07	16.5±0.14
25	ND	ND	ND	ND
Enrofloxacin (64 µg/mL)	31.9±0.14	31.65±0.21	15.85±0.21	17.5±0.35

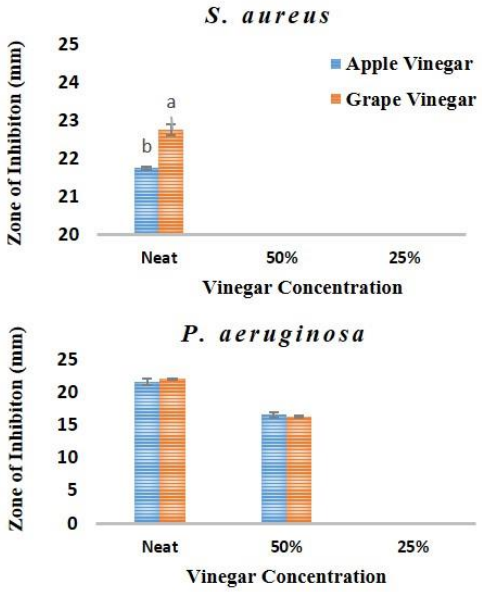

Figure 1. Inhibition zone of *S. aureus* and *P. aeruginosa* strains against apple and grape vinegars. Different superscripts (a,b) indicate that the means are significantly different from each other ($p<0.05$).

Effectiveness of grape and apple vinegar on the biofilm forming ability of selected pathogens is given in Table 3. The solutions containing 50% to 6.25% vinegar samples (both AV and GV) prevented almost 100% biofilm formation in both bacteria. However, solutions containing lower amounts of vinegar inhibited stronger biofilm formation by *P. aeruginosa*.

DISCUSSION

Vinegar is a fermented plant-based product and its content shows differences depending on raw material and techniques used in production process. In this study, the pH values of AV and GV were in line with previous studies (Akbaş et al., 2010; Kahraman et al., 2021). Besides, the vinegar samples complied with regulatory limits for total acidity (total acidity ≥40g/L) which is an important indicator for assessing the quality of vinegar (TSE, 2016).

Previous studies have shown that vinegar samples have high radical scavenging activity and high phenolic content. Aydın and Gökışık (2019) showed that the DPPH activity of the vinegar obtained from Vitis vinifera samples varied between 83.66% and 95.81% and the total phenolic content was 160.23 ± 0.007 µg GAE/ml. In another study, it has been reported that the antioxidant activity of GV and AV samples was 0.119 ± 0.023 and 0.147 ± 0.003 µg TE / mL, respectively. The total phenolic content of both vinegar samples was shown to be 1.025 ± 2.828 and 988 ± 2.828 mg GAE/L (Sengun et al, 2019). In the current study, total phenolic content and antiradical activity of vinegar samples were determined according to spectrophotometric methods. Interestingly,

although high total phenolic content was expected in GV (58.68 ± 2.06 mg gallic acid/g), high phenolic content was obtained from AV (96.11 ± 1.14 mg gallic acid/g). Although GV had a higher phenolic content than AV (Table 1), it had low antiradical activity, which may be due to the presence of various bioactive compounds such as flavonoids that provide antiradical activity (Chen et al., 2016).

Table 3. Effectiveness of different concentration of grape and apple vinegar on the biofilm forming ability of *S. aureus* and *P. aeruginosa*

Vinegar Concentration (%)	Biofilm forming reduction (%)			
	***S. aureus* ATCC 25923**		***P. aeruginosa* ATCC 27853**	
	GV	**AV**	**GV**	**AV**
25.00	98.33±0.40[a]	99.73±1.80[a]	100.75±0.30[a]	100.15±0.52[a]
12.50	98.31±1.29[a]	97.79±0.62[a]	99.40±0.48[a]	99.70±1.04[a]
6.25	97.64±1.52[a]	97.51±2.63[a]	99.40±0.52[a]	98.65±0.92[a]
3.13	49.41±1.82[b]	48.07±1.87[b]	74.66±1.56[b]	65.32±3.82[b]
1.56	28.16±2.84[c]	32.25±2.02[c]	70.59±2.36[b]	63.06±3.09[b]
0.78	17.76±3.31[c]	12.32±3.57[c]	63.65±1.80[b]	62.01±3.12[b]

Each value represents the mean ± standard deviation (SD). Different superscripts within a column (a,b,c) indicate that the means are significantly ($p<0.05$) different from each other. GV: grape vinegar, AV: apple vinegar

Currently, vinegar has gained popularity as an all-natural cleaner due to its contents including organic acids and other compounds with antibacterial activity. Organic acids, such as acetic acid, demonstrate antibacterial activity and their antibacterial activity seems to be associated with altering proton and associated anion concentration in the cytoplasm, resulting in disruption of purine bases and essential enzymes and decrease in bacterial viability (Gómez-García et al., 2019; Lingham et al., 2012). Several studies have reported that vinegar inactivates several bacteria such as *L. monocytogenes*, *Salmonella* Enteritidis, *S. sonnei*, *S. aureus*, *E. coli* and *Enterococcus faecalis*, thus inhibiting growth of and killing most foodborne pathogens (Medina et al., 2007; Chang and Fang., 2007; Zhang et al., 2018; Mohanty et al., 2017). The results obtained in this study are similar to the results obtained from the studies (Baldas and Altuner, 2018; Ousaaid et al., 2021; Yagnik et al., 2018, 2021; Gaber et al., 2020; Bakir et al., 2017; Janchovska et al., 2015). In the current study, generally, we also found that *P. aeruginosa* was more susceptible to vinegars than *S. aureus*, which confirms that of previous study reported that gram negatives are more sensitive than positives (Halstead et al., 2015).

Bacterial biofilms formed by a range of pathogenic microorganisms are a notable challenge in food safety and human health. Inhibiting or preventing biofilm formation has long been an important issue and the most effective way against biofilm formation is to inhibit bacterial growth by using antibacterial agents (Roy et al., 2018). Studies examining the effects of vinegar on biofilm formation are limited. Generally, studies have examined the effects of acetic acid, which is abundant in vinegar, on biofilm formation. Acetic acid is formed during the fermentation of vinegar and present in a 3-5% concentration. 70% apple cider vinegar significantly reduced the biofilm formed by *S. aureus* (Pedroso et al., 2018). Halstead et al. (2015) reported that acetic acid at a concentration of 0.31% statistically significantly inhibited the biofilm formation by *P. aeruginosa*. Tsang et al. (2018) demonstrated that 5% and 3% acetic acid eradicated 96.1% and 85.9% of biofilm-associated methicillin-sensitive *Staphylococcus aureus* (MSSA), respectively. In the present study, both of vinegar solutions (50% to 6.25%) prevented

almost 100% biofilm formation in both bacteria. A positive relation was found between the biofilm reducing ability of vinegar samples and their antibacterial activity in this study.

CONCLUSION

Commercial grape and apple vinegar significantly reduced the viability of *S. aureus* and *P. aeruginosa*, thereby decreasing biofilm formation. Treatment with the vinegar might offer a useful method to decrease the risk of *S. aureus* and *P. aeruginosa* infection either in public spaces or at home.

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Conflict of interest: The authors declared that there is no conflict of interest

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Splenic mass in a dog: clinical case report

ABSTRACT

Splenic masses, the majority of which are formed by hemangiosarcomas, are frequently observed in dogs of older age compared to other species and ages. A 11-year-old non-neutered male Beagle was admitted to the hospital with the complaints of abdominal distension with non-specific findings such as anorexia, stagnation, and weight loss. Severe abdominal distension with pallor of mucous membranes on clinical examinations; leukocytosis with anemia in the hemogram; high BUN and creatinine levels and a significant increase in liver enzymes in serum biochemistry were determined. In the microscopic examination of the aspirate taken by fine needle aspiration of the splenic mass which determined during ultrasonographic examination, a large number of pleomorphic, multinuclear neoplastic cells with eccentric nuclei, and different amounts of eosinophilic cytoplasm were detected. In this case report, in cases of limitations where biopsy or laparotomy could not be performed due to conditions such as the vascular structure of the mass or patient's disapproval; it was demonstrated that mass presence determined by ultrasonographic examination and the number of infiltrating mast cells determined by microscopic examination of the aspirate taken by fine needle aspiration can provide information in determining the benign or malignant character of the mass.

Keywords: Dog, spleen, neoplasia, hemangiosarcoma, ultrasound, aspiration

INTRODUCTION

It has been reported that splenic masses in dogs may be neoplastic such as sarcoma, lymphoma, hemangiosarcoma or non-neoplastic origin such as splenitis, hematoma, lymphoid hyperplastic nodular lesions, and dogs with splenic mass may be asymptomatic as well as with non-specific findings such as lethargy, anorexia, weakness and collapse (Tillson, 2003; Vnuk et al., 2014). Splenic neoplasia is common in elderly and medium to large breed dogs and the hematological and biochemical laboratory findings of are highly variable and non-specific. Although the etiology of the disease is not known exactly, it has been reported that the disorder of the biochemical pathways involved in angiogenesis along with genetic factors plays a role in its development (Thamm, 2007).

In veterinary medicine, abdominal ultrasonography is frequently used in the preoperative evaluation of animals suspected of splenic disease. Splenic masses are often identified by abdominal palpation and/or abdominal ultrasonography/radiography (Tillson, 2003). Although minor changes in splenic composition can be sensitively observed with ultrasonographic imaging, it is not specific for diseases other than splenic abscess or torsion (Vnuk et al., 2014), and the appearance of the spleen differs between diseases (Sato & Solano, 2004).

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Research Article

Erdem GÜLERSOY[1a]
Süleyman Serhat İYİGÜN[2b]
Alper ERTÜRK[2c]
Mahmut OK[2d]

[1]Department of Internal Medicine, Faculty of Veterinary Medicine, Harran University, Şanlıurfa, Turkey

[2]Department of Internal Medicine, Faculty of Veterinary Medicine, Selcuk University, Konya, Turkey

ORCİD-
[a]0000-0001-8511-0150
[b]0000-0002-3270-1931
[c]0000-0002-0284-2004
[d]0000-0002-8210-6735

Correspondence
Erdem GÜLERSOY
egulersoy@yahoo.com

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Therefore, ultrasound-guided fine needle aspiration and biopsy are used to obtain a diagnosis without the need for invasive surgical interventions (Sabattini & Bettini, 2009). Cytological or histological evaluation of splenic tissue samples provides information that can eliminate the need for surgical intervention such as benign character or the presence of systemic disease (Ballegeer et al., 2007). When compared to biopsy technique, fine needle aspiration has a lower risk due to needle's fine characteristic that does not cause ruptur or haemorrhage and its features such as not requiring sedation or anesthesia of the animal (Stockhaus & Teske, 1998).

In this case report, diagnostic methods such as auxiliary hematological and microscopic examinations in the diagnosis of a splenic mass detected in a dog by ultrasonography in conditions of clinical limitations such as lack of biopsy due to vascular structure of the splenic mass or inability to perform laparotomy due to the patient's disapproval are presented.

CASE DESCRIPTION

The material of this case report consisted of 11-year-old non-neutered male Beagle which brought to the Animal Hospital of Selcuk University Faculty of Veterinary Medicine, Department of Internal Medicine, with non-specific findings such as anorexia, stagnation, weight loss, and abdominal distension. The physical examination of the dog revealed dehydration, pallor of the mucous membranes, low body temperature (36.1 °C) and severe abdominal distension. It was determined that palpable lymph nodes such as mandibular and popliteal lymph nodes were indolent and of normal size on palpation. No abnormal sounds and arrhythmias were detected in lung and heart auscultation.

Table 1. Serum biochemistry findings

Serum Chemistry	Values	Range	Direct Bilirubin (mg/dl)	0.8	0-0.3
BUN (mg/dl)	106	4.70-7.30	**Phosphorus** (mg/dl)	15.3	1.8-6.4
Creatinine (mg/dl)	7.3	0.8-1.8	**Albumin** (g/dl)	2.2	2.1-3.9
AST (U/L)	165	10-80	**Cholesterol** (mg/dl)	368	90-205
ALT (U/L)	549	10-80	**Calcium** (mg/dl)	7.4	8-10.7
ALP (U/L)	1148	10-80	**Triglycerides** (mg/dl)	96	10-114
Amylase (U/L)	1799	500-1800	**Magnesium** (mg/dl)	3.6	1.5-3.5 mg/dl
Glucose (mg/dl)	131	70-150	**GGT** (U/L)	36	1-10
LDH (U/L)	201	75-490	**Total Protein** (g/dl)	6.5	5.4-7.8
Total Bilirubin (mg/dl)	1.6	0.1-0.6	**CPK** (U/L)	152	50-450

BUN: Blood urea nitrogen, AST: aspartate aminotransferase, ALT: alanine transaminase, ALP: alkaline phosphatase, LDH: lactate Dehydrogenase, GGT: gamma-glutamyl transferase, CPK: creatine phosphokinase

Heart (124 bpm) and respiratory rate (36 bpm) were within normal reference limits. For further diagnosis, blood gases (ABL90 Flex Radiometer Automatic Analyzer, Denmark), hemogram (MS4e Melet Schloesing Laboratoires, France) and serum biochemistry (BT 3000 plus Biotecnica Instruments SpA autoanalyzer, Italy) analysis along with abdominal ultrasonography (5-7.5 MHz, Mindray DC-6, China) were performed. Hyperlactataemia in blood gases (3.5 mmol/L), leukocytosis (26.26 m/mm^3) and anemia (5.25 M/mm^3) in hemogram, high BUN (106 mg / dl), creatinine (7.3 mg/dl), AST (165 U/L), ALT (549 U/L), ALP (1148 U/L), total bilirubin (1.6 mg/dl), phosphorus (15.3 mg/dl), GGT (36 U/L) together with low albumin (2.2 g/dl) and calcium (7.4 mg/dl) levels in serum biochemistry were determined. In the abdominal ultrasonography performed following blood analysis, the head and tail borders of the spleen and the hyperechoic

capsular area of the spleen could not be visualized in the left hypogastric area, which is the normal anatomical region of the spleen, instead a heterogeneous hypoechoic mass with 9.92 cm diameter with irregular cavernous vascular structure was detected. During the ultrasonographic examination, no abdominal effusion was detected and it was observed that the mass covered the entire splenic area. Considering the vascular characteristic of the splenic mass, transabdominal fine needle aspiration was preferred because of the risk of secondary rupture associated with biopsy.

Table 2. Blood gases and hemogram findings

Blood Gases	Values	Reference	Hemogram	Values	Reference
pH	7.35	7.31-7.42	**WBC** (m/mm^3)	26.26	6-17
pCO_2 (mmHg)	33.4	29-42	**Lym** (m/mm^3)	6.56	0.6-5.1
pO_2 (mmHg)	44.3	85-95	**Mon** (m/mm^3)	0.91	0.1-1.7
K (mmol/L)	3.8	3.6-5.5	**Gra** (m/mm^3)	18.79	3-13.6
Na (mmol/L)	144	139-154	**RBC** (M/mm^3)	5.25	5.5-8.5
Ca (mmol/L)	0.95	2.2-3	**MCV** (fl)	74.6	58-73
Cl (mmol/L)	95	102-120	**MCH** (pg)	25.6	19.5-24.5
Lactate (mmol/L)	3.5	0-2	**MCHC** (g/dL)	25	28-40
Base excess (mmol/L)	-5.8	-4-4	**Hct** (%)	39.1	35-55
HCO_3 (mmol/L)	19.2	17-24	**Hb** (g/dL)	9.8	10-18

pH: Power of hydrogen, pCO2: partial pressure of carbondioxide, pO2: partial pressure of oxygen, K: potassium, Na: sodium, Ca: calcium, Cl: chlorine, HCO3: bicarbonate, WBC: leukocyte, Lym: lymphocyte, Mon: monocyte, Gra: granulocyte, RBC: red blood cells, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, Hct: hemotocrit, Hb: haemoglobin

For fine needle aspiration, the dog was held in the lateral position and the sample was taken with a 22 gauge, 1.5 inch needle under ultrasound guidance without applying negative pressure in the syringe (Figure 1).

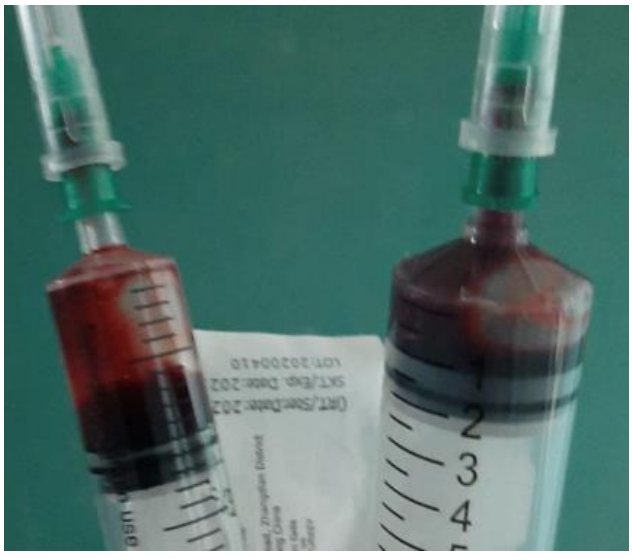

Figure 1. Physical appearance of the aspirate taken from the splenic mass by fine needle aspiration

Wright-Giemsa staining of the aspirate and microscopic examination (Olympus, USA, light microscope x100 magnification) was performed within 1 hr following the procedure. Numerous pleomorphic, multinuclear, neoplastic cells with eccentric nuclei with different amounts of eosinophilic cytoplasm and moderate anisocytosis and anisocaryosis were detected. The dog was humane euthanized with the owner's consent, as the medical condition of the patient did not improve despite palliative treatment.

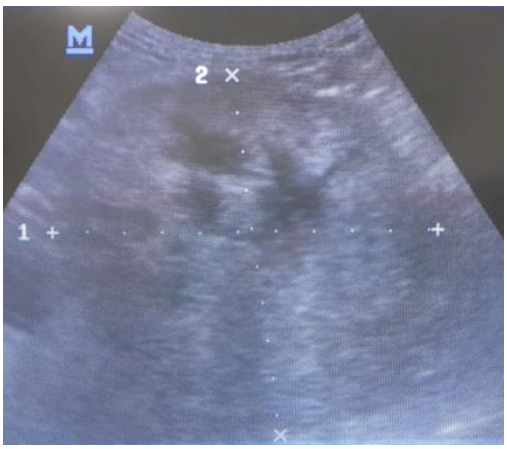

Figure 2. USG image of the splenic mass of 9.92 - 9.29 cm in size (7.5 Mhz).

Considering the emotional state of the owner, necropsy could not be performed. Since the presence of a splenic neoplastic mass was confirmed in accordance with the anamnesis, physical examination, laboratory and imaging techniques, the necropsy of the dog could not be performed, therefore the benign or malignant character of the mass could not be revealed histopathologically. Serum biochemistry findings in Table 1, blood gases and hemogram findings are presented in Table 2, ultrasonographic image of splenic mass in Figure 2, and multinuclear neoplastic cells determined in microscopic examination are presented in Figure 3.

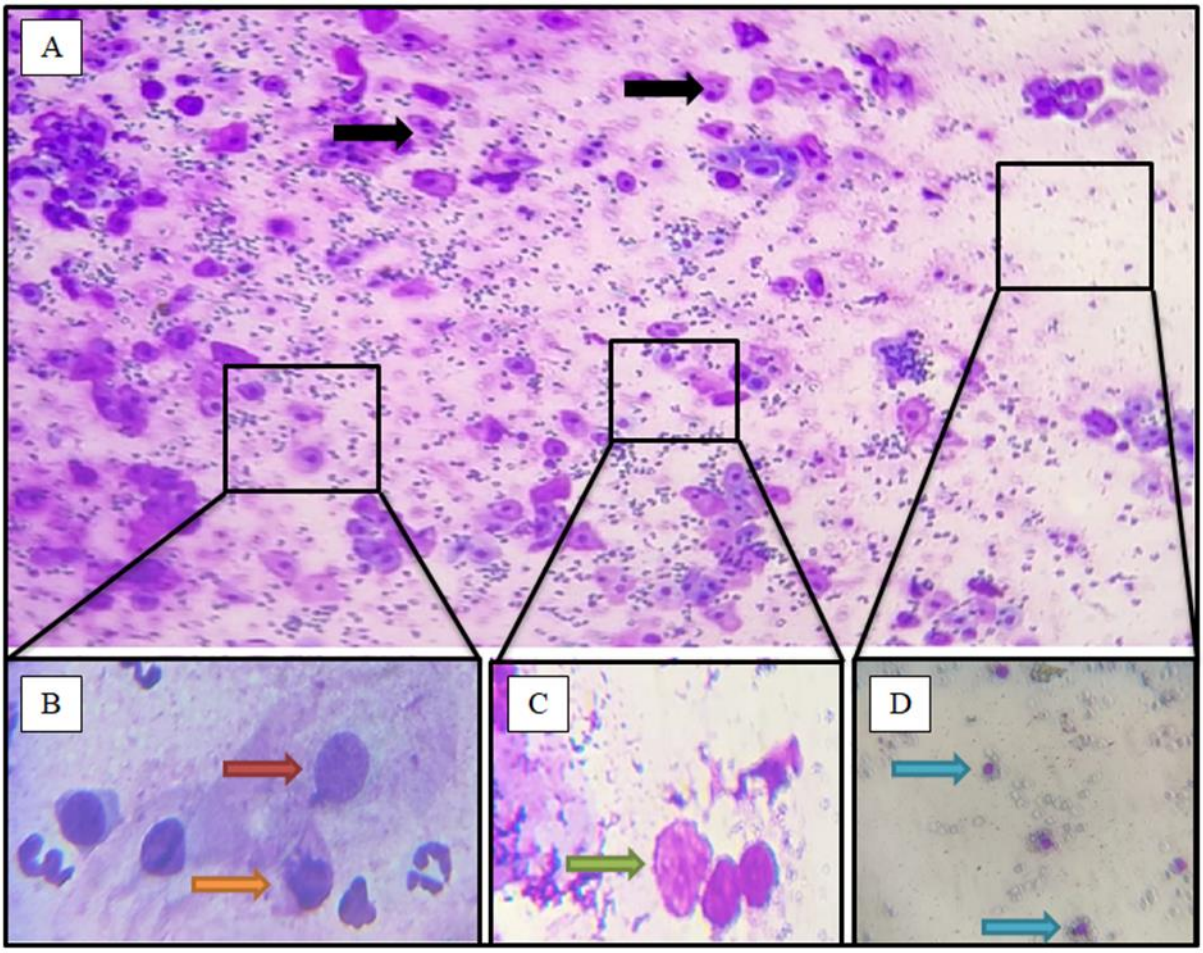

Figure 3. A: Pleomorphic, multinuclear (black arrows) anisocytic neoplastic cells are seen in microscopic examination of the aspirate taken from the spleen by fine needle aspiration, x10 magnification B: Arrows indicate round (red arrow) and eccentric nuclei (orange arrow) of neoplastic cells, x100 magnification with immersion oil C: Neoplastic cells with numerous intracytoplasmic purple granules (green arrow), x100 magnification D: arrows indicate moderate anisocytosis and anisocaryosis (blue arrow), x100 magnification with immersion oil

DISCUSSION

Spleen; is a hematopoietic organ that has many functions such as blood cell production, haemoglobin iron production, erythrocyte destruction, blood filtration, phagocytosis and immune response (Valli, 2007). In addition to conditions such as trauma and torsion, degenerative, inflammatory, hyperplastic and neoplastic diseases of the spleen can be observed (Fry & McGavin, 2012). With appropriate anamnesis and blood analysis including hemogram and serum biochemistry, ultrasonographic imaging and ultrasound-guided biopsy or fine needle aspiration provide important clinical information in the diagnosis of splenic disorders in dogs (Macwilliams, 2008). In the hematochemical analysis of dogs with splenic neoplasia, mild to moderate anemia, leukocytosis, and high ALP levels were

reported (Tillson, 2003). Studies have reported that in hematochemical analysis; regenerative anemia, high ALP, ALT and GGT levels along with weakness, anorexia and abdominal distension in the clinical examination are the most common findings of dogs with splenic mass (Fossum, 2007; Thamm, 2007). In dogs with splenic hemangiosarcoma, increased levels of kidney-related parameters such as BUN, creatinine and liver-related enzymes such as ALP, ALT, AST and GGT have been reported to be associated with tissue hypoxia, hepatic circulatory disorders, cholestasis, dehydration and hemolysis (Gavazza, 2009). Being the mean cell volume MCV, is an important indicator along with MCH and MCHC in anemia cases. If reticulocytes are present, the MCV should increase and MCHC decrease, as reticulocytes are in general larger than the RBCs. It is reported that an increased MCHC is always and artifact and a lower MCHC indicate macrocytic hypochromic regenerative anemia as immature RBCs have less haemoglobin (Gröndahl, 2019). Thus, the low RBC and high MCV together with low MCHC levels detected in the hemogram analysis of the present case indicate that anemia was macrocytic hypochromic regenerative characteristics; elevated BUN and creatinine levels due to dehydration and progressive renal function impairement (Adamidis et al., 2010), elevated levels of liver enzymes in the serum biochemistry due to hepatic circulatory impairment; and that hyperlactatemia is due to tissue hypoxia that develops as a result of decreased tissue oxygenation due to anemia. These findings are consistent with previous reported data in dogs with splenic mass/hemangiosarcoma (Smith, 2003; Tillson, 2003).

In a study, it has been reported that the number of infiltrating mast cells is different between malignant and benign vascular tumors of the spleen, and their number is higher in hemangioma cases compared to hemangiosarcoma cases (Sabattini & Bettini, 2009). In the present case, a large number of pleomorphic, multinuclear, neoplastic cells with eccentric nuclei were detected in the microscopic examination of the aspirate taken from the spleen by fine needle aspiration, but no mast cells were observed. This finding indicates that the mass determined in the present case is neoplastic and may have hemangiosarcoma character due to absence of mast cells (Rodriguez et al., 2020), due to the vascular structure of the mass, lack of biopsy sampling due to the risk of rupture, in cases where laparotomy or necropsy cannot be performed due to the owner's refusal, it shows that the aspirate examination taken with fine needle aspiration can provide important information about the character of the splenic mass (Thamm, 2007; Vnuk et al., 2014).

CONCLUSION

Diagnostic methods such as fine needle aspiration and magnetic resonance imaging are useful for more accurate diagnosis prior to surgical intervention and histopathological examination. In addition to fine needle aspiration technique, which is effective in the early diagnosis of malignant splenic masses, the owner’s acceptance of possible high treatment costs, risks and possible poor prognosis are important factors affecting the survival of the animal.

In conclusion, splenic masses, which are observed more frequently in elderly and medium to large breed dogs, in conditions of clinical limitations such as rupture risk, patient's refusal to take biopsy sample or laparoscopy, abdominal distension in physical examination; macrocytic hypochromic regenerative anemia and leukocytosis in the hemogram; high levels of ALP, ALT and GGT in serum biochemistry; mass presence by ultrasonographic imaging; and the number of infiltrating mast cells in the microscopic examination of the aspirate taken with fine needle aspiration could provide

information about the benign or malignant character of the mass.

ACKNOWLEDGMENT

Ethical approval: A dog brought to the Animal Hospital of the Faculty of Veterinary Medicine for diagnosis and treatment constituted the material of the case report. Informed consent was given to the owner of the patient and permission was obtained.

Conflict of interest: The authors state no conflict of interest.

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Hypoglycemic shock and acute liver injury in a dog associated with xylitol toxicity

Case Report

Durmuş HATİPOĞLU[1a]
Okan KAHRAMAN[2b]

[1]Department of Physiology, Faculty of Veterinary Medicine, Selcuk University, Konya, Turkey
[2]Green Pet Veterinary Polyclinic, Istanbul, Turkey

ORCİD-
[a]0000-0003-3790-7821
[b]0000-0003-0466-1474

Correspondence
Durmuş HATİPOĞLU
drhatip@selcuk.edu.tr

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ABSTRACT

An adult dog were evaluated for treatment loss of consciousness, convulsions, and severe tremors after ingestion of xylitol, a sugar alcohol used as a sweetener in various products. Clinical findings were noted as loss of consciousness, convulsions, and severe tremors, while physio-pathological findings included moderately to severely elevated serum activities of liver enzymes, hypoglycaemia and hypophosphatemia. To correct hypoglycaemia, dextrose was administered intravenously and to provide electrolyte homeostasis Izolen P was administered intravenously. Also, silymarin (Milk Thistle & Beta Glucan Complex, Natur, Turkey) S-adenosylmethionine (Hepatiale Forte Advanced®, Vetexpert, Poland), Vit. E (Evicap®, Koçak Farma, Turkey) and acetylcysteine (Mucomyst®, UPSA, France) were used to correct acute liver damage. Values measured after the applied treatments showed that blood glucose levels and liver enzymes returned to normal, and the patient was discharged. Xylitol causes hypoglycaemia and acute liver damage in dogs after ingestion. As a growing number of products contain xylitol, clinicians should be aware that ingestion of xylitol could have life-threatening effects. This case report also carries the distinction of being the first xylitol toxicity reported in dogs in Turkey.

Keywords: Acute liver damage, dog, hypoglycaemia, liver enzymes, xylitol.

INTRODUCTION

Polyols (sugar alcohols) are nutritious sweeteners obtained by catalytic hydrogenation of the oxo group of natural sugars, i.e. by substituting an aldehyde or keto group with hydroxyl (Ladret et al., 2008). Discovered by Emil Fisher in 1891, xylitol is a 5-carbon sugar alcohol and used as an artificial sweetener (Ur-Rehman et al., 2015). Used as a sugar substitute for the first time in Scandinavian countries during World War II, xylitol was obtained from hardwood species such as Betula pendula (Dunayer & Gwaltney-Brant, 2006). Xylitol has been used as a sugar substitute in diabetic patients in recent years because it tastes similar to sucrose but has fewer calories (Dills, 1989; Janket et al., 2019).

According to a study conducted between 2001 and 2011, xylitol has been reported to be found in various industrial products such as nutritional supplements such as chewing gum, mint candy, lollipop, some prescription medicines, various vitamins (multivitamin tablets, iron, vitamin D chewable tablets, etc.), coenzyme Q10, 5-hydroxytryptophan, chocolate, pudding, fruit preserves, jellies, jelly beans, beverage powders, toothpaste, mouth lozenges, moisturizing mouth sprays and mouthwash solutions (Dunayer, 2006).

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It has been suggested that the xylitol toxicity seen in dogs is related to severe hypoglycaemia caused by insulin secretion (Kuzuya et al., 1969). It is reported that xylitol increases insulin secretion 2.5-7 times more than the same amount of glucose in dogs (Kuzuya et al., 1966). Typical clinical symptoms such as ataxia, seizures and vomiting are observed in xylitol intoxication in dogs. Changes in the nervous system are associated with hypoglycaemia and usually occur approximately 30-60 minutes after xylitol intake. Approximately 0.15 g/kg xylitol has been reported to cause hypoglycaemia (Dunayer & Gwaltney-Brant, 2006). Researchers also state that an increase in liver enzymes occurs 8-12 hours after ingestion of xylene in dogs and severe liver failure is formed (Murphy & Dunayer, 2018).

CASE PRESENTATION

A 10-year-old, 36 kg, spayed female Golden Retriever was brought to the veterinary emergency clinic due to loss of consciousness, convulsions, and severe tremors. After the anamnesis, it was learned that the dog ate one package (180 g) of jelly tots containing xylitol approximately 1-1.5 hours ago. Xylitol was calculated to be approximately 8.2 g/kg of body weight. The dog rectal body temperature was 39.3°C, heart rate was 136 beats/min and respiratory rate was 21 breaths/min. The mucous membranes were pink and the capillary refill time was within the reference range. The abdominal area was saggy and there were no abdominal pain symptoms.

DISCUSSION

The initial diagnosis made included a complete blood count and serum chemical parameters. The blood glucose level found as 41 mg/dl showed that the dog had severe hypoglycaemia and the phosphorus level measured as 0.98 mg/dl showed that the dog had hypophosphatemia. All laboratory findings are shown in Table 1. While normal complete blood count values were noted in the laboratory findings, a slight increase was observed in haemoglobin (Hg) amount and platelet counts; but serum alanine transferase (ALT, 273U/L) and serum aspartate transferase (AST, 242 U/L) were found to be significantly increased (Table 1).

Table 1. Pre-treatment total blood count and serum biochemistry values.

Total blood count values			Serum biochemistry values		
Parameters	**Value**	**Reference ranges**	**Parameters**	**Value**	**Reference ranges**
WBC (10^9/l)	12.19	6.00-17.00	ALT (U/L)*	273	5-60
LYM (10^9/l)	4.08	1.00-4.80	AST (U/L)*	242	5-55
MONO (10^9/l)	1.10	0.20-1.50	GGT (U/L)	5	<=10
GRA (10^9/l)	7.01	3.00-12.00	ALP (U/L)	61	10-150
LY (%)	33.4	12.0-30.0	CREA (mg/dl)	0.70	0.40-1.80
MON (%)	9.0	2.0-6.0	GLU (mg/dl)*	41	60-125
GR (%)	57.5	62.0-87.0	UREA (mg/dl)	31.5	20.0-50.0
RBC (10^12/l)	8.20	5.50-8.50	TBIL (mg/dl)	0.00	<=0.40
HGB (g/dl)	18.4	12.0-18.0	Ca (mg/dl)	8.4	7.5-11.3
HCT (%)	53.08	37.00-55.00	P (mg/dl)*	0.98	2.20-5.50
MCV (fl)	65	60-77	TP (g/dl)	6.6	5.4-7.7
MCH (pg/dl)	22.4	19.5-24.5	ALB (g/dl)	2.7	2.3-3.8
MCHC (g/dl)	34.7	31.0-34.0	TC (mg/dl)	209	<=270
RDWc (%)	18.7		CK (U/L)*	481	20-200
PLT (10^9/l)	542	200-500	AMY (U/L)	727	500-1500
PCT (%)	0.51		GLB (g/dl)	3.90	
MPV (fl)	9.5	3.9-11.1	ALB/GLB	0.69	
PDWc (%)	39.1		(g/dl)		

* Parameters that are outside the reference range.

To correct hypoglycaemia, 5% dextrose (1 ml/kg) and to provide electrolyte homeostasis, Izolen P was administered intravenously in a total of 300 ml. In addition, silymarin (Milk Thistle & Beta Glucan Complex Natur, Turkey) (20-50 mg/kg/day PO), S-adenosylmethionine (Hepatiale Forte Advanced®, Vetexpert, Poland) (17-20 mg/kg/day PO), Vit. E (Evicap®, Koçak Farma, Turkey) (400 IU x 1) and acetylcysteine (Mucomyst®, UPSA, France) were used to correct acute liver damage. Values measured after the applied treatments showed that blood glucose levels and liver enzymes returned to normal, and the patient was discharged (Table 2).

Table 2. Post-treatment total blood count and serum biochemistry values.

Serum biochemistry values		
Parameters	**Value**	**Reference ranges**
ALT (U/L)	55	5-60
AST (U/L)	47	5-55
ALP (U/L)	61	10-150
GLU (mg/dl)	64	60-125
P (mg/dl)*	0.98	2.20-5.50
ALB (g/dl)	2.7	2.3-3.8

* Parameters that are outside the reference range.

Due to the increasing market share of products containing xylitol in the food industry over the past few years, the number of xylitol toxicosis reported in dogs has also increased (Dunayer, 2006; Dunayer & Gwaltney-Brant, 2006; Xia et al., 2009). Although increased insulin releases have been documented in rabbits, goats and cows, it was noted that xylitol does not cause similar insulin release or blood glucose changes in horses, rats, and humans (Kuzuya et al., 1971). It was reported that oral administration of 100, 500, and 1000 mg/kg of xylitol in cats is within reference ranges of both liver enzymes and blood glucose values (Jerzsele et al., 2018). However, xylitol intake in dogs causes a dose-dependent insulin release that is higher than the response to equal doses of glucose (Hirata et al., 1966; Kuzuya et al., 1966). Peak serum insulin concentrations were observed to be 6-times higher than glucose following xylitol intake and thus severe hypoglycaemia was observed in dogs (DuHadway et al., 2015; Kuzuya et al., 1969). It was experimentally reported that dogs that were given 1 or 4 g of oral xylitol per kilogram of body weight showed rapid increases in plasma insulin concentrations within 20 minutes and reached the peak at 40 minutes (Xia et al., 2009). Approximately 80% of xylitol metabolism occurs in the liver (Dunayer, 2004) and where it is rapidly oxidized to D-xylulose, then metabolized to glucose, glycogen, and lactate via the pentose-phosphate pathway (Froesch & Jakob, 1974). The remainder of xylitol (20-30%) is metabolized by the fat stores, erythrocytes, myocardium, kidneys and lungs, where they are then converted into water and carbon dioxide through carbohydrate metabolism (Ur-Rehman et al., 2015). Xylitol itself can directly stimulate pancreatic β-cells to secrete insulin; the increased insulin level then causes significant decreases in blood glucose levels. Hypoglycaemia affects the red blood cell membranes, causing bilirubin to break down and release (Peterson, 2013).

Although no LD_{50} was determined for xylitol intake in dogs, toxicosis has been reported with 0.1 g/kg hypoglycaemia and 0.5 g/kg hepatic necrosis (Piscitelli et al., 2010). Vomiting in dogs due to the development of hypoglycaemia 30-60 minutes after a meal is usually seen as the first sign of xylitol toxicosis, followed by weakness, ataxia and lethargy (Dunayer, 2006). In this case, the dog that swallowed xylitol at a dose of 8.2 g/kg was exposed to a dose

approximately 16.4 times the dose in which hepatic necrosis was seen. Although the first clinical symptoms such as tremor, loss of consciousness, convulsions, and severe hypoglycaemia observed after xylitol intake coincided with the previously reported xylitol toxicosis scenario, no sign of vomiting were observed. Xylitol induces hyperinsulinemia in dogs by stimulating the synthesis and secretion of insulin (DuHadway et al., 2015). The exact mechanism of the xylitol-induced increase in insulin secretion is unclear; however, a study conducted in anaesthetized dogs showed that xylitol directly stimulated insulin secretion by pancreatic islet β cells rather than its metabolites (DuHadway et al., 2015). As expected, increased insulin concentrations after xylitol intake caused a decrease in blood glucose (Table 1). Clinical manifestations such as weakness, depressive activities, tremors, loss of consciousness, and convulsions resulting from a dramatic decrease in blood glucose concentrations were a possible consequence of hypoglycaemia. Intravenous Dextrose and Izolen P, used to prevent and treat hypoglycaemia, yielded successful results (Table 2). The severe increase in liver enzymes such as ALT and AST were similar to previously reported cases and liver enzyme values of dogs for whom experimental xylitol toxicosis was formed (Table 1) (Dunayer & Gwaltney-Brant, 2006; Schmid & Hovda, 2016; Xia et al., 2009). Xylitol is mainly metabolized in the liver (Förster, 1975) and this metabolic process needs ATP (Vincent et al., 1989). When large amounts of xylitol are taken into the bloodstream, ATP in hepatocytes is depleted, resulting in hepatocyte necrosis and thus elevation of plasma ALT and AST (Dunayer& Gwaltney-Brant, 2006). The dramatic increase in ALT and AST is consistent with hepatocellular damage caused by the release of soluble cytosolic enzymes from the liver due to altered cell membrane permeability. As plasma GGT concentration did not change, liver damage was not considered to be associated with effects on the biliary system. S-adenosylmethionine was used to repair acute liver damage that occurred and to support liver function comprehensively and to help regenerate hepatocytes, and vitamin E, a good antioxidant, was used to eliminate the oxidative damage to the liver, and values were found to return to normal (Table 2).

The cause of hepatic necrosis in xylitol toxicosis in dogs is not fully known. Xylitol results in high concentrations of cellular nicotinamide adenine dinucleotide (NAD^+) that produces reactive oxygen species in the liver that can damage cellular membranes and macromolecules and reduce the viability of hepatocytes (Dunayer & Gwaltney-Brant, 2006). NAD^+ is an important cofactor that functions as an electron carrier in the organism and is reduced to NADH by glycolysis in the cytoplasm. (Verdin, 2015). Cytosolic NADH directs the osmotic-chemical synthesis of ATP as an energy storage molecule after it is transported to the mitochondria (Kim et al., 2019). Mitochondria have their own enzymatic mechanism to alleviate and eliminate ROS against cell homeostasis and oxidative stress damage (Moreno-Sánchez et al., 2018). When the mitochondrial ROS level exceeds the antioxidant defence system, the permeability of the mitochondrial inner membrane is lost, and as a result, it can lead to apoptotic cell death (Han et al., 2018; Ralph et al., 2015; Rottenberg & Hoek, 2017). To relieve oxidative stress, glutathione peroxidase (GPx), glutathione reductase (GR), thioredoxin reductase (TrxR), and superoxide dismutase (SOD) are transported from cytosol to mitochondria (Marí et al., 2009; Ribas et al., 2014). Studies have reported that high NADPH levels provide protection against ROS, but low NADPH levels cause cellular damage by causing ROS accumulation (Marí et al., 2009). GSH is required in cell detoxification and many metabolic processes, and acute liver damage caused by oxidative stress in dogs with low glutathione transferase activity or low natural protective mechanisms (Watanabe et al., 2004) may develop due to xylitol toxicity resulting in

NAP^+, the final product in metabolism. Both NADPH / $NADP^+$ and GSH / GSSG ratios are very important for improving oxidative stress (Moreno-Sánchez et al., 2018).

While the use of xylitol in the food industry has increased in recent years, for patients, it is especially critical to be vigilant about the use of xylitol, careful evaluation of product labels, and correct management of the treatment process, especially by veterinarians and dog owners. As a result, there is no antidote to xylitol toxicosis, and emergency treatment methods include stabilizing blood glucose, protecting the liver, and providing more care when needed. Closely monitoring blood glucose levels and providing dextrose supplements as needed is critical in the early stages for treatment modalities of xylitol toxicosis in dogs (Schmid & Hovda, 2016). This report revealed that xylitol toxicosis in dogs affects vital functions, and acute liver insufficiency and severe hypoglycaemia occurring as a result of xylitol toxicosis can be successfully treated with aggressive therapy.

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Socio-economic impacts of COVID-19 in a one health context

ABSTRACT

The last decades experienced a significant increase in the number of infectious disease outbreaks while current economic systems put pressure on the environment and wildlife is being destructed, leading to species to live closer to each other and humans. These diseases including zoonoses cause loss of life and threaten economic development and the integrity of the ecosystems. The recent COVID-19 is a significant example of this situation with a dramatic loss of human life, devastating economies and causing social disruption. The COVID-19 pandemic has also threatened food security, putting millions of people at risk of hunger, disrupted food, and feed supply routes, put pressure on livestock industries, led to a decrease in world meat production, caused trade restrictions, changed consumer habits, affected animal health and animal welfare. In this study, the main drivers of zoonoses, socio-economic impacts of these zoonoses with an emphasis on the COVID-19 pandemic and the necessary actions that need to be taken to prevent further epidemics/pandemics have been discussed in the context of "One Health" approach.

Keywords: Covid-19, one health, preventive health, socio-economy, zoonotic diseases

INTRODUCTION

While the COVID-19 pandemic has spread with a dramatic speed and caused devastating economic and social disruption, the world has already been confronted with a sudden increase in the number of infectious disease outbreaks in the last decades. World Health Organization (WHO) monitored 1483 epidemic events in 172 countries between the years 2011 and 2018. Some of these epidemic diseases such as influenza, Severe Acute Respiratory Syndrome (SARS), Middle East Respiratory Syndrome (MERS), Ebola, Zika, plague and Yellow Fever are fast-spreading and frequently being detected (GPMB, 2019). The global emergence of these diseases is notable in terms of existing human infections of which 60% is reported as zoonoses. Zoonoses are defined as diseases and infections naturally transmitted between people and vertebrate animals. 75% of 30 new human pathogens that have been detected in the last three decades have originated in animals. Around one billion cases of illness and millions of deaths occur every year from zoonoses globally (Jones et al., 2008). In 1918, "Spanish flu", also known as the 1918 flu pandemic, have caused to illness of an estimated 500 million and death of as high as 50 million people (CDC, 2019). Since then, 2.5 billion cases and 2.7 million deaths are caused by zoonoses each year, having serious impacts on health and economies (Njenga, 2020).

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Review Article

Işık ERŞAN [1a]
Arzu GÖKDAİ [2b]
Engin SAKARYA [2c]

[1]Ministry of Food, Agriculture and Livestock General Directorate of EU and External Relations, Ankara, Turkey

[2]Department of Animal Health Economics and Management, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey

ORCİD-
[a]0000-0001-7816-904X
[b]0000-0002-5509-2171
[c]0000-0003-3569-3292

Correspondence
Arzu Gökdai
agokdai@ankara.edu.tr

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Besides fatality, zoonoses jeopardize economic development and the integrity of the ecosystem. In the last twenty years, zoonotic diseases have caused more than $100 billion economic losses, excluding the economic cost of the COVID-19 pandemic, which was foreseen to reach $9 trillion over the next few years (UNEP, 2020). Economic impacts estimated for some of the past epidemics/pandemics are as such 2003 SARS epidemic costing of over US $40 billion (Lee et al., 2004); 2014-2016 West Africa Ebola outbreak causing US $53 billion loss (Huber et al., 2018), the 2009 H1N1 "Swine flu" pandemic causing an economic loss of US $45 to 55 billion (GPMB, 2019). Between the years 2013 - 2014 in Liberia, Gross Domestic Product (GDP) growth declined from 8.7% to 0.7%, due to Ebola and reducing commodity prices, and in Sierra Leone (excluding iron ore), GDP growth decreased from 5.3% to 0.8% (Zafar et al.,2016). The total direct costs of H5N1 "Bird flu" outbreaks have gone beyond US $20 billion since 2003 (Berthe et al., 2017).

Considering 60% of all human infections originate from animals, zoonotic outbreaks have serious economic impacts on the agricultural sector. Fifty percent of reported livestock losses are caused by zoonoses and zoonotic diseases have a higher percentage of animal slaughter (43% of livestock losses) as part of disease control measures compared to other diseases (6% of livestock losses) (Smith et al., 2019). The outbreaks of Ebola, MERS, and SARS devastated agricultural production in the regions that they were spread (Pu and Zhong, 2020).

The aim of this study is to discuss the main drivers of zoonoses, socio-economic impacts of these zoonoses with an emphasis on the COVID-19 pandemic and the necessary actions that need to be taken to prevent further epidemics/pandemics in the context of "One Health" approach.

SOCIO-ECONOMIC IMPACTS of COVID-19

The COVID-19 pandemic has caused a dramatic loss of human life, leading millions of enterprises face an existential danger, putting 3.3 billion workforces at risk of losing their incomes and bringing economic activity to a near-standstill as governments implied movement restrictions to prevent the virus to spread (Chriscaden, 2020, World Bank, 2020). Millions of people are facing the threat of extreme poverty and the number of undernourished people could increase by 132 million more in 2020 (FAO, 2020a).

The World Bank estimated that the global economy has contracted 4.3% in 2020. In advanced economies, the initial contraction was not as severe as expected, but the following recovery has been reduced by re-emergence of COVID19 cases. Meantime, output in China has recovered faster than anticipated due to a particular support from infrastructure spending. China's situation was a special case, disruptions from the pandemic in most of the emerging markets and developing economies were more severe than expected and resulted in deeper recessions and slower recoveries. (World Bank, 2021). As stated by the Economic Commission for Latin America and the Caribbean, COVID-19 will conclude the worst economic and social crisis in decades in the region, with a drop of GDP of -5.3 percent, poverty rate in the region could increase by up to 4.45% points during 2020, which means 2.936 million people (FAO, 2020b).

IMPACT of THE COVID-19 on LIVESTOCK and AGRICULTURE SECTOR

Unlike other infections like the Foot and mouth disease, Avian influenza, E. coli or Listeriosis, the COVID-19 pandemic has not spread directly through livestock and therefore has not directly caused disturbance in food production at farm level. On the other hand, the crisis is threatening the ability of farms to maintain food supply to markets due to closures, work force deficiency

caused by illness and delays in operations due to physical distance and lockdown measures. These factors compromise the capacity of agricultural and food enterprises to carry out operations as always and putting at risk the survival of small agri-food enterprises including farms, traders, manufacturers, distributors, and retailers (FAO, 2020c). The shrinkage in sales due to the COVID-19 has led to increases in factors such as financing cost, logistics, storage, labor, energy, packaging, and distribution. In animal products, significant increases were observed in both sales prices and total costs during this period.

In many countries the COVID-19 spread rapidly between workers in processing plants. There are reports of clusters of COVID-19 cases in these enterprises in many countries such as in Germany, the disease affected at least 1,500 workers in one of the EU's biggest meat processors while in the U.S., there had been more than 39,000 reported positive cases related to meatpacking enterprises as of September 11, 2020 (Marchant- Forde and Boyle, 2020). These outbreaks might be caused by factors such as cold and damp indoor areas are appropriate environments for this virus to spread, the difficulty of workers to stay two meters apart while carrying out operations rapidly moving production lines, and the lack of daylight might be helping the coronavirus to survive (Reuben, 2020). Eighteen processing plants in the United States were closed, that affects more than a third of the beef and pork supply of the country (Felix et al., 2020).

Bovine meat output in the U.S. is predicted to conclude in 2020 with a 5% overall contraction because of labor shortages in meat processing. Not only a decrease of bovine meat especially in the USA and Australia, but also COVID-19 is having a global impact on every meat type as the world meat output was also forecast to fall in 2020 to 333 million tons, 1.7% less than 2019. The contraction would also reflect a decrease in production of pig meat again, mainly in Asian countries with African swine fever outbreaks (FAO, 2020d). There are news reporting milk industry had also been affected by the crisis, as there have been news reported that in the U.S. farmers had to dispose of 14 million liters of milk every day caused by the disruption of supply routes (BBC, 2020). Disruption of supply routes also caused delays for feed sector. In Argentina, which is the top soymeal exporter country, restrictions have decreased soy supply to feed factories considerably (FAO, 2020e).

The current Covid-19 crisis has also affected the consumer habits. Most of the food purchases have now shifted to the supermarkets with the shutdown of cafes, restaurants, canteens and also more people working from home (Hobbs, 2020). Per-capita meat consumption this year is the lowest in nine years and the 3% decline from last year represents the biggest drop since at least 2000 (FAO, 2020d). In the European Union, pork consumption was predicted to fall to a seven-year low in 2020. In China, the news report that there is growing distrust over animal products after the government suggested a link between imported protein and an outbreak in Beijing (Almeida et al., 2020). French shoppers have been choosing more organic food since the start of the pandemic fears took hold of the country (BBC, 2020). Sales of plant-based meat products were increased 264% during a nine-week period from March to April 2020 in the U.S (Lyons, 2020). There has been an increase in egg and poultry meat consumption during lockdowns due to low prices, easy and fast preparation at home etc. (Hafez and Attia, 2020).

Demand have shifted away from higher value items and towards staple and ready-to-eat foods that are available to be stored and there has been a strong increase in online commerce (OECD, 2020). Demand for non-food agricultural products such raw fur skins or wool dropped notably, while increased for staple food, processed fruits and vegetables which reflects the initial panic buying and increase in the home-based consumption. The current pandemic put more downward pressure on world food prices,

which were already on a downward trend at the beginning of 2020, and producer revenues (WTO, 2020).

The beginning of the COVID-19 crisis has led to the governments panic about preserving their own food sources and disrupted global supply chains. As the top global wheat exporter Russia limited its grain exports from April to June 2020, the top global wheat importer Egypt stopped exports of legumes. In Argentina, which is the world's largest soybean products exporter, the roads in soybean production areas were temporarily closed, leading to the country's grain supplies reducing by half until the restrictions were loosened (Torero, 2020). Canadian imports of onions and eggplants from India decreased by 80% in two weeks as air cargo space diminished gradually (Jadhav et al., 2020). Measures implied by governments to control the pandemic have led disruptions to transport and logistics services. Border closures and social distancing requirements, which led to reducing the numbers of inspectors at borders, have increased the duration of customs clearance, and have led to retards, affecting the transit of perishable products (OECD, 2020).

As the crisis might have possible consequences on creating food security risks it might have an impact on animal health as well since veterinary practice have also been affected by the current crisis. According to a survey among practice owners in the U.S. in April 2020, about half of the veterinarians who participated the survey had client visits declined by 50% (AVMA, 2020). According to another survey, which was conducted with 3,258 pet owners from Brazil, the U.S., France, and the UK, more than a quarter of pet owners (27%) delayed or avoided contacting their veterinary practice since the beginning of the Covid-19 pandemic (HealthforAnimals, 2020).

The pandemic also affected animal health in terms of spreading between minks which are farmed for fur production. In Denmark SARS-CoV-2 infection was introduced into mink populations. Scientific investigations also have confirmed that SARS-CoV-2 infection has been reintroduced from mink to humans (OIE, 2021).

Animal welfare problems also occurred due to human cases which points the importance of One Welfare concept along the One Health approach. Since many processing plants were closed because of the clusters among the labors, this situation put pressure on pig and poultry industries. In the U.S., 45% drop in the capacity for pig processing which results 250,000 pigs are not being slaughtered daily, also led to longer transport routes to the plants in function and overcrowding of animals on farm. Some of the pig producers had to cull animals on farm with practices that made the animals feel more pain (Marchant- Forde and Boyle, 2020).

COVID-19 and ENVIRONMENT

This pandemic is reminding us our deteriorated relation with nature as the economic systems are putting pressure on the environment. In the last 50 years, 60% of all wildlife was lost, while the number of emerging diseases has become four times more than in the last 60 years. It is not a coincidence that ecosystem devastation has concurred with a significant boost in these infections. Natural habitats are being destroyed, leading to species to live more closely to each another and to humans, therefore risking that viruses find humans as new hosts, especially as the natural disease resistance which might result from biodiversity is lost (Quinney, 2020). Ecosystem integrity can help controlling diseases through supporting a diversity of species so that it is more difficult for one pathogen to spread faster or dominate (UNEP, 2016).

Land-use and agricultural industry changes are globally the top drivers of zoonoses. Changes in human host behaviors such as travel, conflicts and migration, wildlife trade, globalization, urbanization, and dietary preferences are also drivers of emerging zoonotic diseases (Loh et al.,

2015). Deforestation of land has been related to increased malaria rates, outbreaks of Ebola, Lyme disease and Rabies while Human Nipah virus, was related to intensive of pig farming and Avian Infl¬uenza was related to intensive poultry production (UNEP, 2016). SARS-CoV-2, the coronavirus which caused the COVID-19 pandemics, is closely linked genetically to coronaviruses isolated from bat populations. SARS-CoV, which is the pathogen that caused the SARS outbreak in 2003, is also closely linked to coronaviruses isolated from bats (WHO, 2020).

While many of these recent events have been caused by environmental degradations, these diseases have impacts on the environment as well. The Covid-19 pandemics have affected the environment in both positive and negative ways.

According to the NASA, deforestation degrees are changing in some regions, air pollution is declining, quality of water is advancing, and snow is becoming more reflective in some places since the beginning of the pandemic (Bates, 2020). After increasing for decades, global carbon dioxide emissions dropped by 6.4%, or 2.3 billion tons, in 2020, as the COVID-19 pandemic suppress economic and social activities (Tollefson, 2021). Emissions from road transport and aviation account for the largest share of the global decrease, due to the restrictions of governments.

The decline of emissions appears more in the US (–12%) and EU27 countries (–11%) and least pronounced in China (–1.7%) (UEA, 2020). According to the World Air Quality Index, air quality (NO2, CO, PM2.5, and O3) data for the January 2019–April 2020 period, shows there was a significant decrease in the levels of NO2, CO, and PM in 2020, compared to their levels in 2019 (Habibi et al., 2020). The observations of global nitrogen dioxide (NO2) during March, April, and May 2020, have revealed reductions between 10%-30% in polluted areas of Europe, North America, and Asia (EC, 2020). This drop in NO2 is related to the significant decline in land and air transportation, industry, and electricity generation during the lockdown. According to analyses carried out in some European cities including Dublin, Madrid, Rome and Brussels, there has been a significant reduction in sound levels or noise due to reduction in road and air traffic (Aletta et al., 2020, Asensio et al., 2021, Basu et al., 2021, Gibney, 2020).

The first studies on water quality suggest an improvement resulting from the lockdowns. The pollutants' concentration in the Vembanad Lake, which is the longest lake in India, dropped by 16% compared to the previous year (Yunus et al., 2020). On the other hand, an increase in the concentrations of microplastics in the waters is also possible, since from the beginning of the COVID-19 outbreak plastic-based personal protective equipment production has increased rapidly (Singh et al., 2020, Tedesco, 2020). As the consumer demand for e-commerce and home delivery increased globally as a result of lockdown, waste generation has increased. However, waste management and recycling activities has been limited because of disease control measures in many countries (Zambrano-Monserrate et al., 2020).

GENERAL EVALUATION and CONCLUSION

The COVID-19 pandemic has highlighted the importance of a One Health approach, which recognizes the health of people, animals and the environment are closely interconnected, as the solution to reduce the threat of possible pandemics in the future. This approach has gained importance in recent years due to the changes in interactions between people, animals and the environment that have led to emerging and re-emerging global diseases. Generally, environment health initiatives have been less represented than animal, livestock, and human health initiatives in global programs for the prevention and control of zoonoses. However,

environment is crucial for the One Health approach since land use change is the top driver to new outbreaks of zoonoses. Landscape policies and investments should be made with a One Health approach to take the opportunities to address the biggest challenges of our time (Njenga, 2020).

Likewise, animal health is an important component of the One Health approach and therefore Veterinary Services should have to cooperate and collaborate with Public Health authorities and those responsible for wildlife, to reduce the impact of the COVID-19. There are good examples of this approach, such as Veterinary Services in some countries have supported the public health services by testing samples from humans, while in some other countries veterinary practitioners have donated their protective equipment and ventilators. According to the OIE, Veterinary Services should be considered as essential services and national authorities should continue in the activities related to animal health, welfare and veterinary public health, under appropriate protocols (OIE, 2021).

Global based analyses suggest high return on investment in human and animal health systems in low- and middle-income countries to prevent possible future pandemics and epidemics, assume that US$1.8 billion to US$4.5 billion annual expenditure would result as a benefit between US $30-60 billion per year in avoided cost (Machalaba et al., 2017).

Nature provides extensive opportunities for businesses and governments since every dollar invested in restoration of nature could provide minimum $9 of economic benefits (Nature4Climate, 2020). In addition, shifts in farming and food production practices could reveal $4.5 trillion a year in business opportunities by 2030 and save trillions of dollars economic cost caused by social and environmental harms (FOLU, 2019).

Pointing to the likely cost of COVID-19 of $8-16 trillion globally by July 2020, estimations suggest that costs in the U.S. might be up to $16 trillion by the end of 2021. The experts estimate the cost of reducing risks to prevent pandemics is 100 times less costly than responding to these pandemics, highlighting the importance of "providing strong economic incentives for transformative change" (Rukikaire, 2020).

Recent studies have estimated that the cost of preventing further pandemics over the next decade by protecting wildlife and forests equates to just 2% of the financial damage caused by the COVID-19 (Dobson et al., 2020).

Even though the impact of the pandemic will differ between countries, it will probably increase poverty and inequalities globally and will make achieving the "Sustainable Development Goals" of the United Nations more crucial and urgent than ever (UN, 2020). It is also an opportunity to advance transformations in the food and agriculture sector to make it more resilient to the challenges in the future such as climate change (OECD, 2020).

Therefore, policymakers need to consider close interactions between humans, animals and ecosystem with the "One Health" approach and take into account "Prevention is better than cure" approach for health policies and investments in order to be prepared for the future challenges and to help sustainable and equitable distribution of resources.

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On behalf of all the co-authors hereby, I declare that the manuscript entitled "Socio-economic Impacts of COVID-19 in a One Health Context" does not require the ethical statement.

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Veteriner protozoolojide aşı uygulamalarına güncel yaklaşım

Current approach to vaccine applications in veterinary protozoology

ÖZET

Hayvanlarda görülen protozoer enfeksiyonlar, önemli üretim kayıplarına neden olur ve birçok protozoan parazit türü, zoonotik öneme sahiptir. Protozoer hastalıklarla mücadele etmenin en yaygın yolu, antiprotozoal ilaçların kullanılmasına dayanmaktadır. Bununla birlikte, gıda için yetiştirilen hayvanlarda antiprotozoan ilaç direnci ve ilaç kalıntılarının varlığı dünyanın çeşitli yerlerinde protozoan kontrol programları için ana sorunlardan biri olarak ortaya çıkmıştır. Protozoer enfeksiyonları kontrol etmenin en verimli ve uygun maliyetli yolu, bu tür enfeksiyonları önlemek için hayvanları aşılamaktır. Aşılamanın başlangıç maliyeti yüksek olmasına rağmen, hayvanların aşılanmasından kaynaklanan uzun süreli bağışıklık, bu tür enfeksiyonları kontrol altına almak için daha ucuz ve etkili bir alternatif sunmaktadır. Bu derlemede veteriner protozoolojide aşı uygulamalarının mevcut durumu gözden geçirilmiştir.

Anahtar Kelimeler: Aşı, protozoa, kontrol, parazit

ABSTRACT

Protozoan infections in animals are liable for significant production losses, and lots of protozoan parasites are of zoonotic importance. The use of anti-protozoal drugs is the most common way to treat protozoan diseases. Nevertheless, anti-protozoan drug resistance and the prevalence of drug residues in food-producing animals in different parts of the world have emerged as one of the key problems for protozoan control programs. Vaccinating animals to control certain infections is the most reliable and cost-efficient way to control protozoan infections. Although the initial cost of vaccination is high long-term immunity from animal vaccination provides a cheaper and more reliable solution to infection control. With this review, the current status of vaccine applications in veterinary protozoology has been reviewed.

Keywords: Vaccine, protozoa, control, parasite

GİRİŞ

Hayvanlarda görülen protozoer enfeksiyonlar diğer paraziter hastalıklarda olduğu gibi, birçok ülkede tıbbi ve ekonomik yönden önemli sorunlara sebep olmaktadır. Bu önemli hastalıkları tedavi etmek için kullanılan etkili ilaçların mevcudiyetine rağmen, başarılı aşıların geliştirilmesine olan ihtiyaç devam etmekte, küreselleşmenin bu kadar hızlı seyrettiği dünyamızda, bu ihtiyaç giderek artmaktadır (Behr vd., 2016).

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Review Article

Ahmet GÖKSU[1a]
Hatice ÇİÇEK[1b]

[1]Department of Parasitology, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey

ORCİD-
[a]0000-0001-6040-761X
[b]0000-0002-8957-7273

Correspondence
Ahmet GÖKSU
ahmetgoksu@aku.edu.tr

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İnsan nüfusunun 2050 yılına kadar tahmini 9 milyar olacağı ve artan nüfusun beslenmesinde gıda üretiminin %50 oranında artırılmasının gerektiği, bunun ise temiz, sağlıklı ve sürdürülebilir hayvansal gıda üretimiyle karşılanabileceği tahmin edilmektedir (Upadhayay vd., 2012; Fitzpatrick, 2013).

Medikal önemi olan protozoonlar Eucoccidia (Cins: *Babesia, Theileria, Plasmodium, Cryptosporidium, Eimeria, Toxoplasma*) ve Kinetoplastida (Cins: *Trypanosoma* ve *Leishmania*) takımlarında yer almaktadır. Günümüzde protozoer enfeksiyonların tedavi ve kontrolünde ilaç kullanımı oldukça yaygındır. Kullanılan ilaçlara karşı oluşan direnç gelişimi aşı çalışmalarına olan ilgiyi artırmıştır. Protozoer enfeksiyonların önlenmesi ve tedavisinde her iki yaklaşımın da tamamlayıcı olduğu kabul edilmektedir (Cerami ve Warren, 1994; Milon, 1994; Tanner ve Evans, 1994).

Hayvancılık sektöründe paraziter hastalıklar açısından önemli sorunlar büyük çiftlik işletmelerinde görülmektedir. Bu işletmelerde uzun mevsimsel süre boyunca parazitik hastalıkların varlığı, yüksek miktarlarda antiparazitik ilaç kullanımını gerektirmekte bu durum süt, sütten elde edilen ürünler ve ette ilaç kalıntıları ile ilişkili sorunlara yol açmaktadır. Ayrıca, bu tür kemoprofilaksi ve kemoterapinin uzun süreler boyunca pratikte uygulanması zordur (Behr vd., 2016). Uzun süre ilaç kullanımına bağlı gelişen direnç aşıların önleyici etkilerinden faydalanmayı önemli hale getirmiştir (Sharman vd., 2010). Moleküler tekniklerin ilerlemesi, yeni aşıların geliştirilmesini olanaklı hale getirmiştir.

Bu makalede veteriner protozoolojide kullanılan ve geliştirilmekte olan aşıların mevcut durumunu değerlendirilmiştir.

VETERİNER PROTOZOOLOJİDE MEVCUT AŞI UYGULAMALARI

Günümüzde çoğunluğu serbest yaşayan yaklaşık 65.000 protozoa türünün varlığı bildirilmiştir. Hem omurgalı hem de omurgasız hayvanlarda yalnızca 7000 protozoan türü parazit olarak yaşamaktadır (Verma vd., 2012). Protozoan parazitler, hayvanlarda yaygın morbidite ve mortaliteye neden olan önemli etkenlerden biri olarak kabul edilir. Bunların yanında, protozoan parazitlerin çoğu doğada zoonotiktir. Bu nedenle hem ekonomi hem de halk sağlığı açısından yüksek öneme sahiptirler. Theileriosis, babesiosis, toxoplasmosis, coccidiosis, cryptosporidiosis gibi hastalıklar yaygın olarak görülmektedir. Bunlardan Toxoplasmosis ve cryptosporidiosis gibi enfeksiyonlar yaygın görülen zoonozlardandır (Lightowlers, 1994; Meeusen vd., 2007).

Aşılar, ölü, inaktive edilmiş organizmalar veya bunlardan elde edilen saflaştırılmış ürünlerdir. Protozoan enfeksiyonlarına karşı kullanılmakta olan, inaktif, canlı zayıflatılmış, alt ünite, DNA, rekombinant vektör, yenilebilir aşılar gibi çeşitleri bulunmaktadır. Türkiye'de ve dünyada çeşitli etkinliklerde birçok veteriner hekimlik aşıları ruhsatlandırılmıştır. Protozoan enfeksiyonlarına karşı kullanılan lisanslı ticari aşılar ve çeşitleri Tablo 1'de gösterilmiştir.

Çiftlik hayvanlarının protozoan hastalıklarına karşı mevcut olan aşıların büyük kısmı canlı organizmalara dayanmaktadır. Ancak son yıllarda inaktive edilmiş alt ünite aşıların geliştirilmesi ve ticarileştirilmesinde ciddi ilerlemeler kaydedilmiştir (Lightowlers, 1994; Sharman vd., 2010). Hayvanlarda doğal olarak oluşan protozoan hastalıklar için geliştirilen başarılı aşılar, insan aşı araştırmalarına da rehberlik etmektedir (Mcallister, 2014). Parazitler güçlü bir humoral ve hücresel bağışıklık tepkisine neden olurlar. Ancak bu, konağın yeniden enfeksiyona karşı tam korunmasını sağlayamamaktadır. Parazit protozoonlar, yalnızca konağın immün yanıtlarından kaçınmak için değil, aynı zamanda immünokompetan konakta hayatta kalmalarını ve bulaşmalarını kolaylaştırmak için mekanizmalar geliştirmişlerdir.

Tablo 1. Protozoan enfeksiyonlarına karşı kullanılan lisanslı ticari aşılar ve çeşitleri

Ticari ismi	Üretici	Hedef Enfeksiyon	Hayvan türü	Aşı tipi	Referanslar
Leishmune®	Zoetis, Brezilya	Leishmaniasis (*L. infantum*)	Köpek	Alt ünite	Borja-Cabrera vd., 2002
Leish-Tec®	Hertape Calier, Brezilya	Leishmaniasis (*L. infantum*)	Köpek	Rekombinant	Fernandes vd., 2008
CaniLeish®	Virbac, Fransa	Leishmaniasis (*L. infantum*)	Köpek	İn vitro aksenik kültür	Lemesre vd., 2005
LetiFend®	LetiPharma, İspanya	Leishmaniasis (*L. infantum*)	Köpek	Rekombinant DNA	Cotrina vd., 2018
Coccivac B, D®	Intervet/Merck Animal Health	Kanatlı koksidiyozu	Broyler ve yumurtacı tavuklar	Canlı sporlanmış	Johnson ve Reid, 1970
Coccivac-T®	Intervet /Merck Animal Health	Koksidiyozis	Hindi	Canlı sporlanmış	Mathis ve McDougald, 1989
Nobilis® COX ATM	Intervet (Hollanda)	Koksidiyozis	Broyler	Canlı sporlanmış	Vermeulen vd., 2001
Eimeriavax® 3m, 4m, Tamm, Plus	Eimeria Pty, Avustralya	Koksidiyozis	Broyler, damızlık ve yumurtacılarda	Canlı sporlanmış	Gore vd., 1983; Mcdonald ve Shirley, 2009
Immucox® C_1, C_2	Vetech Laboratories, Kanada	Koksidiyozis	Broyler ve damızlık tavuklarda	Canlı sporlanmış	Lee, 2006
Livacox® D, T, Q	Biopharm, Çekya	Koksidiyozis	Broyler ve damızlık tavuklarda	Canlı atenüe	Shirley ve Bedrnik 1997
CoxAbic®	Novartis AH, İsviçre	Koksidiyozis	Broyler ve damızlık	Alt ünite	Mcallister, 2014; Wallach, 2002; Sharman vd., 2010
Toxovax® (Yeni Zellanda ve Avrupa)	MSD Animal Health, ABD	Toxoplasmosis (*T. gondii*)	Koyun	Canlı atenüe	O'Connell vd., 1988
Giardiavax®	Zoetis, Brezilya	Giardiasis (*G. lamblia*)	Köpek	İnaktive	Olson vd., 2001
Bovilis® Neoguard	Intervet International B.V., Hollanda	Neosporosis (*N. caninum*)	İnek	İnaktive	Meeusen vd., 2007
Babesia canlı aşıları	Devlet tarafından finanse edilen laboratuvarlar, Avustralya, Arjantin, Güney Afrika ve İsrail	Babesiosis (*B. bovis, B. bigemina*)	Sığır	Canlı atenüe	OIE, 2014
[1]Tayledoll®, [2]Teylovac®, [3]Rakshavac-T®,	[1]Dollvet, Türkiye, [2]Vetal, Türkiye, [3]Indian Immunologicals Ltd., Hindistan	Tropikal theileriosis (*T. annulata*)	Sığır	Canlı attenüe (*Theileria annulata* makroşizontları ile enfekte lenfoid hücre)	Altuğ vd., 2013; Singh vd., 2014
Muguga kokteyli	VetAgro Tanzania Ltd., (Kenya ve Tanzanya)	Şark sahil humması (*T. parva*)	Sığır	Canlı virülent	Patel vd., 2011

Rekombinant DNA teknolojisi ve monoklonal antikorların 1980'lerin başında geliştirilmesiyle, immün (humoral ve/veya hücresel) tepkilerin hedefi olan bireysel antijenler belirlenmiştir.

Karşılık gelen genleri tanımlamak, klonlamak, dizilemek ve çeşitli patojenik parazitlere karşı olası aşı adayları olmalarını sağlamak için önemli miktarlarda rekombinant veya sentetik antijen üretimi mümkün hale geldiğinden son yıllarda aşı çalışmaları hızla ilerlemektedir (Behr vd., 2016).

Leishmania aşıları

Leishmaniasisin önlenmesi için farklı aşı türleri geliştirilmiştir (Nagill ve Kaur, 2011). Köpeklerde az sayıda aşı adayı test edilmiş olup dört aşı canine visceral leishmaniasise karşı ticari lisans almıştır. İlki Quil-A® ile adjuvanlanmış yarı saflaştırılmış bir fukoz-mannoz ligand antijeni (FML) olan Leishmune® aşısıdır. Etkinliği açısından faz III gerekliliklerini karşılamadığı için lisansı 2014 yılından beri askıya alınmıştır (Borja-Cabrera vd., 2002). Leish-Tec® ise saponin ile adjuvanlanmış bir rekombinant protein A2 içeren Brezilya'da satılan ticari bir aşıdır. Avrupa'da kullanılan (CaniLeish®, Virbac, France) aşısı ise, *L. infantum* sekret/ekstret ürünlerinden (LiESAp) oluşur ve QA-21 ile adjuvanlanır ve son olarak, aktif bileşen olarak Protein Q içeren LetiFend®, Avrupa Komisyonundan pazarlama izni almıştır (Cotrina vd., 2018; Fernandes vd., 2008; Lemesre vd., 2005; Lemesre vd., 2007).

Antikoksidiyal aşılar

Eimeria türlerinin canlı ookistleri aşı üretimi için kullanılan kanatlıların dışkılarından elde edilir. Kanatlı çiftliklerinde, koruyucu bağışıklığı sağlamak için yavruların karışık türlerdeki koksidiyal ookistlerle ağızdan aşılanması tekniği kullanılır. Günümüzde, canlı koksidiyoz aşılarının antikoksidiyal ilaçlar kullanımından önce uygulanması önerilmektedir (Williams, 2002).

Coccivac®-B

Bu canlı ve zayıflatılmış aşı, ilk olarak 1940'ların sonlarında, mevcut antikoksidiyal ürünler piyasaya sürülmeden önce geliştirilen izolatlardan üretimiştir. *Eimeria acervulina, E. mivati, E. maxima* ve *E. tenella* suşlarından hazırlanmıştır. Coccivac B aşısı, mevcut antikoksidiyallerin performansını eski haline getirmek için değerli bir aşıdır. Kanatlılar üzerinde yapılan deneysel bir çalışmada, bu aşı beş farklı *E. tenella* suşu ile çeşitli koruma seviyeleri göstermiştir (Chapman, 1994; Williams vd., 2000; Awad vd., 2013).

CoxAbic®

Kümes hayvanları için ticari olarak temin edilebilen ilk alt ünite aşıdır ve E. *maxima*'nın makrogametositinden izole edilmiş saflaştırılmış antijenlerini içermektedir. Gametosit antijeni kullanılarak yapılan aşılama, antikor yanıtının üretilmesine neden olmaktadır. CoxAbic®, doz başına diğer koksidiyoz aşı türlerine göre daha pahalı olmasına rağmen aşılanan her tavuk, çok sayıda yavruya bağışıklığı geçirmektedir (Mcallister, 2014; Sharman vd., 2010; Wallach, 2002).

Livacox® D, T, Q

Evcil kanatlılar için geliştirilen canlı atenüe koksidiyoz aşılarıdır. Yumurtacılar için Livacox® D, broylerler için Livacox® T, damızlık ve yumurtacı yarkalar için Livacox® Q kullanılmaktadır (Shirley ve Bedrnik 1997).

Paracox®-8

Ookist süspansiyonundan oluşan canlı zayıflatılmış oral bir aşıdır. Tavukların farklı *Eimeria* türlerine karşı aktif immünizasyonu için kullanılır (Shirley vd., 2005).

Immucox® C1, C2

Oral yolla uygulanan *Eimeria* spp. canlı ookist aşısı, sağlıklı broylerlerde koksidiyozise karşı bağışıklık geliştirmelerine yardımcı olmak için tasarlanmıştır. Kanatlı hayvanlara üretim süreçleri boyunca tek sefer uygulanarak koruyucu bağışıklık sağlayan bir aşıdır (Lee, 2006).

Eimeriavax® (3m, 4m, Tamm, plus)

Bu aşı, PBS içinde süspanse edilmiş *E.acervulina* suşu RA, *E. maxima* suşu MCK + 10, *E. necatrix* suşu mednec3 + 8 ve *E. tenella* suşu Rt3 + 15'in canlı ookistlerinden oluşur (Gore vd., 1983; Mcdonald ve Shirley, 2009).

Nobilis® COX ATM

İyonofor bileşiklerin varlığında bile benzersiz bir aktif olma özelliğine sahip canlı bir aşıdır. Aşı, iyonofor bileşiğe toleranslı *E. acervulina, E. tenella* ve *E. maxima* suşlarını içerir. Bu aşının en önemli avantajlarından biri, bağışıklık üretiminin yanı sıra, bağışıklığın zayıf olduğu 3-4 haftalık yaşlarda bile iyonofor bileşiklerinin kullanımına izin vermesidir (Vermeulen vd., 2001).

Coccivac-T®

Eimeria dispersa, E. meleagrimitis, E. adenoides ve *E. gallopavonis*'in sporlanmış ookistlerini içeren canlı bir aşıdır. Günlük hindi palazlarına sprey şeklinde uygulanır. Coccivac T'deki Eimeria suşları, modern iyonofor ve kimyasal antikoksidiyal ürünlerin ortak kullanımından önce izole edilmiş ve tüm antikoksidiyallere karşı oldukça hassas olduğu belirtilmiştir (Mathis ve McDougald, 1989).

Coccivac-D®

Farklı *Eimeria* türlerini içeren canlı, sporlanmış bir ookist aşısıdır. Tam bağışıklık sağlamak için, orijinal koksidiyal ookist dozu sürüde en az dört yaşam döngüsünü tamamlamalıdır (Johnson ve Reid, 1970).

Koksidiyozis aşı adayları

Eimeria acervulina'nın rekombine edilmiş bir merozoit antijeni olan p250 hastalığa karşı %50 koruma sağlamaktadır (Kim vd., 1989). MZ5-7 ise *Eimeria tenella* MZ5-7 ve tavuk IL-17 genini birlikte eksprese eden bir DNA aşısıdır. MZ5-7 geni, 2. nesil merozoitin yüzey proteini MZP5-7'yi kodlar. MZ5-7, aşı için etkili bir aday antijendir ve sitokinin antijen ile birlikte ekspresyonu, DNA aşısını güçlendirmek için alternatif bir yöntemdir (Xu ve Li, 2011).

pcDNA-Gam56

pcDNA-Gam56 ile aşılanmış gruplar, yüksek lenfosit proliferasyonu ve antikor seviyeleri gösterirken aynı zamanda vücut ağırlığında artış ve büyük oranda ookist saçılımının azalması (%53,7) görülmüştür (Xu vd., 2013).

TA4

İnterlökin-2 ile birlikte eksprese edilen *E. tenella*'ya karşı geliştirilen bir DNA aşısıdır. *Eimeria tenella, E. necatrix, E. maxima* ve *E. acervulina*'ya karşı koruyucu bağışıklık oluşturabilir. Bulgular, yapılandırılmış multivalent epitop DNA aşılarının, *Eimeria* türlerinin meydana getirdiği enfeksiyonlara karşı potansiyel multivalent aday aşılarından biri olduğunu göstermektedir (Song vd., 2015).

EtMIC2

Bitkinin eksprese ettiği rekombinant EtMIC2'nin hem humoral hem de hücre aracılı immün yanıtları ortaya çıkarma yeteneğinden faydalanılmıştır. EtMIC2 uygulanan kanatlılarda karşılaştırıldığında, azalmış ookist çıkışı ve artan kilo artışı gösterilmiştir (Sathish vd., 2011).

Ayrıca *Eimeria* türlerine karşı başarıyla test edilmekte olan antikoksidiyal alt ünite aşı adayları şunlardır; Apikal membran antijeni 1, mikronem proteini 2-3, bağışıklık haritalanmış protein 1, laktat dehidrogenaz, rhomboid benzeri proteinler, SO7 (Mehlhorn, 2016).

Toxoplasma gondii aşıları

S48 suşu (Toxovax®)

Farelerde (x3000) tekrarlanan pasajlarla üretilir. Parazitin bradizoit veya ookist formların oluşumunu engellemektedir. Abort ve yenidoğan ölümlerini %75 oranında azalttığı bildirilmiştir (O'Connell vd., 1988).

TS-4 ve T-263

Sıcaklığa duyarlı bir suş olan TS-4, kist oluşumuna ve konjenital toksoplazmoza karşı koruma sağlamaktadır (Bourguin vd., 1993). T-263 ise *Toxoplasma gondii*'nin ookist oluşturmayan, canlı mutant bradizoitidir. T-263'ün uygulanması, kedilerde ookist saçılımının azalmasını sağlayarak koruyucu etki oluşturur (Verma ve Khanna, 2013). Toksoplazmozise karşı aşı geliştirmek amacıyla gen düzenlemesine dayalı canlı zayıflatılmış, nanopartikül, ekzozom ve karbonhidrat bazlı aşıların geliştirildiği teknikler ve uygulamalar son yıllarda kullanılmaya başlanmıştır (Wang vd., 2019).

Giardia aşıları

Giardiavax®

Köpekler için hazırlanmış, kültür yöntemiyle elde edilen bir trofozoit aşısı olup, *G. lamblia* enfeksiyonunun ve saçılmasının önlenmesinde etkili bulunmuştur. Aşı, koyunlardan izole edilen *G. duodenalis*'ten elde edilmiştir. Deri altına 1 mL dozunda uygulanır. İlk doz 8 haftalıkken ve ikinci doz ise ilk uygulamadan 2-4 hafta sonra verilir. Ardından aşı her yıl tekrarlanır (Olson vd., 2001). Başka bir çalışmada zayıflatılmış *Salmonella typhimurium* tarafından taşınan α1-Giardin ve CWP2'nin bivalent DNA aşısı kullanılarak *Giardia lamblia* ile enfekte olmuş farelerin aşılanması sonucunda, dışkıdaki trofozoitlerin ve kistlerin azaldığı tespit edilmiştir (Feng vd., 2016).

Neospora caninum aşıları

İdeal bir aşının hem enfeksiyona hem de klinik belirtilere karşı koruma sağlaması gerekir, bu nedenle, fötusa zararı olmayan hücre aracılı bağışıklık tepkisini oluşturabilecek bir aşıya ihtiyaç vardır (Goodswen vd., 2013). *Neospora caninum*'a karşı geliştirilen öldürülmüş taşizoit bazlı aşının, süt sığırlarında abortusun önlenmesinde etkili olduğu bildirilmiştir (Weston vd., 2012; Williams vd., 2007). Tutunmada, istila etmede veya diğer parazit-konak-hücre etkileşimi süreçlerinde önemli role sahip olan proteinler, bu önemli protozoan parazite karşı etkili bir aşı geliştirilmesi için uygun hedeflerdir. *Neospora* enfeksiyonuna karşı bu şekilde koruma sağlanabileceği bildirilmiştir (Hemphill vd., 2013). Sığır neosporosisine karşı küresel aşı pazarının çok büyük olduğu öne sürülse de henüz aborta neden olan neosporosisin bulaşmasını önleyecek derecede etkili bir tedavi veya aşı bulunmamaktadır (Weston vd., 2012).

Bovilis® Neoguard

Tek lisanslı *N. caninum* aşısı, inaktive edilmiş *N. caninum* taşizoitleri, %10 havlogen adjuvantı, %5 stabilizörler ve %5 fosfat tamponlu salinden oluşan (Bovilis® Neoguard Intervet International B.V., Hollanda) aşısıdır. Abort oranlarını %50'ye kadar düşürmekle beraber 1 yıla kadar koruyuculuk sağlayabilmektedir (Romero vd., 2004; Weston vd., 2012). Son zamanlarda, soya lesitin / b-glukan adjuvantı (sNcAg/AVEC) ile taşizoit özütü aşısının çözünür bir fraksiyonu, gebe sığırlarda immünojeniklik ve yüksek IFN- γ tepkilerinin indüksiyonunu gösterdiği bildirilmiştir (Mansilla vd., 2015).

Canlı taşizoit aşıları

Nc-Nowra, Nc-Spain1H ve Arjantin izolatı Nc-6 gibi doğal olarak zayıflatılmış veya virülensi daha düşük izolatlardan oluşan canlı taşizoit aşıları, *N. caninum* antikor yanıtlarında önemli bir artış sağlamakta ve abort oranlarını önemli ölçüde düşürmektedir. Bununla birlikte, canlı aşıların kullanılmasının, canlı parazitlerin toplu olarak korunması ve aşılamadan sonra patojenliğin tersine dönme riski gibi bazı doğal dezavantajları vardır. Bu nedenle etkisizleştirilmiş veya alt ünite aşıların daha uygun seçenekler olduğu düşünülmektedir (Hecker vd., 2013; Reichel vd., 2015). Ayrıca, oligo-mannoz mikrozomunda (M3-NcGRA7) hapsolmuş rekombinant NcGRA7 (50–200 lg) ve rNcSAG1, rNcHSP20 ve rNcGRA7 dâhil olmak üzere bakteriyel olarak eksprese edilen ve

saflaştırılmış rekombinant proteinler, enfeksiyonu önleyemedikleri için çok az umut vadetmektedir. Bu nedenle, *Neospora*'nın canlı zayıflatılmış suşları (Ca2+-bağımlı protein kinaz 2 eksik taşizoitler dâhil) gibi yeni, daha verimli bir aşı yaklaşımının tanımlanması, neosporosise karşı uzun süreli koruma sağlamak için kritik görülmektedir (Nishimura vd., 2013; Wang vd., 2018).

Babesia aşıları

Pirodog®/Nobivac® Piro: İn vitro kültürlerin (*B. canis* ve *B. rossi*) süpernatantlarının %80 koruma sağlayan çözünebilir parazit antijenidir (Schetters, 2005). Köpeklere 6 aylıkken verilir ve ilk aşılamadan 3 ila 6 hafta sonra ve daha sonra ise kas içi yolla 6 ayda bir yeniden aşılama gereklidir. Her iki aşı da enjeksiyon bölgesinde bir miktar lokal reaksiyon oluşturur ancak bu reaksiyon Nobivac® Piro aşısı kullanımında daha fazladır (Freyburger vd., 2011). Aşı Avrupa'nın bazı bölgelerinde pazarlanmıştır fakat aşının artık üretilmediği bildirilmiştir (Mcallister, 2014).

Sığırlarda *Babesia bovis* ve *B. bigemina*'nın canlı zayıflatılmış suşlarından oluşan aşılar, birçok ülkede enfekte olmuş donör hayvanların kanlarından veya in vitro kültür yoluyla üretilmektedir. Bu aşılar hayvanlar enfekte kenelerin bulunmadığı bir bölgeden kene ile enfekte olmuş bir alana taşındığında önerilmektedir. Zayıflatılmış B. bovis etkenleri, splenektomize edilmiş öküzlerde virülans bir suşun 20 ile 30 arasında seri ve hızlı geçişlerinden üretilir. B. bigemina'nın zayıflatılması benzer bir prosedürle yapılır, fakat bu durumda buzağılar arasında yavaş ve art arda geçişler kullanılır (Florin-Christensen vd., 2014).

Babesia bovis ve *B. bigemina* canlı aşılarının çoğu, özellikle Avustralya, Arjantin, Güney Afrika ve İsrail'de hayvancılık endüstrilerine hizmet olarak devlet destekli üretim tesislerinde üretilmektedir. Bu iki parazitin her biri tarafından enfekte olan eritrositlerin karıştırıldığı bivalent bir formül olarak veya trivalent bir formül olarak hazırlanmaktadır (OIE, 2014).

Canlı aşılar genellikle 4-10 aylık buzağılara güvenli uygulanırken bazen yaşlı hayvanların aşılanması sonrasında klinik hastalık gelişebilmektedir. Bundan dolayı hayvanlar belli bir süre gözetim altında tutulmalı ve yan etkiler gözlenirse antiprotozoer bir ilaçla tedavi edilmelidir. Normalde ömür boyu süren koruyucu bağışıklık 3-4 hafta içinde gelişmektedir (De Waal ve Combrink 2006). Mevcut aşıların klinik sığır babesiosis vakalarını önlemedeki genel etkinliğine rağmen, hastalığa karşı koruma sağlayan, daha güvenli, kullanımı ve üretimi daha kolay olan gelişmiş alt ünite aşılarının geliştirilmesine önemli derecede ihtiyaç vardır (Ganzinelli vd., 2018).

Şimdiye kadar, herhangi bir alt ünite aşısı ruminantlardaki *Babesia* türlerinde doğal konakçılara koruyucu bağışıklık kazandırmada başarılı olamamıştır. Yine de patojenite mekanizmaları ve konak-patojen etkileşimlerini ifade eden moleküller dahil olmak üzere bu ve ilgili parazitler hakkında hızla artan bilgi düzeyi göz önüne alındığında, alt ünite aşılarında istenen seviyelere çok kısa süre içerisinde ulaşılabilir (Florin-Christensen vd., 2014). Ayrıca kene önleyici aşılar bu tip aşılara göre daha uygun fiyatlı ve çevre dostu bir çözüm sunmaktadır. Kene kontrolü ve aşılamayı içeren uyumlu önlemler ve bu hastalıklara dirençli çiftlik hayvanlarının kullanımı, hastalıkların önlenmesinde muhtemelen en doğru sonuçları üretecektir (Ganzinelli vd., 2018). Babesia enfeksiyonlarından korunma amacıyla geliştirilmekte olan aşı adayları Tablo 2'de sunulmuştur.

Theileria aşıları

Theileria parva ve *T. annulata* için canlı aşılar 40 yıldan fazla bir süre önce üretilmiştir. Birçok alanda kullanımlarını sınırlandıran dezavantajları bulunmaktadır (Nene vd., 2016; Radley vd., 1975). *Theileria* türleri, sığır makrofajları veya lenfoid hücreleri içinde in

vitro kültüre adapte edilebilir. Pek çok suş, hücre kültüründe uzun süreli bir geçişle zayıflatılabilmektedir (Gubbels vd. 2000). Aşının koruyucu etkisinin, sahada en az 19 ay sürdüğü bildirilmiştir. Aşı etkinliğinin süresinin, enfekte kenelere tekrar tekrar maruz kalmaya bağlı olup olmadığı sorgulanmalıdır (Yin vd., 2008). *Theileria lestoquardi* (*Theileria hirci*) için benzer aşılar, koyunlarda enfeksiyondan korunmak için İran, Irak ve Bulgaristan'da kullanılmaktadır (Ali vd., 2008).

Tablo 2. *Babesia* enfeksiyonlarından korunma amacıyla geliştirilmekte olan aşı adayları

Aşı Adayları	Aşı tipi	Hedef Türler	Referanslar
GASA-1	Alt ünite	*Babesia bovis*	(Flores vd., 2020)
Canine Babesiosis Antigen (CBA)	Rekombinant	*Babesia canis*	(Moubri vd., 2018)
GPI bağlı proteinler	Rekombinant	*Babesia microti, Babesia divergens*	(Wieser vd., 2019)
6-Cys genleri	Canlı aşı	*Babesia bovis*	(Alzan vd., 2019)

Theileria annulata şizont aşısı

Tarım ve Orman Bakanlığı Pendik Veteriner Kontrol Araştırma Enstitüsü tarafından ve bazı özel firmalar tarafından üretilen atenüe *T. annulata* şizont aşısı 1982 yılından bu yana ülkemizde üretilmekte ve sahada uygulanmaktadır (Altuğ vd., 2013; Özkoc ve Pipano, 1981). Hindistan'da ise aynı tekniklerle üretilen uzun süreli in vitro pasajlarla zayıflatılmış, lenfoblast hücre kültüründe üretilen canlı şizontları içeren (Rakshavac-T®, Indian Immunologicals Ltd., India) aşı kullanılmaktadır (Singh vd., 2014).

SPAG-1 ve p67

Theileria annulata ve *Theileria* parva'nın her ikisi de yaşam döngüsünün sporozoit aşamasında sırasıyla SPAG-1 ve p67 olarak adlandırılan ana yüzey antijenine sahiptir. Her durumda, bu antijenler aşı adaylarıdır. Daha önceki çalışmalarda bir derece homolog korumayı indükledikleri bildirilmiştir (Hall vd., 2000).

Tams1

İyi karakterize edilmiş bir *T. annulata* antijeni olan Tams1, bugüne kadar çalışılan tüm *Theileria* türleri üzerinde tanımlanmış olan ana merozoit piroplazma yüzey antijeni (mMPSA) ailesinin bir üyesidir (Katzer vd., 2002). Aşının, klinik olarak önemli herhangi bir anormallik veya yan etki olmaksızın sığırlarda enfeksiyonun önlenmesinde etkili olduğu kanıtlanmıştır (Esmaelizad vd., 2011).

Muguga kokteyli

Theileria parva'ya karşı Doğu Afrika Veteriner Araştırma Örgütü (şimdiki ismiyle Kenya Tarım Araştırma Enstitüsü) tarafından geliştirilen multivalent bir aşı olan Muguga kokteyli Kenya ve Tanzanya'da kullanılması için lisans almıştır (Patel vd., 2011).

Cryptosporidiosis aşıları

Liyofilize C. parvum ookisti

Liyofilize ookistlerle oral olarak aşılama sonrasında, ortalama diyare ve ookist çıkarma sürelerinde kısalma saptanmıştır (Harp ve Golf, 1995).

Aşı adayları

Cryptosporidiosis enfeksiyonundan korunmada sporozoitlerin yüzeyinde bulunan, Gp15, Gp40 ve Gp900 ve düşük karmaşıklıkta asidik proteine (Cp23) sahip glikoproteinler mevcut aşı adaylarıdır (Haserick vd., 2017).

Cpgp40/15 ve Cpgp40

Cpgp40/15'in İndirekt immünofloresans analizi ile hücre içi gelişim sırasında parazitofor vakuol membranı (PVM) ile ilişkili olduğu gösterilmiştir. Bu antijen gruplarına karşı gelişen anti-gp40/15 ve anti-gp40 antikorları, in vitro olarak *C. parvum* enfeksiyonunu nötralize etme yeteneği gösterdiği bildirilmiştir. Konağın istilasında veya PVM'de Cpgp40/15'in (veya gp40/15 kompleksinin) spesifik rolünü ve fonksiyonel mekanizmasını tam olarak anlamak ve gp40/gp15'in cryptosporidiosis için bir aşı adayı olarak uygulanabilirliğini belirlemek için daha fazla araştırma gerekmektedir (Cui vd., 2020).

İnaktive edilmiş, zayıflatılmış ve canlı tam hücre aşıları, günümüzün geleneksel aşı sistemlerinin bir parçasıdır. Bu tam hücre aşılarıyla karşılaştırıldığında, aşı geliştirmede çoklu epitop tabanlı araştırma yaklaşımları önem kazanmaktadır. *Cryptosporidium parvum*'a karşı çoklu alt ünite aşı tasarımı için immünoinformatik çalışmalar devam etmektedir (Dhal vd., 2019).

SONUÇ

Aşılama, dünya çapında halk sağlığı üzerinde oldukça büyük bir etkisi olan etkili bir tıbbi müdahale olarak kendini kanıtlamıştır. Evcil hayvanların sağlığı ve üretkenliği, dünya çapında protozoanların neden olduğu paraziter hastalıklarla önemli derecede etkilenmektedir. Ayrıca birçok durumda zoonoz olan bu parazitler insan sağlığını da etkilemektedir. Hayvanlarda ilaca dirençli parazitlerin yaygın olarak görülmesi, paraziter hastalıkların kontrolünü tehdit etmektedir. Aşılama aynı zamanda halk sağlığını iyileştirmeyi de hedeflediğinden ucuz ve etkili bir aşının geliştirilmesi önem arz etmektedir. Ekonomik olarak uygun ve etkili protozoan aşıları geliştirme çabaları, yeni aşı adayları olabilecek yeni antijenleri keşfetme stratejileri hala devam etmektedir. Yakın gelecekte, veteriner sağlık hizmetlerinde hayvanların paraziter hastalıklarına karşı geliştirilecek yeni aşıların kullanımının, hayvancılık verimliliğinin artırılmasına önemli katkı sağlaması beklenmektedir. Ayrıca bu alanda gerçekleşen ilerlemeler insanlar için yapılan aşı geliştirme çalışmaları için de yol gösterici olacaktır. Sonuç olarak, gelecekte paraziter hastalıklara karşı aşılamanın parazitik enfeksiyonların kontrolünde en iyi alternatiflerden biri olacağı öngörülmektedir.

TEŞEKKÜR / AÇIKLAMALAR

Çıkar çatışması: Yazarlar herhangi bir çıkar çatışması olmadığını bildirmişlerdir.

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Measurement of heart frequency with practical ECG device in horses

Letter to Editor

Cenker Çağrı CINGI[1a]
Abdullah Ruhi SOYLU[2b]

[1]Department of Internal Medicine, University of Afyon Kocatepe, Afyonkarahisar, Turkey

[2]Department of Biophysics, Hacettepe University, School of Medicine, Ankara, Turkey

ORCİD-
[a]0000-0001-6286-6553
[b]0000-0001-9886-9983

Correspondence
Cenker Çağrı CINGI
cagricingi@aku.edu.tr

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Dear Editor,

Electrocardiography (ECG) is the recording of the electrical activity shaped as a result of myocardial depolarization and repolarization through electrodes placed in different parts of the body (Turgut, 2017). The real-time first negative wave, first positive wave and first negative wave after positive wave (QRS wave) is detected. Therefore, adaptive QRS size, high frequency noise and the combined adaptive threshold method, which evaluates all of the adaptive slew-rate (revolution) methods should be used through the recording simultaneously (Christov, 2004). The aim of this study is to determine the efficiency of the practical ECG device, which was developed by us and under patent protection, in detecting heart rate in horses.

This study was carried out on six Thoroughbred British Horses aged between 2-5 which continued race life. Datas were taken from the resting horses. Datas measured with the dual-channel three-electrode portable ECG device which developed by ourselves for practical measurements with the weight of 180 g (Table 1, Figure 1). The ECG device can record potentials between -10 mV and +10 mV with 1000 Hz sampling frequency and 15 bit analog-to-digital converter (ADC) quality. Cardiac potentials were recorded from the skin surface with Ag/AgCl electrodes. A Java-based smartphone application developed by us was used to record, display and store ECG signals. This software runs under the Android operating system and is compatible with the ECG hardware that we have developed. As a result, it was seen that practical ECG device measurements and smart phone software were successful in recording data and the device could be used safely in race horses (Figure 2).

Table 1. Heart Beat per minute in Horses

Horse No	Heart Beat/min
1	42
2	60
3	71
4	39
5	40
6	54

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Figure 1. Practical ECG Device

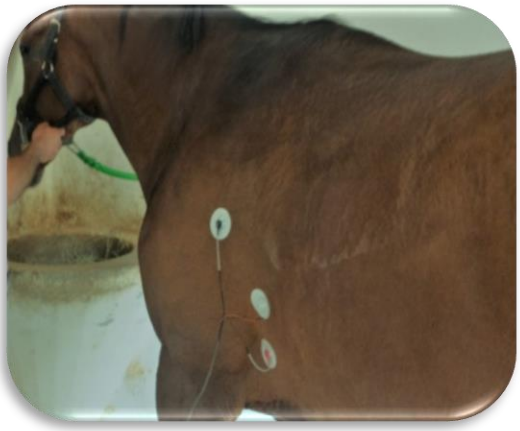

Figure 2. Placement of the ECG device in the equine body

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Ethical approval: Ethics committee approval was not enrolled in the present study, but it has been denoted that there is no need for approval of the ethics committee in non-experimental clinical veterinary practices index of Article 2 (b) of the Regulation on Working Procedures and Principles of Animal Experiments published in the Official Newspaper dated 15.02.2014 with no 28914 as was expressed. In the present study sera samples were withdrawn from sick animals, in an attempt to control their health status since it was understood that there was informed consent form in the study, ethics committee approval was not required for this study.

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

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