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RESEARCH ARTICLES

SHORT COMMUNICATION

CASE REPORT

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RESEARCH ARTICLE

Seroprevalence of Paratuberculosis in Cattle in Ardahan Region

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ABSTRACT

In this study, it was aimed to determine the prevalence of paratuberculosis the dairy cattle of Ardahan province. 11 focuses randomly selected from the Ardahan center and its districts and a total of 400 cattle blood sera from 22 farms in these centers constituted the study material. In the study, commercial ELISA antibody test kit was used to investigate "*Mycobacterium avium subsp. Paratuberculosis"* (MAP) antibodies in cattle blood serum samples. As a result of the analysis, 17 of 400 animals were positive for MAP and prevalence in Ardahan province and its vicinity was determined as 4.25% (17/400). Paratuberculosis was detected in 9 of 22 farms sampled. This result has been predicted that pTB is subclinical in dairy cattle in Ardahan region and may cause economic losses. Considering that this disease is zoonotic, it can be said that public health may also be affected. Therefore, it will be beneficial to carry out more studies on pTB.

Keywords: Cattle, Johne's Disease, Paratuberkulosis, Seroprevalence

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Ardahan Yöresindeki Sığırlarda Paratüberküloz'un Seroprevalansı

ÖZ

Bu çalışmada Ardahan yöresindeki süt sığırlarında paratüberküloz'un (pTB) prevalansının belirlenmesi amaçlandı. Ardahan merkez ve ilçelerinden rastgele seçilen 11 odak ve bu odaklardaki 22 işletmeden alınan toplam 400 sığır kan serumu çalışmanın materyalini oluşturdu. Çalışmada, sığır kan serum örneklerinde "*Mycobacterium avium subsp. Paratuberculosis"* (MAP) antikorlarının araştırılması amacıyla ticari Enzyme Linked Immunosorbent Assay (ELISA) antikor test kiti kullanıldı. Analiz sonucunda 400 hayvandan 17'sinde MAP yönünden pozitiflik belirlendi. Ardahan ili ve çevresindeki yaygınlık oranı %4,25 (17/400) olarak tespit edildi. Örnek alınan toplam 22 çiftliğin 9'unda paratüberküloz'un varlığı tespit edildi. Bu sonuçla, Ardahan yöresindeki süt sığırlarında pTB'un subklinik olarak bulunduğu ve ekonomik kayıplara yol açabileceği öngörülmüştür. Bu hastalığın zoonotik olduğu düşünüldüğünde halk sağlığının da etkilenebileceği söylenebilir. Bu nedenle pTB ile ilgili daha fazla çalışma yapılması faydalı olacaktır.

Anahtar Kelimeler: Johne's Hastalığı, Paratüberküloz, Seroprevalans, Sığır

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GİRİŞ

Paratüberküloz Norveç, İsveç ve Avustralya'daki bazı bölgeler hariç dünyanın birçok ülkesinde görülen yaygın bir hastalıktır. Paratüberkülozis ya da diğer ismi olan Johne's hastalığı "*Mycobacterium avium subsp. paratuberculosis"* (MAP)'in neden olduğu kronik, granülomatöz enteritis ile karakterize, hayvanların et ve süt veriminin azalmasının yanında, reprodüktif verimin önemli bir şekilde düşmesine ve büyük ekonomik kayıplara neden olan oldukça bulaşıcı bir enfeksiyondur (Gilardoni ve ark. 2012, Makav ve ark. 2013, Lingling Li ve ark. 2017, William ve ark. 2018). Etken ilk olarak Alman bilim adamları Johne ve Frothingham tarafından 1895'de izole edilmiştir. Son yıllarda yapılan fenotipik ve genotipik çalışmalar sonucunda tespit edilen etkenin *Mycobacterium avium*'a çok yakın olduğu ortaya konulduğundan ayrı bir tür olarak değil *M. avium*'un bir alt türü olması ve adının "*Mycobacterium avium subspecies paratuberculosis"* olması gerektiği ileri sürülmüştür (Hurley ve ark. 1988, Thorel ve ark. 1990, Johne ve ark. 1895, Collins ve ark. 2003, Abendan ve ark. 2013). Hastalık 2-6 yaş ergin ineklerde kendini gösterir. Fakat etken buzağıların ilk aylarında hayvanları enfekte edebilir (Civelek 2018). *Mycobacterium avium subsp. paratuberculosis*'in konakçı yaygınlığı oldukça geniştir. Genellikle süt sığırlarında karşılaşılan bir etken olarak görülmektedir. Fakat deve, manda ve muflonlarda da paratüberküloz tespit edilmiştir. Aynı zamanda bazı hayvan türlerinde de (tavşan, ayı, tilki, rat, fare, yaban domuzu ve kuşlar) paratüberküloz tespit edilmiştir (Selbitz 2002, Machackova ve ark. 2004, Alvarez ve ark. 2005, Florou ve ark. 2005, Judge ve ark. 2005, Sivakumar ve ark. 2005). Hastalığın ortaya çıkışını hızlandıran hayvanın doğum yapması, nakiller, beslenme yetersizlikleri ve fazlalıkları, yüksek süt verimi, paraziter enfestasyonlar ve aynı zamanda ortaya çıkan başka hastalıklar, nemli ya da mineral bakımından yetersiz meralarda otlatma gibi predispoze nedenlerdir (Baumgartner ve ark. 2006). Paratüberküloz'un teşhisinde, kesin bir tanı yöntemi bulunmamakla birlikte kan testleri, kültür ve mikroskobik muayene yöntemleri kullanılmaktadır. Rektum mukozası kazıntısı ve gaitadan alınan örneğin Ziehl-Neelsen tekniği ile boyanması, pTB basillerinin tespit edilmesinde en basit yöntemdir (Yazıcıoğlu 2011). Ayrıca ELISA subklinik enfekte hayvanların tespitinde, diğer testlere göre en güvenilir metot olduğu bildirilmiştir (Stricklands ve ark. 2005). ELISA, MAP'a karşı oluşan antikorların belirlenmesini sağlar. Günümüzde bu hastalığın tanısında en yaygın kullanılan metottur (Kalis ve ark., 1999; Jubb ve ark., 2004; Yıldırım ve Civelek, 2013).

MATERYAL VE METOT

Bu çalışma, Kafkas Üniversitesi Hayvan Deneyleri Yerel Etik Kurul Başkanlığı'ndan alınan onay (KAÜ-HADYEK 2016-126) sonrası yürütülmüştür. Sunulan çalışmada Ardahan ili merkezi ve ilçelerinden rastgele seçilen 11 odak belirlendi ve bu belirlenen odaklardan 22 işletmede bulunan klinik muayene sonucunda sağlıklı olan 2 yaş ve üzerinde toplam 400 sığır kan serumu çalışmanın materyalini oluşturdu. *Vena coccygea*'dan 10 mL antikoagulantsız tüplere (Hemelab) kan örneği alındı. Kan örnekleri alındıktan sonra Kafkas Üniversitesi Veteriner Fakültesi İç Hastalıkları Anabilim Dalı laboratuvarında 3000 devirde 10 dakika santrifüj edildikten sonra elde edilen serum örnekleri mikrosantrifüj tüplerine konuldu ve etiketlenerek ölçüm yapılıncaya kadar -20ºC'de muhafaza edildi. Çalışmada sığır kan serum örneklerinde MAP antikorlarının araştırılması amacıyla ticari ELISA antikor test kiti kullanıldı (*Mycobacterium paratuberculosis* Antibody Test Kit, 06-07130-26, IDEXX). Serum örnekleri ve test kontrollerine ait değerler excel dosyasına aktarılarak, testin validitesi, ilgili hesaplamalar ve sonuçların yorumlanması üretici firmanın talimatları doğrultusunda gerçekleştirildi.

İstatistiksel Değerlendirme

ELISA sonuçlarına ait pozitif ve negatif veriler Microsoft Office Excel programı kullanılarak % olarak hesaplandı.

BULGULAR

Analiz sonucunda 400 hayvandan 17'sinde MAP yönünden pozitiflik tespit edildi ve Ardahan ili ve çevresindeki yaygınlık oranı %4,25 (17/400) olarak tespit edildi. Elde edilen sonuçlar Tablo 1'de verilmiştir.

Paratüberküloz açısından pozitif bulunan vakaların odak/köy ve işletmelere göre dağılımı Tablo 2'de sunulmuştur. Örnek alınan toplam 22 işletmenin 9'unda paratüberküloz varlığı tespit edildi. Paratüberküloz pozitif 17 vakanın ırk olarak dağılımı; 12 simental veya melezi ve 5 montofon veya melezi olarak tespit edildi (Tablo 3). Buna göre pozitif vakaların paratüberküloz oranı simental veya melezlerinde %70,58 (12/17), montofon veya melezlerinde %29,42 (5/17) olarak gerçekleşti. Paratüberküloz pozitif vakaların yaşa göre dağılımı Tablo 4'te sunulmuştur. Pozitif 17 vakanın yaşa göre dağılımı; pozitif vakanın 12'si 2 yaşında veya 2 yaş ile 6 yaş arasında, 1'i 6 yaşında veya 6 ile 8 yaş arasında ve 4'ü 8 yaşında veya 8 yaşından büyük olarak tespit edilmiştir.

Tablo 1: Ardahan yöresinde paratüberküloz seroprevalansı.
Table 1: Paratuberculosis seroprevalence in Ardahan region.

	Hayvan Sayısı	Yüzdesi (%)
Pozitif (+)	17	4,25
Negatif (-)	383	95,75
Toplam	400	100

Tablo 2: Seropozitif hayvanların işletmelere göre yaygınlık oranı.
Table 2: Prevalence rate of seropositive animals by enterprises.

Odak/Yerleşim	İşletme Kodu	Örnek Sayısı	Pozitif Örnek Sayısı
Ardahan/Beşiktaş Köyü	1	29	0
	2	31	0
Göle/Sürügüden Köyü	10	25	5
	3	49	0
Göle/Yanatlı Köyü	19	40	0
	11	7	1
Göle/Mollohasan Köyü	20	23	0
Göle/Tahtakıran Köyü	9	12	0
Göle/Okçu Köyü	21	7	1
Göle/Merkez	22	10	0
Damal	12	15	4
	13	23	1
	14	18	0
Posof/Çamyazı Köyü	15	14	2
	16	12	1
	17	12	1
	18	19	1
Çıldır/Göleben Köyü	4	22	0
	6	10	0
	8	6	0
Çıldır/Gölebakan Köyü	7	5	0
	5	11	0
Toplam	22	400	17

Tablo 3: Seropozitif hayvanların ırklara göre dağılımı.
Table 3: Distribution of seropositive animals by race.

Irk	Hayvan sayısı	Pozitif Hayvan sayısı	Pozitif Hayvan Yüzdesi
Simental veya melezi	150	12	%70,58
Montofon veya melezi	150	5	%29,42
Yerli ırk veya melezi	50	0	0
Toplam	400	17	100

Tablo 4: Seropozitif hayvanların yaşa göre dağılımı.
Table 4: Distribution of seropositive animals by age.

Hayvanın yaşı	Hayvan sayısı	Pozitif hayvan sayısı	Pozitif Hayvan Yüzdesi
≥2 - <6	252	12	70,59
≥6- <8	45	1	5,89
≥8	103	4	23,52
Toplam	400	17	100

TARTIŞMA

Dünyada paratüberküloz'un prevalansı ile ilgili çalışmalar mevcuttur. Bu durum dünyanın diğer yerlerinde de paratüberküloz'un önemli olduğu ve hala sorun oluşturduğunu göstermektedir. Genel olarak, Avrupa ülkelerindeki yaygınlığının %0 ile %19,8 arasında değiştiği belirlenmiştir (Nielsen ve ark. 2009). Ardahan yöresinde de prevalans oranı (%4,25) bu aralık içerisindeydi. Paratüberküloz'un prevalansının, yapılan çalışmalara göre ülkeden ülkeye hatta kıtadan kıtaya değişiklik gösterdiği saptanmıştır. Bu durum kullanılan tanı yöntemlerinin farklılığı, ülkeden ülkeye göre yetiştirici bilincinin farklığı, yetiştirilen toprakların asit ve alkali farkı, bakım ve hijyen farklılıklarından kaynaklanabileceği düşünüldü. İç Anadolu'da yapılan bir çalışmada paratüberküloz'un seroprevalansı %2,7 olarak saptanmıştır (Vural ve ark. 1988). Türkiye'de yapılan diğer çalışmalarda; Elazığ Bölgesinde %3,4 ile %5 (Çetinkaya ve ark. 2000) ve Uşak Bölgesinde %2,5 ile %20 (Yıldırım ve ark. 2013) arasında değiştiği, ayrıca Trakya bölgesinde %0 (Ikız ve ark. 2005) ve Burdur Bölgesinde %6,2 (Öztürk ve ark. 2010) olarak bulunmuştur. Makav ve ark. (2013), yaptıkları bir çalışmada paratüberküloz'un seroprevalansını Kars yöresinde ELISA yöntemiyle %3,5 olarak bulmuşlardır. Sunulan bu çalışmada da Ardahan yöresinde ELISA yöntemiyle seroprevalans %4,25 olarak bulundu. Sunulan çalışmadaki bu oran İç Anadolu, Elazığ ve Kars yöresinde yapılan çalışmalara yakın bulunmuştur. Paratüberküloz sığırlarda kronik ishal ve verim kayıplarına yol açan, MAP'ın neden olduğu bakteriyel bir hastalıktır. İşletme bazında önemli ekonomik kayıplara neden olmaktadır. Paratüberküloz'da kandaki antikor seviyesi hastalığın şiddeti ve yaşla artmaktadır. O nedenle 2 yaşın altındaki hayvanlarda güç teşhis edileceği bildirilmiştir (Whittington ve ark. 2001). Sunulan bu çalışmada da 2 yaşın üstündeki hayvanlarda kan örnekleri alınarak ELISA yöntemiyle MAP antikorları araştırılmıştır. Çünkü daha önceden yapılan çalışmalar da 2 yaşın altında seroprevalansın düşük olduğu 2 yaşın üzerinde yüksek olduğu bildirilmiştir (Whittington ve ark. 2001, Sweeney ve ark. 2011). Yapılan bir çalışmada Burdur yöresinde hastalığın en yüksek yaygınlık oranını ise 3 yaşındaki sığırlarda %19,7 belirlemişlerdir (Öztürk ve ark. 2010). Kars yöresinde yapılan bir çalışmada Paratüberküloz'un en yüksek yaygınlık oranının yaşı 7 ve/veya 7 üzeri olarak gruplandırılan sığırlarda %5,1 olduğu belirlenmiştir (Makav ve ark. 2013). Sunulan çalışmada ise paratüberküloz açısından 2-6 yaşları arasında sığırlarda en yüksek oranda seropozitiflik (%4,7) bulunmuştur. Bu yaş aralığında seropozitifliğin daha yüksek olmasının nedeni, numune alınan sığırların büyük çoğunluğunun 2-6 yaş aralığında olması, daha ilerki yaşlı numune sayısının az olması neticesinden kaynaklanabileceği düşünüldü. Sunulan çalışma ve belirtilen çalışmaların en yüksek seropozitiflik yaşı 2 ve üzerinde olmasıyla birbirleriyle benzerlik göstermiş, ayrıca litaratür bilgilerle uyumlu bulunmuştur (Whittington ve ark. 2001, Sweeney ve ark. 2011).

Burdur yöresinde pTB'nin prevalansı Holştayn ırkı sığırlarda %6,2 olarak belirlenmiştir (Öztürk ve ark. 2010). Kars yöresinde yapılan bir çalışmada ise üç ayrı ırkta yaygınlık oranları değerlendirildiğinde pozitif vakaların %35,7'si simental ve melezi, %50'si montofon ve melezi ve %14,3'ü yerli ırk ve melezi olarak tespit edildi (Makav ve ark. 2013). Bu çalışmada ise pozitif vakaların oranları simental ve simental melezlerinde %70,58 ve montofon veya melezlerinde %29,42 oranında bulundu. Yerli ırklarda herhangi bir pozitifliğe rastlanmadı. Sunulan çalışmanın sonuçları ile yapılan çalışmaların kültür ırklarında oranın yüksek olmasıyla benzerlik göstermiştir. Bu durum kültür ırklarının hastalığa yerli ırklara göre daha duyarlı olabileceğini göstermektedir.

SONUÇ

Sonuç olarak Ardahan yöresindeki süt sığırlarında pTB nin seroprevalansı %4,25 olarak bulundu. Bu sonuç Ardahan yöresindeki sığırlarda pTB'un subklinik olarak bulunduğunu ortaya koymaktadır. Bu durum hastalığa karşı ülkemizde olduğu gibi Ardahan yöresinde de bu hastalığa karşı kontrol önlemlerinin alınması gerektiğinin göstergesi olabilir. Sunulan bu çalışma da pTB hastalığının kültür ırkı sığırlarda daha yaygın olduğunu, dolayısıyla kültür ırklarının daha duyarlı olduğunu ortaya koymuştur. Ayrıca bu hastalığın zoonotik olduğu düşünüldüğünde halk sağlığının da etkilenebileceği söylenebilir. Bu nedenle pTB ile ilgili daha fazla çalışma gerçekleştirilmesi fayda sağlayacaktır.

Çıkar Çatışması: Yazarlar, çıkar çatışması olmadığını beyan eder.

KAYNAKLAR

Abendan N, Sevilla IA, Prieto JM, Garrido JM, Juste RA, Alonso-Hearn M. Mycobacterium aviumsubspeciesparatuberculosis isolates from sheep and goats show reduced persistence in bovine macrophages than cattle,bison, deer and wild boar strains regardless of genotype. Vet Microbiol. 2013; 163:325-334.

Alvarez J, De Juan L, Aranaz A. A survey on paratuberculosis in wildlife in Spain. In:8th International Colloquium on Paratuberculosis. Copenhagen, Denmark, 2005.

Baumgartner W. ve Khol JL. Paratuberculosıs (Johne´S Disease) in ruminants -an ongoing story, Slov Vet Res. 2006; 43(1):5-10.

Civelek T. Paratüberküloz 'sığırlarda paratüberküloz'. http://www.turancivelek.net/FileUpload/ks117047/File/ptb.pdf. Erişim tarihi: 14.02.2018.

Collins M. and Manning E. "Johne's Information Center" The University of Wisconsin-School of Veterinary Medicine, 13 March 2003.

Çetinkaya B, Muz A, Ertaş HB, Öngör H, Sezen İY, Gülcü HB. Süt ineklerinde paratüberküloz prevalansının polimeraz zincir reaksiyonu (PZR) ile saptanması. Turk J Vet Anim Sci. 2000; 24:371-379.

Florou M, Leontides L, Billinis C. Isolation of Mycobacteium avium subspecies paratuberculosis from non-ruminant wildlife in Greece. In: 8th International Colloquium on Paratuberculosis. Copenhagen, Denmark, 2005.

Gilardoni LR, Paolicchi FA, Mundo SL. Bovine paratuberculosis: a review of the advantages and disadvantages of different diagnostic tests. Rev Argent Microbiol. 2012; 44:201-215.

Hurley SS, Splitter GA. and Welch RA. Deoxyribonucleic acid relatedness of Mycobacterium paratuberculosis to other members of the family Mycobacteriaceae. Int. J. Syst. Bact. 1988; 38:143-146.

Ikiz S, Bagcigil AF, AK S, Ozgur NY, Loaz A. Paratuberculosis in cattle in Turkey detected by PZR. Medycyna Wet. 2005; 61:881-883.

Johne HA, Frothingham L. Ein eigenthumlicher fall von tuberculose beim rind (a particular case of tuberculosis in a cow). Deut Z Tiermed Vergl Pathol. 1895; 21:438–454.

Jubb TF, Sergeant ES, Callinan AP, Galvin J. Estimate of the sensitivity of an ELISA used to detect johne's disease in Victorian dairy cattle herds. Aust Vet J. 2004; 82: 569-73.

Judge J, Kyriazakis I, Greis A. Clustering of Mycobacterium avium subsp. paratuberculosis in rabbits and the environment: how hot is a hot spot? Appl Environ Microbiol. 2005; 71:6033-6038.

Kalis CHJ, Hesselink JW, Barkema HW. Comparison of culture of individual and strastegically pooled bovine faecal samples for Mycobacterium avium subsp. parastuberculosis. In: Manning, E.J.B., Collins, M.T. (Eds). Proceedings of the 6th International Colloquium on Paratuberculosis. Madison. Wisconsin. 1999; 344-8.

Lingling L, John PB, Joseph JC, Arlo R, Yrjo TG, Robab K, Megan S, Jessica RB, Vivek K. Identification of sero-reactive antigens for the early diagnosis of Johne's disease in cattle. J. Pone. 2017; 9(1):1-18.

Machackova M, Svastova P, Lamka J. Paratuberculosis in farmed and free-living wild ruminants in the Czech Republic. Vet Microbiol. 2004; 101:225-34.

Makav M. ve Gökçe E. Kars yöresi sığırlarında subklinik paratuberkülozun seroprevalansı. Kafkas Univ Vet Fak Derg. 2013; 19(5):913-916.

Nielsen SS, Toft N. A review of prevalences of paratuberculosis in farmed animals in Europe. Prev. Vet. Med. 2009; 88:1-14.

Öztürk D, Pehlivanoğlu F, Tok AA, Günlü S, Güldalı Y, Turutoğlu H. Seroprevalence of paratuberculosis in the Burdur province (Turkey), in dairy cattle using the enzyme linked immunosorbent assay (ELISA). Israel J Vet Med, 2010; 65:53-57.

Selbitz HJ. Bakterielle Krankheiten der Tiere. In: Rolle M, Mayr A, eds. Medizinische Mikrobiologie, Infektions- und Seuchenlehre. 7. Aufl. Enke Verlag, pp 562-3, Stuttgart, 2002.

Sivakumar P, Tripathi BN, Singh N. Detection of Mycobacterium avium subsp. paratuberculosis in intestinal and lymph node tissues of water buffaloes (Bubalis bubalois) by PCR and bacterial culture. Vet Microbiol. 2005; 108:263-70.

Stricklands J, Scott HM., McJordan, ER. Effects of seasonal climatic conditions on the diagnosis of Mycobacterium avium subspecies paratuberculosis in dairy cattle. J Dairy Sci. 2005; 88: 2432-40.

Sweeney RW. Pathogenesis of paratuberculosis, In: Collins, T.M. (Eds) Johne's Disease. Vet. Clin. Food Anim. 2011; 27:525-535.

Thorel MF, Krichevski M and LevyFrebault VV. Numerical taxonomy of mycobactin-dependent mycobacteria, emended description of Mycobacterium avium, and description of Mycobactearium avium subsp. avium subsp. nov., Mycobacterium avium subsp. paratuberculosis subsp. nov and Mycobacterium avium subsp. silvaticum subsp. nov. Int. J. Syst. Bacteriol. 1990; 40:254-260.

Vural B, Atala N. Serological study on bovine paratuberculosis in central Anatolia using the microcomplement fixation and tube complement fixation tests. Etlik Vet. Mikrobiol Derg. 1988; 6:87-97.

Whittington RJ ve Sergeant ESG. Progress towards understanding the spread, detection and control of Mycobacterium avium subsp paratuberculosis in animal populations, Australian Veterinary Journal. 2001; 79:267-278.

William CD and Kun TP. progress towards control of a mycobacterial pathogen, mycobacterium aviumsubsp. paratuberculosis, the causative agent of johne's disease in cattle and humans. Food Hyg. 2018; 33(4):221-228.

Yazıcıoğlu Ö. Paratüberküloz, http://www.bornovavet.gov.tr/paratuberkuloz.htm. Erişim Tarihi: 03.05.2017.

Yıldırım D. Civelek T. Prevalence of subclinical paratuberculosis in dairy cattle in Uşak Region. Kafkas Univ Vet Fak Derg. 2013; 19(1):121-126.

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RESEARCH ARTICLE

Molecular Detection and Characterization of Infectious Laryngotracheitis Virus in Backyard Chickens in Turkey

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ABSTRACT

Gallid herpesvirus I (GaHV1) is etiological agent of infectious laryngotracheitis (ILT) and ILT is one of the important disease that included in respiratory infections of chickens. Few studies have been conducted in Turkey and there is no data about the existence of ILT in backyards. The purpose of the study was to document the detection and characterization of GaHV1 in backyard chickens using PCR, RFLP and sequencing. Of the163 tracheal swap samples which were taken from 43 backyard flocks 5 (3.07%) were found to be positive for ILT infection. Positivity was 4.65 % (2/43) at flock level. The nucleic acid sequences of the ICP4 gene compared with ILTV sequences in GenBank and the level of identity differed from 96.82 to 100%. When the sequences of samples compared with TCO strains 99.77-100% homology was observed. The virtual RFLP analysis with the *Hga*I restriction enzyme characterized strains as having a pattern similar to the vaccine strain TCO. This is the first study that presents presence and characterization of GaHV1 in backyards. Large scale studies are needed to estimate prevalence of ILT in Turkey. Chickens should be monitored and growers should avoid to contacting vaccinated birds with non-vaccinated chickens, to control ILTV outbreaks.
Keywords: Backyard, GaHV1, infectious laryngotracheitis, RFLP, sequencing

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Türkiye'deki Köy Tavuklarında İnfeksiyöz Laringotrakeitis Virüsünün Moleküler Tespiti ve Karakterizasyonu

ÖZ

Gallid herpesvirüs I (GaHV1), infeksiyöz laringotrakeitisin (ILT) etiyolojik ajanıdır ve ILT, tavukların solunum yolu enfeksiyonları arasında yer alan önemli hastalıklardan biridir. Türkiye'de çok az çalışma yapılmış ve köy tavuklarında ILT'nin varlığına dair veri bulunmamaktadır. Çalışmada, köy tavuklarında PCR, RFLP ve sekans verileri kullanarak GaHV1'in tespitini ve karakterizasyonu amaçlandı. Toplamda 43 köy tavuğu işletmesinden alınan 163 trakeal swap örneğinin 5'inde (% 3,07) ILT enfeksiyonu pozitif bulundu. Sürü düzeyinde pozitiflik % 4,65 (2/43) idi. ICP4 geninin nükleik asit dizileri GenBank'taki ILTV dizileri ile karşılaştırıldığında benzerlik % 96.82 ile % 100 arasında değiştiği görüldü. Örneklerin sekans dizileri TCO suşları ile karşılaştırıldığında % 99.77-100 homoloji gözlemlendi. *Hga*I enzimi ile yapılan sanal RFLP analizinde, çalışmadaki suşlar TCO aşı suşuna benzer bir paterne sahip olarak karakterize edildi. Bu araştırma, köy tavuklarında GaHV1'in varlığını ve karakterizasyonunu ortaya koyan ilk çalışmadır. Türkiye'de ILT prevalansını tahmin etmek için büyük ölçekli çalışmalara ihtiyaç vardır. ILTV salgınlarını kontrol etmek için tavuklar izlenmeli ve yetiştiriciler aşılanmamış tavuklarla aşılanmış olanların temas etmesinden kaçınmalıdır.
Anahtar Kelimeler: GaHV1, infeksiyöz laringotrakeitis, köy tavuğu, RFLP, sekans

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INTRODUCTION

Infectious laryngotracheitis (ILT) is one of the important disease that included in contagious respiratory infections of chickens. *Gallid herpesvirus I* (GaHV1) also known as infectious laryngotracheitis virus is responsible for infection and classified in the family *Herpesviridae*, subfamily *Alphaherpesvirinae*, genus *Iltovirus*, with a linear double-stranded DNA which is approximately 150 kb size (King, et al. 2018, Thureen and Keeler 2006). Infection causes production loss, low growth rates, and mortality. Inflammation predominantly affects mucosal surfaces such as conjunctiva, larynx, trachea and hemorrhagic, necrotic tracheal lesions may be seen in diseased flocks. Chickens that are infected with ILT show clinical signs including lacrimation, nasal discharge, conjunctivitis, respiratory distress, dyspnea, coughing, bloody mucus secretion, decreased egg production and dead (Guy and Garcia 2008).

The respiratory and ocular routes are important ways of transmission between birds although vertical infection has not been verified yet. Main sources of viruses are known as sick chickens, latent carriers, contaminated litter and drinking water. Biofilm formation of some bacteria may be affective on remaining of virus in environment (Ou and Giambrone 2012).

Live-attenuated vaccines have been developed and widely used against the disease. Strains were derived by serial passages in chicken embryos (CEO; chicken-embryo origin) or in cell culture (TCO; tissue culture origin) and used in commercial layers, layer breeders, and broiler breeders (Oldoni and García 2007). Several CEO and TCO vaccines are commercially available. Besides their protection, these vaccines may revert to virulence due to bird to bird passage or reactivation of latent strains (Guy et al. 1990, Hughes et al. 1991, Kotiw et al. 1995). It has been reported that vaccinated birds may be a source of disease for non-vaccinated flocks (Blacker et al. 2011).

Virus isolation, serological and molecular tests are widely used for the detection of infection. Enzyme-linked immunosorbent assay (ELISA), serum neutralization tests, agar gel immunodiffusion and fluorescent antibody tests can be applied to investigate antibodies in serum samples (OIE 2019). Serological tests and virus isolation methods are time consuming and require skilled personnel.

Polymerase chain reaction (PCR) is more sensitive than serological tests and preferred as an alternative to virus isolation for diagnosis of ILT because of the rapid, specific, and simplex properties of the method (Creelan et al. 2006, Oldoni and García 2007). Primers targeting the infected-cell protein 4 (ICP4) gene regions are used in many studies for the detection of virus by PCR and giving trustful results (Blakey et al. 2019, Bayoumi et al. 2020). DNA detection has been successfully performed to identify agent in clinical samples including trachea, larynx and conjunctiva. GaHV1 has also been detected in internal organs of chickens including heart, liver, spleen, lung, kidney, thymus, glandular stomach, duodenum, pancreatic gland, small intestine, large intestine, cecum, cecal tonsil, bursa fabricius and brain by PCR method (Zhao et al. 2013)

It is difficult to differentiate whether the new outbreaks are caused by vaccine strains or field strains. Sequencing and restriction fragment length polymorphism (RFLP) methods were performed to clarify the epidemiology of infections. RFLP involves differentiation of strains by restriction enzyme cleavage patterns of digested genes (Oldoni and García 2007, Yan et al. 2016).

Few studies have been conducted in broiler breeders, layer hens in Turkey and there is no data about the existence of ILT in backyards. The purpose of the study was to document the detection and characterization of GaHV1 in backyard chickens using PCR and sequencing.

MATERIAL and METHODS

Sampling

Tracheal swap samples were taken from chickens which have nasal discharge, dyspnea and coughing. During the study 163 animals were sampled between November 2018 and March 2019. Specimens were collected from 43 backyard flocks located in 12 different location around Sivas province. Chicken numbers were between 10 and 30 in each flock. The examined flocks were at 25-35 weeks age and all flocks were not vaccinated against ILT.

DNA extraction and PCR

Tracheal swabs were suspended in 2 ml of 1 M sterile PBS and centrifuged for 10 min at 3,500 rpm to remove large cellular debris. DNA was extracted from the supernatants using a GF-1 Viral Nucleic Acid Extraction Kit (Vivantis Technologies, Malaysia) according to the manufacturer's instructions. Eluted nucleic acids were kept at −80ºC until use.

The ICP4 gene specific primers (ILTF 5' CCTCGACGCCGAGTAATTT 3' and ILTR 5' GAGCGAGTCGATGACCGTAT 3') designed by Wanasawaeng and Chansiripornchai (2010) were used. Amplification was performed in Bio-Rad T100™ Thermal Cycler in a reaction volume of 50 µl, containing 5 µl of 10X PCR buffer, 5 µl of 25 mM MgCl, 10 mM of deoxynucleotide triphosphate, 5 U of Taq DNA polymerase (Vivantis, Germany), 10 pmol each primer and 25 ng template DNA. The PCR was incubated through one cycle of 95 C for 3 min, then 40 cycles of 94 C for 30 sec, 52 C 30 sec and 72 C for 1 min , and finally one cycle of 72 C for 10 min. The amplified products were detected by staining with ethidium bromide (0.5 mg/ml) after electrophoresis at 80 V for 2 h (7 V/cm) in 1.5% agarose gels.

Sequencing and RFLP analysis

PCR amplicons (440 bp) of three positive field samples were sequenced. Two DNA samples from F location and one from H location were randomly selected and investigated further by partial sequence analysis. Phylogenetic analyses and molecular relationship of viruses were interpreted with Geneious Prime Version 2020.2.2 software (Kearse et al. 2012).

RFLP analysis was implemented to the partial sequences of ICP4 gene of strains using the in silico Virtual Gel plugin for Geneious Version 2020.2.2 (Kearse et al. 2012). For this purpose a previous Turkish strain (MH921826.1), a TCO vaccine strain (JN580312.1), two CEO vaccine strains (HM230782.1 and EU104900.1) and three strains (MN717261.1, MN717262.1 and MN717263.1) obtained from present study were investigated.

RESULTS

PCR findings

According to the Wanasawaeng and Chansiripornchai (2010) the primers should have provide amplicons of 428 bp, 440 bp and 450 bp from CEO, TCO and filed isolates, respectively. Only 440 bp amplicons obtained in this study. Of the 163 DNA samples, 5 (3.07%) were found to be positive for ILT infection. Four samples were belong to one flock in F location and one sample was belong to a flock in F location. Positivity was 4.65 % (2/43) at flock level (Table 1).

Phylogenetic analysis findings

Partial ICP4 sequences of three positive field samples were used for subsequent phylogenetic analysis. Two samples from F location and one from H location were compared and investigated. The partial nucleotide and amino acid sequences of chosen samples were deposited to the GenBank with accession numbers as follows: MN717261 (ILTV/TUR/7), MN717263 (ILTV/TUR/74) and MN717262 (ILTV/TUR/66). Sequences of two samples gathered from same flock in F location (MN717261, MN717263) were homolog with each other. The last one in H location was (MN717262) showed one nucleotide difference than others. The nucleic acid sequences of the ICP4 gene compared with overall the ILTV sequences in GenBank and the level of identity differed from 96.82 to 100%. When the sequences of samples taken in the present study compared with TCO strains 99.77-100% homology was observed. Besides, samples showed 96.82-97.32 homology with CEO strains because of the presence of deletions including 12 nucleotides in CEO strains. The phylogenetic tree data showed that the ICP4 genes of TCO like strains and CEO like strains (Table 2) were divided into two different branches (Fig. 1).

RFLP findings

Selected strains were further analyzed by RFLP using *Hga*I restriction enzyme with the in silico Virtual Gel plugin for Geneious Version 2020.2.2. The DNA sequences of same region of the gene of previous Turkish strain (MH921826.1), TCO vaccine strain (JN580312.1), CEO vaccine strains (HM230782.1 and EU104900.1) and three strains (MN717261.1, MN717262.1 and MN717263.1) detected in present study were digested in silico to characterize isolates. Two different RFLP patterns were observed for CEO, TCO and Turkish field strains. The CEO vaccine strain was digested to 414 bp and 14 bp fragments. The TCO vaccine strains and Turkish field strains showed three DNA fragments of 250, 176 and 14 bp (Fig. 2).

Table 1. Distribution of samples and flocks

Location	Flocks/Positive flocks	Samples/Positive samples
A	4/-	10/-
B	3/-	8/-
C	5/-	20/-
D	3/-	13/-
E	2/-	10/-
F	5/1	23/4
G	3/-	11/-
H	6/1	19/1
I	4/-	14/-
J	2/-	9/-
K	5/-	21/-
L	1/-	5/-
Total	43/2	163/5

Table 2. GenBank accession numbers of GaHV1 strains used in genetic analysis.

Strain	Accession Number
ILTV/TUR/7/Turkey (*present study*)	MN717261.1
ILTV/TUR/66/Turkey (*present study*)	MN717262.1
ILTV/TUR/74/Turkey (*present study*)	MN717263.1
TR2-70-1/Turkey	MH921826.1
Composite of 6 (RefSeq)/USA	NC006623.1
ILTV.29/19/Australia	MK894996.1
SA2/Australia	JN596962.1
ILTVclass10/Australia	KR822401.1
S2.816/Australia	MF417807.1
CSW-1/Australia	JX646899.1
2009/1643/Russia	MF405079.1
VFAR-043/Peru	MG775218.1
USDA/USA	JN542534.1
2/A/04/BR/USA	EU104912.1
TCO_IVAX/VACCINE/USA	JN580312.1
ILTV/Brazil/2012/472-3/Brazil	KP768193.1
V1-99/Australia	JX646898.1
CL9/Australia	JN804827.1
ILTV.358/19/Australia	MK895003.1
0206/14/Ko/South_Korea	MH937564.1
KR_1020170134159-A/15/VACCINE/South_Korea	LY517296.1
2013/2701/Russia	MF405080.1
WG/China	JX458823.1
Jiang-2011-3/China	JN969100.1
193435/07/Italy	KP677883.1
CEO_Intervet/VACCINE/Italy	HM230782.1
ILT.77.IR_Iran	KX344451.1
63140/C/08/BR/USA	JN542536.1
CEO_VACCINE/USA	EU104900.1

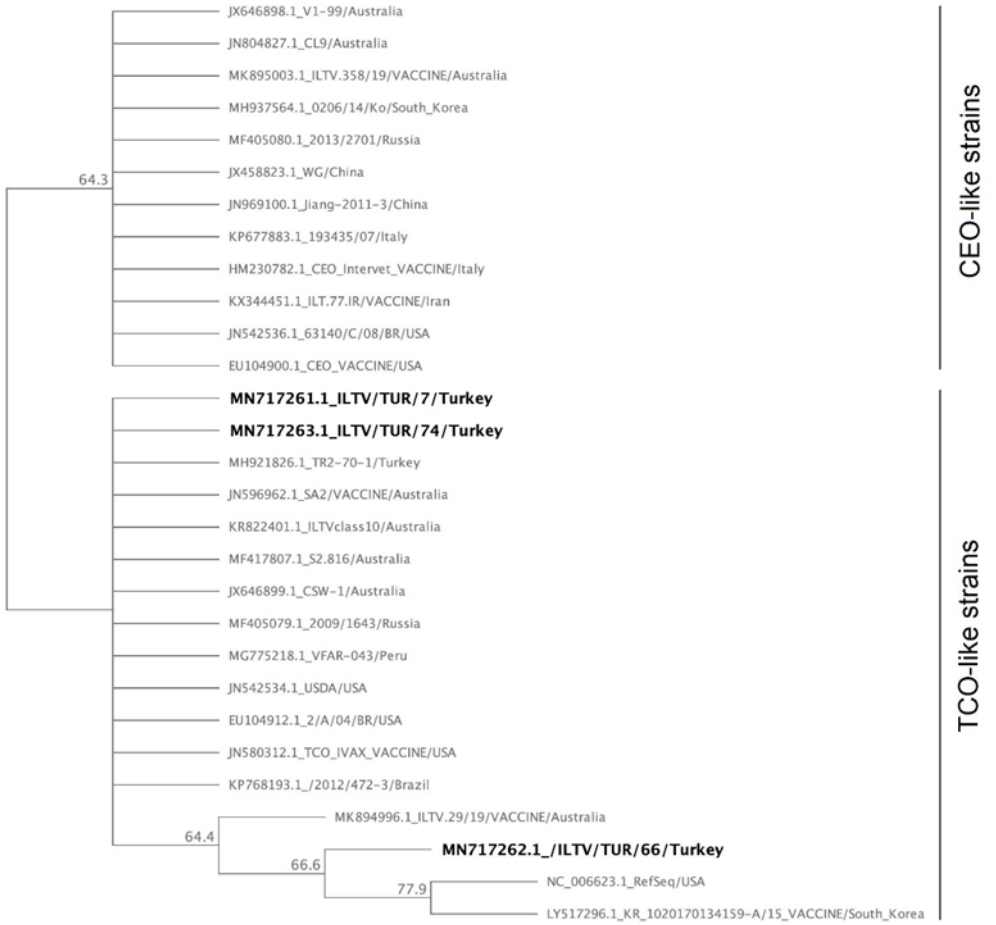

Figure 1. The phylogenetic tree of ICP4 genes of TCO like strains and CEO like strains.

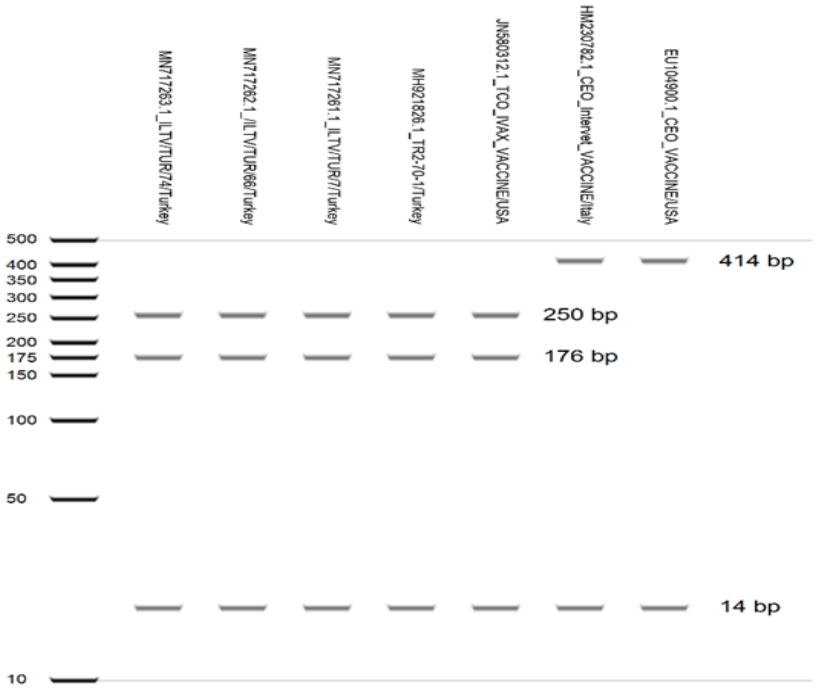

Figure 2. The restriction model of gel electrophoresis of the strains analyzed in Virtual Gel plugin for Geneious. Lane 1: Ladder, Lane 2-8: The RFLP patterns of CEO, TCO and Turkish field isolates cut by *Hga*I.

DISCUSSION

This is the first study that presents the situation of ILT infection in backyards. Tracheal swab samples were collected and examined in a four month period by PCR. The frequency of the disease in backyard chickens estimated as 3.07% and two flocks were positive (4.65%) within the 43. Results of present study showed that GaHV1 is not widely distributed in backyard chicken flocks in Turkey. So far, only a few studies have been conducted in Turkey although the ILT is one of the well-known respiratory diseases of poultry and seen throughout the world. It was firstly reported in Elazığ province in nine white leghorns (Gülaçtı et al. 2007). In 2016 five tracheal samples of broiler breeders (located in Mediterranean region) have been examined and three of them have been detected as positive for GaHV1 (Kaya and Akan 2018). Aras et al. (2018) have investigated commercial layer hens serologically in a six month period in Konya and they have indicated the disease as 64% (16/25) and 42.6% (266/625) at flock and samples levels, respectively. There was no vaccination history for ILT in these three studies. They have reported that ILT was observed in layers and broiler breeders. However, no adequate data are available for the potential of ILT at the national level in Turkey, and it is not easy to estimate the degree of threat that it acts to the poultry population. Similarly, none of the flocks had been vaccinated against the ILT in the present study. The positivity rates of backyards were quite lower than Aras et al (2018) and this difference may be related with the sensitivity of ELISA tests. Virus isolation and serological tests (fluorescent antibody technique, enzyme-linked immunosorbent assay, serum neutralization, and agar gel immunodiffusion) have been used for diagnoses but, these methods are laborious, time consuming and the sensitivities of serological tests are lower than PCR (Alaraji et al. 2019).

ILT continue to keep its importance throughout the world. It had been firstly reported in the USA in 1925 and over 100 countries have declared the disease so far (Menendez et al. 2014). Countries including USA, Canada, Brazil, Europe, Australia, China, Egypt, South Asia, Lebanon and Saudi Arabia have notified ILT outbreaks (Gowthaman et al. 2020). Lastly, the disease have been reported in Iraq (Alaraji et al. 2019) and Namibia (Molini et al. 2019). In Turkey, the frequency of the disease that determined in present study is lower than other countries. In previous studies samples have been taken from commercial flocks in poultry industry (layers, broilers) although the material of this study was backyards. Besides, we know that many countries had been demonstrating live ILT vaccination and vaccines are not fairy innocent. Vaccines may cause latent infections (Hughes et al. 1991) and infected birds may be a source of disease (Blacker et al. 2011) for non-vaccinated flocks in those countries. In latent or some recovered birds, the virus may pass a non-infectious state and reside in, so the chickens appear to be healthy (Williams et al. 1993). ILT spreads from bird to bird aerogenically, via fomites. Vertical transmission or wild bird infection has not been reported yet (Gowthaman et al. 2020).

In present study the ICP4 gene of GaHV1 characterized partially and confirmed that the viral agent present in backyard chickens in Turkey. Genetic relationships of GaHV1 strains and phylogenetic positions of Turkish strains were also demonstrated. The viruses detected in Turkey, shared a high nucleotide similarity (96.82 to100 %). The sequence analysis shows that the strains detected in this study shares a high similarity with the other GaHV1 strains worldwide. Two clusters observed when the sequences of USA, Australia, Russia, Peru, Brazil, South Korea, China, Italy, Iran and Turkey compared (fig 1). Broilers, broiler breeders, layers, and layer breeders are usually vaccinated with CEO and/or TCO vaccines in many countries. The sequences of ICP4 genes of CEO and TCO vaccine strains deposited to the GenBank had been investigated and differences had been reported between CEO and TCO vaccine strains (Wanasawaeng and Chansiripornchai 2010). The nucleotides at the position of 272-283 were missing in the CEO vaccine strain and this deletion had been used for the differentiation of strains by RFLP. The PCR-RFLP patterns of several genome regions have been very useful to search genetic diversity within the GaHV1 genome, and to distinguish filed and vaccine strains (Bayoumi et al. 2020). The virtual RFLP analysis characterized all of the strains (detected in present study) as having a pattern similar to the vaccine strain TCO. Digestion with the *Hga*I restriction enzyme showed 414 and 14bp bands for the CEO vaccines. TCO vaccine and the tested samples represented three bands (250, 176 and 14bp in length). Wanasawaeng and Chansiripornchai (2010) declared that 428, 440 and 450 bp amplicons should be provided from CEO, TCO and filed isolates, respectively with recommended primer set. A 440 bp amplicons and TCO like RFLP patterns (three bands: 250, 176 and 14bp) were observed. Samples detected in present study were not field isolate (not amplified 450 bp bands) besides sequences of the Turkish strains were very similar with TCO vaccine strains in GeneBank and were located in TCO-like strains (fig 1). These may be explained with the circulation of a TCO vaccine strain and it may be passed from commercial birds to backyards. In contrast, studies conducted in US indicated that most of ILTV outbreaks are caused by CEO vaccine isolates that circulating in longlived bird operations and spill-over broiler populations (Oldoni and García 2007).

In conclusion, this study revealed that ILT has migrated to backyard chickens. This is the first report

of presence and characterization of GaHV1 in backyards. Chicken meat and eggs, which contain high-quality protein, vitamins, and minerals for the human are the important and cheapest source of animal proteins. Although the commercial poultry industry dealing with this necessity, people raising their own birds in rural and suburban areas all over the world. Besides, poultry products are important commodity of Turkish livestock economy. Large scale studies including commercial layer hens, broilers and backyards are needed to estimate prevalence and incidence of ILT infections in Turkey. It is not clear to understand the direction of the disease which may be transmitted from commercial chickens to backyards or opposite. Therefore characterization of Turkish ILTV strains (by sequencing or RFLP) is essential to clarify the epidemiology of infections and to compare with vaccine strains applied in our country. Additionally chickens should be monitored and growers should avoid to contacting vaccinated or recovered birds with non-vaccinated chickens, to control ILTV outbreaks. Management, biosecurity, rapid detection, effective vaccination protocols, collaboration of the government-poultry industry-veterinarians-growers are critical for the prevention and the control of the disease.

Conflict of Interest: The authors declare that they have no conflict of interest.

REFERENCES

Alaraji F, Hammadi H, Abed AA, Khudhair YI. Molecular detection and phylogenetic tree of infectious laryngotracheitis virus in layers in Al-Diwaniyah province, Iraq. Veterinar World. 2019; 12(4): 605-608.

Aras Z, Yavuz O, Gölen GS. Occurrence of infectious laryngotracheitis outbreaks in commercial layer hens detected by ELISA. Journal of Immunoassay and Immunochemistry. 2018; 39:2, 190-195. doi: 10.1080/15321819.2018.1428991.

Bayoumi M, El-Saied M, Amer H, Bastami M, Sakr EE, El-Mahdy M. Molecular characterization and genetic diversity of the infectious laryngotracheitis virus strains circulating in Egypt during the outbreaks of 2018 and 2019. Arch Virol. 2020; 165(3):661-670. doi:10.1007/s00705-019-04522-4.

Blacker H, Kirkpatrick N, Rubite A, O'Rourke D, Noormohammadi A. Epidemiology of recent outbreaks of infectious laryngotracheitis in poultry in Australia. Australian Veterinary Journal. 2011; 89(3):89-94. doi:10.1111/j.1751-0813.2010.00665.x.

Blakey J, Stoute S, Crossley B, Mete A. Retrospective analysis of infectious laryngotracheitis in backyard chicken flocks in California, 2007-2017, and determination of strain origin by partial ICP4 sequencing. J Vet Diagn Invest. 2019; 31(3):350-358. doi:10.1177/1040638719843574.

Creelan JL, Calvert VM, Graham DA, McCullough SJ. Rapid detection and characterization from field cases of infectious laryngotracheitis virus by real-time polymerase chain reaction and restriction fragment length polymorphism. Avian Pathol. 2006; 35:173-179.

Gowthaman V, Kumar S, Koul M, Dave U, Murthy T, Munuswamy P, Tiwari R, Karthik K, Dhama K, Michalak I, Joshi SK. Infectious laryngotracheitis: Etiology, epidemiology, pathobiology, and advances in diagnosis and control - a comprehensive review. The veterinary quarterly. 2020; 40(1):140–161. doi:10.1080/01652176.2020.1759845.

Gülaçtı I, Bulut H, Eröksüz Y, Çeribaşı AO. Outbreak of clinical infectious laryngotracheitis in Turkey. Vet. Rec. 2007; 160: 554–555. doi: 10.1136/vr.160.16.554.

Guy JS, Barnes HJ, Smith L. Increased virulence of modifed-live infectious laryngotracheitis vaccine virus following bird to-bird passage. Avian Dis. 1990; 35:348 https ://doi.org/10.2307/15911 88.

Guy JS, Garcia M. Laryngotracheitis. In: Disease of poultry, Ed; Saif YM, Fadly AM, Glisson JR, McDougald LR, Nolan LK, Swayne DE, 11th ed. Ames: Iowa State University Press, 2008; pp. 137-152 35.

Hughes CS, Williams RA, Gaskell RM, Jordan FTW, Bradbury JM, Bennett M, et al. Latency and reactivation of infectious laryngotracheitis vaccine virus. Arch Virol. 1991; 121:213-218.

Kaya BI and Akan M. First report of avian infectious laryngotracheitis infection in broiler breeders in Turkey. Ankara Üniversitesi Veteriner Fakültesi Dergisi. 2018; 65: 331-334.

Kearse M, Moir R, Wilson A, et al. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 2012; 28(12):1647-1649. doi:10.1093/bioinformatics/bts199.

King AMQ, Lefkowitz EJ, Mushegian AR. et al. Changes to taxonomy and the international code of virus classification and nomenclature ratified by the international committee on taxonomy of viruses. Arch Virol. 2018; 163, 2601–2631. https://doi.org/10.1007/s00705-018-3847-1.

Kotiw M, Wilks CR, May JT. The effect of serial in vivo passage on the expression of virulence and DNA stability of an infectious laryngotracheitis virus strain of low virulence. Vet Microbiol. 1995; 45:71–80. https://doi.org/10.1016/0378-1135(94)00115-D.

Menendez KR, Garcia M, Spatz S, Tablante NL. Molecular epidemiology of infectious laryngotracheitis: a review. Avian Pathol. 2014; 43(2):108–117.

Molini U, Aikukutu G, Khaiseb S. et al. Investigation of infectious laryngotracheitis outbreaks in Namibia in 2018. Trop Anim Health Prod. 2019; 51: 2105–2108 https://doi.org/10.1007/s11250-019-01918-x.

Oldoni I, García M. Characterization of infectious laryngotracheitis virus isolates from the US by polymerase chain reaction and restriction fragment length polymorphism of multiple genome regions. Avian Pathol. 2007; 36: 167-176.

Ou SC, Giambrone JJ. Infectious laryngotracheitis virus in chickens. World J Virol. 2012; 1(5): 142-149. doi: http://dx.doi.org/10.5501/wjv.v1.i5.142.

Thureen DR and Keeler CL. Psittacid herpesvirus 1 and infectious laryngotracheitis virus: Comparative genome sequence analysis of two avian alphaherpesviruses. J. Virol. 2006; 80:7863–7872.

Wanasawaeng W and Chansiripornchai N. Molecular classification of infectious laryngotracheitis virus from chick embryo origin vaccine, tissue culture origin vaccine and field isolates. Thai J. Vet. Med. 2010; 40:393–398.

Williams RA, Bennett M, Bradbury JM, Gaskell RM, Jones RC, Jordan FTW. Demonstration of sites of latency of infectious laryngotracheitis virus using the polymerase chain reaction. *Journal of General Virology*. 1993; 73**:** 2415–242.

World Organisation for Animal Health (OIE). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2019 Chapter 3.3.3. Avian infectious laryngotracheitis. OIE, Paris. Available at: https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.03.03_AVIAN_INF_LARYNGO.pdf. Accession date: 02.07.2020.

Yan Z, Li S, Xie Q, Chen F, Bi Y. Characterization of field strains of infectious laryngotracheitis virus in China by restriction fragment length polymorphism and sequence analysis. J Vet Diagn Invest. 2016; 28(1):46-49. doi:10.1177/1040638715618230.

Zhao Y, Kong C, Cui X, Cui H, Shi X, Zhang X, Hu S, Hao L, Wang Y. Detection of infectious laryngotracheitis virus by real-time PCR in naturally and experimentally infected chickens. PLoS One. 2013; 8(6):e67598.

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RESEARCH ARTICLE

Protective Effect of Resveratrol and N-Acetylcysteine Combination Against Locomotor Hyperactivity Induced by MK-801

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ABSTRACT

N-Methyl-D-Aspartate (NMDA) receptors are one of the most important elements of the glutamatergic system. The hypofunction of this receptor causes locomotor hyperactivity. The chemical agent MK-801, which is an NMDA receptor antagonist, also causes locomotor hyperactivity in rodents. In the present study, it is aimed to find the lowest protective dose of neuroprotective resveratrol and N-acetyl-cysteine (NAC) combination on increased locomotor activity using MK-801 in mice. For this purpose, 84 female mice were used and 14 groups of equal number of mice were formed. Locomotor hyperactivity was created in two parts as acute (1 day drug administration) and sub-acute (4 days drug administration) phases. After drug administrations, animals were subjected to open field testing. According to the results, the drug combination was successful in reducing locomotor hyperactivity in the acute phase than in the sub-acute phase. It was observed that the intraperitoneal administration of both low doses of the combination, 40mg/kg resveratrol + 20mg/kg NAC and 20mg/kg resveratrol + 10mg/kg NAC, successfully prevented the locomotor hyperactivity in the acute phase. As a result, it was concluded that the combination of antioxidants has an effect on acutely formed locomotor hyperactivity.
Keywords: Antioxidant, Locomotor Hyperactivity, MK-801, N-acetylcysteine, Resveratrol

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Resveratrol ve N-Asetilsistein Kombinasyonunun MK-801' le İndüklenen Lokomotor Hiperaktivite Karşısında Koruyucu Etkisi

ÖZ

N-Methyl-D-Aspartate reseptörleri glutamaterjik sistemin önemli bir öğesidir. Bu reseptörün hipofonksiyonu lokomotor hiperaktiviteye sebep olur. NMDA reseptörlerinin antagonisti olan MK-801 isimli kimyasal da lokomotor hiperaktiviteye sebep olmaktadır. Sunulan bu çalışmada nöroprotektif özellikleri olduğu bilinen resveratrol ve N-asetilsistein isimli iki antioksidan kombinasyonunun farelerde MK-801'le indüklenen lokomotor hiperaktivite üzerinde etkin olan en düşük dozu araştırılmıştır. Bu amaçla 84 dişi fare eşit olarak 14 gruba bölünmüştür. Lokomotor hiperaktivite akut (1 gün ilaç uygulanması) ve sub-akut (4 gün ilaç uygulanması) faz olmak üzere iki bölümde oluşturuldu. İlaç uygulamalarından sonra hayvanların katettikleri mesafe açık alan test düzeneğinde 10 dakika boyunca kaydedilmiştir. Ölçüm sonuçlarına göre antioksidan kombinasyonunun akut fazda sub-akut faza göre başarılı olduğu gözlenmiştir. Kombinasyonun her iki düşük dozu olan 40mg/kg resveratrol + 20mg/kg NAC ve 20mg/kg resveratrol + 10mg/kg NAC'ın intraperitoneal uygulamalarının akut fazda lokomotor hiperaktiviteyi başarıyla engellediği gözlendi. Sonuç olarak antioksidan kombinasyonunun akut oluşan lokomotor hiperaktivite karşısında etkisinin olduğu sonucuna varıldı.
Anahtar Kelimeler: Antioksidan, Lokomotor Hiperaktivite, MK-801, N-asetilsistein, Resveratrol

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INTRODUCTION

The glutamate is the major neurotransmitter in the brain. The N-Methyl-D-Aspartate (NMDA) receptors are receptors which glutamate binds. Hypofunction of this receptor leads to mental illness such as schizophrenia (McArthur 2012). The hypofunction that has occurred causes an increase in locomotor activity other than schizophrenia. MK-801 is one of the NMDA receptor antagonists and its applications cause compulsive climbing, biting and falling to the side in rodents (Kruk-Slomka et al. 2016, Xiu et al. 2014, 2015, Yu et al. 2011). In addition to these symptoms, increased locomotor activity is also observed (Xiu et al. 2014, 2015, Yu et al. 2011). Since glutamate cannot bind to the NMDA receptor, it begins to accumulate in the extracellular space, thus triggering oxidative stress. The oxidative stress is the abundant production of the reactive oxygen species (ROS) in the tissues. ROS accumulation disrupts the antioxidant system and destroys nerve cells and myelin layer in the brain (Genius et al. 2013, Lin and Lane 2019, Ozyurt et al. 2007).

The combination of resveratrol and N-acetylcysteine (NAC) is known to build a defence mechanism against oxidative stress (García-Alcántara et al. 2018). The resveratrol is an organic and polyphenol non-flavonoid antioxidant. It is found in the vegetables and fruits (Gupta 2016). Since the brain contains high amounts of fat and needs oxygen, it is highly likely to be affected by oxidative stress. (Rege et al. 2013, Venturini et al. 2010). Resveratrol crosses the blood brain barrier and increases the release of endogenous antioxidant enzymes that maintain the oxidation balance within the cell (Bastianetto et al. 2015, Gerzson et al. 2014, Venturini et al. 2010). It is known that the resveratrol increases the neuronal plasticity, strengthens the memory, and improves the macular degeneration, stroke and dementia in the elderly (Bastianetto et al. 2015, Monserrat et al. 2016). However, resveratrol acts as a neuroprotector against various toxins that affect the brain and cause Alzheimer's and Parkinson's disease (Giovinazzo and Grieco 2015, Jeon et al. 2012, Pasinetti et al. 2014, Rege et al. 2013).

Besides the resveratrol, the NAC is also quite effective on the brain (Dean et al. 2004, 2011). The NAC also crosses the blood-brain barrier, protects the nerve cells and improves the demyelination (Adair et al. 2001, Dean et al. 2004, 2011, Farr et al. 2003). Immediately after intraperitoneal (i.p.) administration, NAC reaches high values in the brain and continues this for 48 hours (García-Alcántara et al. 2018). It neutralizes and reduces the ROS by boosts the endogenous antioxidant mechanism (Tardiolo et al. 2018). NAC has a healing and protective role in mental illnesses such as schizophrenia and Alzheimer's (Adair et al. 2001, Lin and Lane 2019, Dean et al. 2011, Farr et al. 2003). It also plays a role in NMDAR recovery (Himi et al. 2003, Janaky et al. 2007, Varga et al. 1997). Recent human trials of NAC have shown that it has a curative effect on the negative symptoms of schizophrenia. (Bulut et al. 2009).

In the present study, a positive control group was also formed. Clozapine used in the positive control group is an atypical antipsychotic drug. The use of this drug is in the treatment of anxiety-induced psychological illnesses. However, it is known that clozapine has an inhibitory effect on the locomotor hyperactivity initiated by NMDA receptor antagonist (Gattaz et al. 1994, Gururajan et al. 2012, Pinar et al. 2015).

In the present study, the open field test was used to determine the degree of locomotor activity in mice (Akillioglu et al. 2012, Pinar et al. 2015). The effect of antioxidants on the locomotor hyperactivity induced by MK-801 has been demonstrated in previous studies. However, no study was found to obtain a low protective dose by combining antioxidants. Therefore, the aim of this study is to try to find the lowest useful dose of antioxidant combination by inducing locomotor hyperactivity in mice.

MATERIAL and METHODS

The trial was performed on the 84 female balb/c mice. All mice were obtained from the Experimental Animal Unit of Afyon Kocatepe University after approval of the Ethical Committee for Experimental Animals of the same university (AKUHADYEK-55–18).

First, all mice were evenly divided into 14 groups and kept in quarantine for one week before the trial. They were fed ad libitum with commercial rat feed and tap water. All mice in the control group were received 10ml/kg saline intraperitoneally (i.p.) (As drugs are dissolved in this liquid). The doses of the resveratrol, NAC, clozapine and MK-801 in this study were selected from the previous studies (Atalay et al. 2017, Fukami et al. 2004, Gattaz et al. 1994, Xiu et al. 2015). The groups created are shown in Table 1.

Table 1: Information about groups and drugs. IP: intraperitoneally.

Group	Agent+Dosage	Drug Administration (Day)	Administration Way	Administration
1	Control-saline (10 ml/kg)	1	IP	Acute
2	Clozapine (5mg/kg)	1	IP	Acute
3	MK-801 (1mg/kg)	1	IP	Acute
4	Clozapine (5mg/kg) / MK-801 (1mg/kg) Positive control	1	IP	Acute
5	Resveratrol (50mg/kg)	1	IP	Acute
6	NAC (100mg/kg)	1	IP	Acute
7	Resveratrol (50mg/kg) + NAC (100mg/kg) / MK-801 (1mg/kg)	1	IP	Acute
8	Resveratrol (40mg/kg) + NAC (80mg/kg) / MK-801 (1mg/kg)	1	IP	Acute
9	Resveratrol (20mg/kg) + NAC (40mg/kg) / MK-801 (1mg/kg)	1	IP	Acute
10	Resveratrol (10mg/kg) + NAC (20mg/kg) / MK-801 (1mg/kg)	1	IP	Acute
11	Resveratrol (50mg/kg) + NAC (100mg/kg) / MK-801 (1mg/kg)	4	IP	Sub-acute
12	Resveratrol (40mg/kg) + NAC (80mg/kg) / MK-801 (1mg/kg)	4	IP	Sub-acute
13	Resveratrol (20mg/kg) + NAC (40mg/kg) / MK-801 (1mg/kg)	4	IP	Sub-acute
14	Resveratrol (10mg/kg) + NAC (20mg/kg) / MK-801 (1mg/kg)	4	IP	Sub-acute

The injections were administered to the 1, 2, 3, 5, 6th groups in the morning and after 30 minutes, they were subjected to open field testing. In the 4th group, the clozapine was injected late in the morning and the MK-801 early in the afternoon and the open field test was performed after 30 minutes. 7, 8, 9 and 10th groups received the resveratrol with NAC combination late in the morning and MK-801 early in the afternoon and they were subjected to open field testing after 30 minutes. These groups were terminated on the same day. 11, 12, 13 and 14th groups were also received resveratrol with NAC combination late in the morning and MK-801 early in the afternoon for 4 days and after the final MK-801 injection all mice were tested in the open field device.

After drug administrations all mice were placed in the open field device. This device is made of stainless steel and measures 60cmx60cmx24cm. The basement of the device was divided into 36 equal squares and the movements of the mice were recorded by video camera for 10 minutes. The motor activity degree of the mice were determined by counting the squares passed by the animal (Akillioglu et al. 2012, Al-Amin et al. 2000, Furuie et al. 2013, Kocahan et al. 2012, Xiu et al. 2014, 2015).

The data obtained from experimental animals were evaluated and analysed by SPSS 21.0 by using one-way analysis of variance (ANOVA) and was expressed as means and standard deviations. The LSD and non-parametric Kruskal-Wallis tests were performed for the analysis. A difference in the mean values of $P < 0.05$ was considered to be significant for both tests.

RESULTS

When the data of the MK-801 applied group were analysed, it was seen that MK-801 caused locomotor hyperactivity ($P < 0.05$). It was observed that the movements of the mice in the clozapine (group 2), resveratrol (group 5) and NAC (group 6) groups did not differ from the control group ($P > 0.05$). It was observed that clozapine suppressed the locomotor hyperactivity initiated by MK-801 in the group in which MK-801 was injected with clozapine (Group 4) ($P < 0.05$) (Table 2).

It was observed that the combination of antioxidants suppressed locomotor hyperactivity in groups in which MK-801 was acutely applied (groups 9 and 10) ($P < 0.05$). Especially in the 9th group, the locomotor hyperactivity value was very close to the controls. It was found that the antioxidant combination was particularly effective against acute applications of MK-801. The antioxidant combination was quite effective even at low doses (Table 2).

However, the effect of the combination was not at the desired level against sub-acute applications of MK-801 ($P > 0.05$). The values of the 11, 12 and 13th groups were better than the 14th group. However, the data of the last group was very close to that of the MK-801 group (Table 2).

Table 2: Groups and means of squares passed in open field test.

Groups	Mean	Coefficient of error	P_1; P_2
1	178.67 c	21.849	
2	175.50 c	29.095	
3	514.17 a	122.526	
4	229.17 c	53.398	
5	144.33 c	41.185	
6	128.17 c	27.952	0.000*
7	300.67 bc	72.980	0.000*
8	300.17 bc	20.243	
9	208.33 c	50.038	
10	264.17 c	52.811	
11	365.67 ab	55.892	
12	344.00 ab	65.248	
13	428.83 ab	88.521	
14	504.33 a	68.576	

[a,b,c]: In the same column values with different letters show statistically significant differences ($P < 0.05$).
P_1 is the importance level for the varians analyse.
P_2 is the importance level for the Kruskal-wallis.

DISCUSSION

The most important finding of this study was that the antioxidant combination tested was effective on locomotor hyperactivity created by using MK-801 in mice even at low doses in the acute phase. Since the accumulation of MK-801 in the body increased in the subacute phase, the effect of the antioxidant combination was weakened. In a presented study, the protective effect of caffeic acid phenethyl ester (CAPE), which is a very powerful antioxidant produced from propolis, on locomotor hyperactivity using MK-801 on rats was tested. In that study, it was observed that CAPE application suppressed locomotor hyperactivity formed in rats (Ozyurt et al. 2007). In another study, the effectiveness of melatonin hormone against increased locomotor activity using MK-801 was investigated. As it is known, melatonin hormone is a very powerful antioxidant that is secreted from the pineal gland at night and protects the tissues against oxidative stress. In that study, using the melatonin hormone showed a suppressive effect on the increased locomotor activity by MK-801 (Ozyurt et al. 2014).

1-Methyl-1,2,3,4-tetrahydroisoquinoline is a substance produced in the brain and the regulator of the dopaminergic system. The protective property of this substance, which is also a powerful neuroprotective, against the locomotor hyperactivity created by MK-801, was tested. According to the results of the open field test, it was found that this substance also had a suppressive effect on locomotor hyperactivity (Pietraszek et al. 2009). In another study presented, the protective effect of caffeine administration on the locomotor hyperactivity induced by MK-801 was observed. In that study, different doses of caffeine were tried in mice and it was observed that a dose of 1mg/kg improved locomotor hyperactivity and the symptoms completely disappeared after 1 week (De Oliveira et al. 2005).

Apart from antioxidants, regular exercise has benefits on locomotor activity. Exercise is also an important form of treatment for neurological diseases. In a study conducted to understand this, a voluntary wheel was placed in mouse cages for two weeks and it was found that exercise attenuates the locomotor hyperactivity effect created by MK-801 (Kim et al. 2014).

Clozapine, used as a positive control in this study, is an important drug used in neural diseases. Ventral hippocampus defects in newborns can cause symptoms similar to schizophrenia. To test this, MK-

801 was applied to young rat pubs with induced defects in their ventral hippocampus, and severe hyperlocomotion was observed. In this case, clozapine administration reduced the resulting hyperlocomotion to the level of the control group (Al-Amin et al. 2010).

Moreover, the resveratrol has a protective effect on the excitotoxicity induced by the NMDAR. Repeated doses of resveratrol have a curative effect on the locomotor hyperactivity induced by methamphetamine (Miller et al. 2013). Apart from the resveratrol, NAC also has an ameliorative effect on glutamatergic dysfunction. The administration of various doses of NAC has a dose-dependent suppressive effect on methamphetamine-induced acute hyperlocomotion (Fukami et al. 2004). In addition, the combination of resveratrol and NAC improved ototoxicity in the cochlea. The 5 days administration of 10 mg/kg resveratrol + 400 mg/kg NAC has the inhibitory effect on the secondary effect of the aminoglycosides in the ototoxicity (García-Alcántara et al. 2018).

CONCLUSION

In conclusion, in this study, it was tried to create locomotor hyperactivity by administrating MK-801 and the protective effect of antioxidant combination on the locomotor hyperactivity was investigated. It was determined that the combination of antioxidants applied had a protective effect against acutely induced locomotor hyperactivity at both low doses (40mg/kg resveratrol + 20mg/kg NAC and 20mg/kg resveratrol + 10mg/kg NAC). However, the combination was found to have no protective effect against MK-801 administered sub-acutely for 4 days. For the sub-acute and more advanced stages, the dose of the combination can be increased or different antioxidant combinations can be tried. As a result, it was concluded that the combination of antioxidants has an effect on acutely formed locomotor hyperactivity.

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Conflict of Interest: The authors declare that they have no conflict of interest.

KAYNAKLAR

Adair JC, Knoefel JE, Morgan N. Controlled trial of N-acetylcysteine for patients with probable Alzheimer's disease. Neurology. 2001; 57 (8): 1515-1517.

Akillioglu K, Babar Melik E, Melik E, Kocahan S. The investigation of neonatal MK-801 administration and physical environmental enrichment on emotional and cognitive functions in adult Balb/c mice. Pharmacology Biochemistry and Behavior. 2012; 102 (3): 407-414.

Al-Amin HA, Weinberger DR, Lipska BK. Exaggerated MK-801-induced motor hyperactivity in rats with the neonatal lesion of the ventral hippocampus. Behavioural Pharmacology. 2000; 11 (3-4): 269-278.

Atalay T, Gulsen I, Colcimen N, Alp HH, Sosuncu E, Alaca I, Ak H, Ragbetli MC. Resveratrol treatment prevents hippocampal neurodegeneration in a rodent model of traumatic brain injury. Turkish Neurosurgery. 2017; 27 (6): 924-930.

Bastianetto S, Ménard C, Quirion R. Neuroprotective action of resveratrol. Biochimica et Biophysica Acta - Molecular Basis of Disease. 2015; 852 (6): 1195-1201.

Bulut M, Savas HA, Altindag A, Virit O, Dalkilic A. Beneficial effects of N-acetylcysteine in treatment resistant schizophrenia. The World Journal of Biological Psychiatry. 2009; 10 (4–2): 626-628.

De Oliveira RV, Dall'Igna OP, Tort ABL, Schuh JF, Neto PF, Santos Gomes MW, Souza DO, Lara DR. Effect of subchronic caffeine treatment on MK-801-induced changes in locomotion, cognition and ataxia in mice. Behavioural Pharmacology. 2005; 16 (2): 79-84.

Dean O, Giorlando F, Berk M. N-acetylcysteine in psychiatry: Current therapeutic evidence and potential mechanisms of action. Journal of Psychiatry and Neuroscience. 2011; 36 (2): 78-86.

Dean O, van den Buuse M, Copolov D, Berk M, Bush A. N-acetyl-cysteine treatment inhibits depletion of brain glutathione levels in rats: implications for schizophrenia. International Journal of Neuropsychopharmacology. 2004; 7 (Suppl. 2): 262.

Farr SA, Poon HF, Dogrukol-Ak D, Drake J, Banks WA, Eyerman E, Butterfield DA, Morley JE. The antioxidants α-lipoic acid and N-acetylcysteine reverse memory impairment and brain oxidative stress in aged SAMP8 mice. Journal of Neurochemistry. 2003; 84 (5): 1173-1183.

Fukami G, Hashimoto K, Koike K, Okamura N, Shimizu E, Iyo M. Effect of antioxidant N-acetyl-L-cysteine on behavioral changes and neurotoxicity in rats after administration of methamphetamine. Brain Research. 2004; 1016 (1): 90-95.

Furuie H, Yamada K, Ichitani Y. MK-801-induced and scopolamine-induced hyperactivity in rats neonatally treated chronically with MK-801. Behavioural Pharmacology. 2013; 24 (8): 678-683.

García-Alcántara F, Murillo-Cuesta S, Pulido S, Bermúdez-Muñoz JM, Martínez-Vega R, Milo M, Varela-Nieto I, Rivera T. The expression of oxidative stress response genes is modulated by a combination of resveratrol and

N-acetylcysteine to ameliorate ototoxicity in the rat cochlea. Hearing Research. 2018; 358: 10-21.

Gattaz, WF, Schummer B, Behrens S. Effects of zotepine, haloperidol and clozapine on MK-801-induced stereotypy and locomotion in rats. J Neural Transm Gen Sect. 1994; 96 (3): 227-232.

Genius J, Geiger J, Dölzer AL, Benninghoff J, Giegling I, Hartmann AM, Möller HJ, Rujescu D. Glutamatergic dysbalance and oxidative stress in in vivo and in vitro models of psychosis based on chronic nmda receptor antagonism. PLoS One. 2013; 8 (7): e59395.

Gerszon J, Rodacka A, Puchała M. Antioxidant properties of resveratrol and its protective effects in neurodegenerative diseases. Advances in Cell Biology. 2014; 2: 97-117.

Giovinazzo G, Grieco F. Functional properties of grape and wine polyphenols. Plant Foods for Human Nutrition. 2015; 70 (4): 454-462.

Gupta RC. Nutraceuticals : Efficacy, safety and toxicity. UK, Academic Press-Elsevier. 2016.

Gururajan A, Taylor DA, Malone DT. Cannabidiol and clozapine reverse MK-801-induced deficits in social interaction and hyperactivity in Sprague–Dawley rats. Journal of Psychopharmacology. 2012; 26 (10): 1317-1332.

Himi T, Ikeda M, Yasuhara T, Murota SI. Oxidative neuronal death caused by glutamate uptake inhibition in cultured hippocampal neurons. J Neurosci Res. 2003; 71 (5): 679-688.

Janáky R, Dohovics R, Saransaari P, Oja SS. Modulation of [3H] dopamine release by glutathione in mouse striatal slices. Neurochem Res. 2007; 32 (8): 1357-1364.

Jeon BT, Jeong EA, Shin HJ, Lee Y, Lee DH, Kim HJ, Kang SS, Cho GJ, Choi WS, Roh GS. Resveratrol attenuates obesity-associated peripheral and central inflammation and improves memory deficit in mice fed a high-fat diet. Diabetes. 2012; 61 (6): 1444-1454.

Kim TW, Kang HS, Park JK, Lee SJ, Baek SB, Kim CJ. Voluntary wheel running ameliorates symptoms of MK-801-induced schizophrenia in mice. Molecular Medicine Reports. 2014; 10 (6): 2924-2930.

Kocahan S, Babar E, Melik E, Akillioglu K. The effect of the interaction between N-methyl-D-aspartate receptor blockade and growth environment during the last maturation period of the nervous system on anxiety-related behaviour in adulthood in the rat. Neurochemical Journal. 2012; 6 (3): 194-201.

Kruk-Slomka M, Budzynska B, Slomka T, Banaszkiewicz I, Biala G. The influence of the CB1 receptor ligands on the Schizophrenia-like effects in mice induced by MK-801. Neurotoxicity Research. 2016; 30 (4): 658-676.

Lin CH, Lane HY. Early identification and intervention of schizophrenia: Insight from hypotheses of glutamate dysfunction and oxidative stress. Frontiers in Psychiatry. 2019; 10: 1-9.

McArthur R. Translational neuroimaging 1st edititon tools for CNS drug discovery, development and treatment. ScienceDirect. 2012.

Miller DK, Oelrichs CE, Sage AS, Sun GY, Simonyi A. Repeated resveratrol treatment attenuates methamphetamine-induced hyperactivity and [3H]dopamine overflow in rodents. Neuroscience Letters. 2013; 554: 53-58.

Monserrat Hernández-Hernández E, Serrano-García C, Antonio Vázquez-Roque R, Díaz A, Monroy E, Rodriguez-Moreno A, Florán B, Flores G. Chronic administration of resveratrol prevents morphological changes in prefrontal cortex and hippocampus of aged rats. Synapse. 2016; 70 (5): 206-217.

Ozyurt B, Ozyurt H, Akpolat N, Erdogan H, Sarsilmaz M. Oxidative stress in prefrontal cortex of rat exposed to MK-801 and protective effects of CAPE. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2007; 31 (4): 832-838.

Ozyurt H, Ozyurt B, Sarsilmaz M, Kus I, Songur A, Akyol O. Potential role of some oxidant/antioxidant status parameters in prefrontal cortex of rat brain in an experimental psychosis model and the protective effects of melatonin. European Review for Medical and Pharmacological Sciences. 2014; 18 (15): 2137-2144.

Pasinetti GM, Wang J, Ho L, Zhao W, Dubner L. Roles of resveratrol and other grape-derived polyphenols in Alzheimer's disease prevention and treatment. Biochimica et Biophysica Acta - Molecular Basis of Disease. 2014; 1852 (6): 1202-1208.

Pietraszek M, Michaluk J, Romańska I, Wąsik A, Golembiowska K, Antkiewicz-Michaluk L. 1-Methyl-1,2,3,4-tetrahydroisoquinoline antagonizes a rise in brain dopamine metabolism, glutamate release in frontal cortex and locomotor hyperactivity produced by MK-801 but not the disruptions of prepulse inhibition, and impairment of working memory in rat. Neurotoxicity Research. 2009; 16 (4): 390-407.

Pinar N, Akillioglu K, Sefil F, Alp H, Sagir M, Acet A. Effect of clozapine on locomotor activity and anxiety-related behavior in the neonatal mice administered MK-801. Bosnian Journal of Basic Medical Sciences. 2015; 15 (3): 74-79.

Rege SD, Kumar S, Wilson DN, Tamura L, Geetha T, Mathews ST, Huggins KW, Broderick TL, Babu JR. Resveratrol protects the brain of obese mice from oxidative damage. Oxidative Medicine and Cellular Longevity. 2013; 419092: 2013.

Tardiolo G, Bramanti P, Mazzon E. Overview on the effects of N-acetylcysteine in neurodegenerative diseases. Molecules. 2018; 23 (12): 3305.

Varga V, Jenaei Z, Janáky R, Saransaari P, Oja SS. Glutathione is an endogenous ligand of rat brain N-methyl-D-aspartate (NMDA) and 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors. Neurochem Res. 1997; 22 (9): 1165-1171.

Venturini CD, Merlo S, Souto AA, Fernandes MDC, Gomez R, Rhoden CR. Resveratrol and red wine function as antioxidants in the central nervous system without cellular proliferative effects during experimental diabetes. Oxidative Medicine and Cellular Longevity. 2010; 3 (6): 434-441.

Xiu Y, Kong X, Zhang L, Qiu X, Gao Y, Huang CX, Chao FL, Wang SR, Tang Y. The myelinated fiber loss in the corpus callosum of mouse model of schizophrenia induced by MK-801. Journal of Psychiatric Research. 2015; 63: 132-140.

Xiu Y, Kong XR, Zhang L, Qiu X, Chao FL, Peng C, Gao Y, Huang CX, Wang SR, Tang Y. White matter injuries induced by MK-801 in a mouse model of schizophrenia based on NMDA antagonism. Anatomical Record. 2014; 297 (8): 1498-1507.

Yu J, Qi D, Xing M, Li R, Jiang K, Peng Y, Cui D. MK-801 induces schizophrenic behaviors through downregulating Wnt signaling pathways in male mice. Brain Research. 2011; 1385: 281-292.

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RESEARCH ARTICLE

Evaluation of Factors Affecting Elective Course Preferences: Example of Faculty of Veterinary Medicine Validity and Reliability Study

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ABSTRACT

This study aims to determine the factors affecting the elective course preferences of veterinary faculty students. Developing a scale (Elective Course Preference Attitude Scale) that provides an evaluation of the elective course preferences of students of veterinary medicine students is also aimed. 354 students studying at Selçuk University, Faculty of Veterinary Medicine in the 2019-2020 academic year participated in the study. With regard to constructing validity, Cronbach's alpha (α) coefficient was used for the reliability analysis. For validity analysis, factor analysis was applied. In light of the data obtained from the study, it can be suggested that the "*Elective Course Preference Attitude Scale*" is a valid and reliable tool in the evaluation of the elective course preferences of veterinary medicine students, and this scale can also be used in the evaluation of the elective course preferences of students of other departments.

Keywords: Elective course, Scale, Student, Veterinary faculty

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Seçmeli Ders Tercihlerine Etki Eden Faktörlerin Değerlendirilmesi: Veteriner Fakültesi Örneği Geçerlik ve Güvenirlik Çalışması

ÖZ

Bu çalışma ile veteriner fakültesi öğrencilerinin seçmeli ders tercihlerine etki eden faktörlerin belirlenmesi amaçlandı. Ayrıca veteriner fakültesi öğrencilerinin seçmeli ders tercihlerinin değerlendirilmesini sağlayan bir ölçeğin (Seçmeli Ders Tercihi Tutum Ölçeği) geliştirilmesi hedeflendi. Çalışmaya, Selçuk Üniversitesi Veteriner Fakültesi'nde 2019-2020 Eğitim-Öğretim döneminde öğrenim görmekte olan 354 öğrenci katıldı. Yapı geçerliliği için; güvenirlik analizlerinde Cronbach alfa (α) katsayısı kullanıldı. Geçerlik analizi için faktör analizi uygulandı. Çalışmada elde edilen veriler neticesinde "Seçmeli Ders Tercihi Tutum Ölçeği" nin veteriner fakültelerinde öğrenim görmekte olan öğrenciler için güvenilir ve geçerli olduğu; veteriner fakültesi öğrencileri seçmeli ders tercihlerinin değerlendirilmesinde kullanılabileceği gibi diğer bölümlerde öğrenim görmekte olan öğrencilerin seçmeli ders tercihlerinin değerlendirilmesinde kullanılabilecek bir ölçek olduğu da ileri sürülebilir.

Anahtar Kelimeler: Seçmeli ders, Ölçek, Öğrenci, Veteriner Fakültesi

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INTRODUCTION

In the world, the rapid change in science and technology affects people's lifestyles, the structure as well as the needs of the society, and the required human qualities and necessitates the training of individuals who are equipped in many aspects with different knowledge and skills (Durmuşçelebi and Mertoğlu 2018). In the process of training qualified manpower, one way to provide students with a better learning environment and opportunities is the elective courses, which the students can choose in accordance with their professional interests and personal skills during their university education (Dündar 2008, Durmuşçelebi and Mertoğlu 2018).

The criteria considered by the students in the selection of elective courses are generally subjective (Dündar 2008). Some subjects interest students more than the others because of several reasons. So, in return, universities offer many elective course alternatives to their students. The selection of the most suitable of the alternative courses for students is a complex decision process that requires the consideration of multiple factors and criteria (Ersöz et al. 2011). In this process, making the best choice in a situation where many criteria are at play is difficult as these criteria sometimes may be inconsistent (Kutlu et al. 2012). The findings obtained in different studies show that among the primary criteria for elective courses are contribution to professional life, course credit, and opinions on the lecturer (Tezcan and Gümüş 2008).

Allowing students to choose their own courses apart from the compulsory ones is also compatible with today's democratic understanding. Offering different alternatives to students will also enable them to develop positive attitudes towards the university. Elective courses contribute to students' cognitive (knowledge, skill) affective (interest, attitude), and social development. Students with different interests, needs, and abilities are offered different course options in the programs, and elective courses are also expected to accommodate students with the qualifications to acquire professional skills. In a world that is changing at an incredible pace, it is of great importance for students to develop their life skills in order to keep up with this rapid change (Ersöz et al. 2011).

In the light of this information, it was aimed to develop a scale that enables the evaluation and determination of the factors affecting the choice of elective courses of veterinary faculty students.

MATERIAL and METHODS

Data collection form

A data form whose power analysis was made before starting the study (96.32% power) and in the preparation of which different sources (Tezcan and Gümüş 2008, Kutlu et al. 2010) were also utilized and consisting of 28 questions was applied in person to 354 bachelor students of Selçuk University, Faculty of Veterinary Medicine between the dates 4 and 12 April 2020.

Statistical analyses

In the study, reliability and validity analyzes were made for the "*Elective Course Preference Attitude Scale*". Cronbach alpha (α) coefficient was used for reliability analysis and factor analysis to determine validity. Suitability for factor analysis was evaluated using Bartlett's test of sphericity, and the sufficiency of the sample size was evaluated using the Kaiser-Meyer-Olkin (KMO) sampling adequacy scale.

Descriptive statistics were given for categorical and continuous variables in the study. In the evaluation of the data, SPPS 25 Released 2017. IBM SPSS Statistics for Windows (Version 25.0. Armonk, NY: IBM Corp.), statistical software package was used. $p < 0.05$ and p 0.01 level was considered statistically significant

RESULTS

Of the 354 Selcuk University Faculty of Veterinary Medicine students participating in the study, 60.2% are male and 39.8% are female. While the study was participated by senior year students the most (27.7%), it was participated the least by the junior year students (10.5%) (Table 1). There are no items with a total correlation value of less than 0.20 in the data form. Therefore, since all 28 items were determined to have a high level of reliability, no items were removed (Table 2).

For the reliability of the data form, since each item of the scale is measured by using a 5-point Likert scale, in terms of Cronbach's Alpha, the form is reliable with regard to internal consistency (α) Cronbach's alpha reliability coefficient value for the 28-item data form used in the research (α) was calculated as 0.906 (Table 3).

In terms of the validity study of the data form, factor analysis was performed with the Varimax method for the data collected on the items in the form and the findings are presented in Table 4.

In the study, the Kaiser-Meyer-Olkin sampling adequacy was found to be 0.909, the chi-square value of Bartlett's sphericity 4921,612, the degree of freedom 378, and $p = 0.001$.

When the total variance was analyzed, it was determined that according to the application data, there were 5 factors for 28 items, and they explain 59,089% of the measurement made by this scale. (Table 4).

Tukey's range test was applied to obtain a total scale score by the addition of the item scores. Considering the additivity line, p was determined as > 0.05 (Table 5).

The enthalpy–entropy chart was used in the study (Figure 1). In the graph, the cut-off point of the

eigenvalues represents the 5th main component. Therefore, the basic component may not be taken by determining 5 factors. However, since the study aimed to explain a larger part of the total variability, a 5th main component was included.

In the study, no item was removed due to low factor load found in the load factor analysis. Questions 15 to 20 cover Factor 1 (Additive Factor), 21 to 25 cover Factor 2 (Personal Factor), 1 to 10 cover Factor 3 (Structural Factor), 11 to 14 cover Factor 4 (Instructor Factor), and 26 to 28 cover Factor 5 (Environmental Factor). The sub-items collected in the factors were taken into consideration in the naming of each factor. The lowest item load was determined as 0,400 and the highest item load was determined as 0.829 (Table 6).

Table 1: Socio-Demographic data

		n	**%**
CR	Term 1	90	25,4
	Term 2	74	20,9
	Term 3	37	10,5
	Term 4	98	27,7
	Term 5 (Intern)	55	15,5
Gender	Female	141	39,8
	Male	213	60,2
Total		354	100

Table 2: Item-based reliability coefficients and item-total correlation of the scale

	Average to be valid if an item is removed from the scale	**Variance to be valid if an item is removed from the scale**	**Total Item Correlations**	**Reliability to be valid if an item is removed from the scale Cronbach's α Coefficient**
[1. The "content of the course" affects my elective course choice.]	99,9605	320,078	0,535	0,902
[2. The "course selection system" affects my elective course choice.]	100,2288	326,880	0,380	0,905
[3. The "way the course is taught (traditional, student research, presentations, etc.)" affects my elective course choice.]	99,9915	317,300	0,571	0,901
[4. The "class hours (whether the class is in the morning or afternoon)" affect my elective course choice.]	100,5593	329,029	0,243	0,908
[5. The "similarity to courses I have taken and was successful at until now" affects my elective course choice.]	100,2684	320,814	0,509	0,902
[6. The "previous elective course(s)" affects my elective course choice.]	100,3136	320,403	0,491	0,903
[7. "Whether the course is applied or not" affects my elective course choice.]	100,0395	319,120	0,555	0,902
[8. "Whether the course is up-to-date" affects my elective course choice.]	100,0198	317,362	0,619	0,900
[9. "Whether the course encourages to conduct research" affects my elective course choice.]	100,4294	320,642	0,518	0,902
[10. "The view of the instructor towards absenteeism" affects my elective course choice.]	99,7316	331,851	0,230	0,908

[11. "My views about the instructor of the course" affect my elective course choice.]	99,4068	325,823	0,461	0,903
[12. "The academic career of the lecturer of the course" affects my elective course choice. (Prof. Dr. – Assoc. Prof. Dr. – Dr. Lecturer)".]	100,7345	324,609	0,355	0,906
[13. "The lecturers I consult" affect my elective course choice.]	100,8277	320,698	0,477	0,903
[14. The "examination type (written, oral, test, etc.)" affects my elective course choice.]	99,6073	328,789	0,357	0,905
[15. The "possible contribution of the course to my professional life" affects my elective course choice.]	99,6808	318,994	0,594	0,901
[16. The "possible contribution of the course to my academic development" affects my elective course choice.]	99,9181	317,503	0,592	0,901
[17. The "possible contribution of the course to my personal development" affects my elective course choice.]	99,8559	317,047	0,647	0,900
[18. The "possible contribution of the course to my knowledge of general culture" affects my elective course choice.]	99,9463	318,085	0,598	0,901
[19. The "possible contribution of the course to my theoretical knowledge" affects my elective course choice.]	100,0000	316,601	0,630	0,900
[20. The "possible contribution of the course to my practical knowledge" affects my elective course choice.]	100,0339	317,642	0,587	0,901
[21. "My personal interests" affect my elective course choice.]	99,6582	318,390	0,645	0,900
[22. "My personal skills" affect my elective course choice.]	99,9492	315,924	0,668	0,900
[23. "My expectations" affect my elective course choice.]	99,7994	320,778	0,597	0,901
[24. "My expectations about academic life" affect my elective course choice.]	100,3305	316,262	0,586	0,901
[25. The "possibility that the course will raise my grade point average" affects my elective course choice.]	99,6836	328,846	0,318	0,906
[26. The "students who have taken that course before" affect my elective course choice.]	99,7994	326,535	0,358	0,905
[27. The "courses my friends will select" affect my elective course choice.]	100,0960	329,532	0,281	0,907
[28. The "opinions of the people from the later years even if they did not take the course" affect my elective course choice.]	100,2655	325,686	0,336	0,906

Table 3: The total reliability coefficient

	Number of Item	**Cronbach's α**
Data form used in the study	28	0,906

Table 4. Data form validity coefficient

Sum of Squares of Factor Loads as a Result of Varimax Rotation			
Factor	**Total**	**% of Variance**	**Cumulative Variance %**
1	7,319	26,140	26,140
2	3,462	12,363	38,504
3	2,377	8,490	46,994
4	1,750	6,251	53,245
5	1,636	5,844	59,089

Table 5. Tukey's Test of Additivity

			Sum of Squares	**Df**	**Mean Square**	**F**	**Sig**
Between population			112,549	105	1,072		
Within population			111,317	10	11,132	31,393	,000
	Residual	Nonaddivity	,425[a]	1	,425	1,198	,274
		Balance	371,894	1049	,355		
		Total	372,319	1050	,355		
	Total		483,636	1060	,456		
Total			596,185	1165	,512		

Table 6. Factor loadings

Elective Course Preference Attitude Scale	**Factor 1: Additive Factor**	**Factor 2: Personal Factor**	**Factor 3: Structural Factor**	**Factor 4: Instructor Factor**	**Factor 5: Environmental Factor**
[1. The "content of the course" affects my elective course choice.]			0,613		
[2. The "course selection system" affects my elective course choice.]			0,557		
[3. The "way the course is taught (traditional, student research, presentations, etc.)" affects my elective course choice.]			0,572		
[4. The "class hours (whether the class is in the morning or afternoon)" affect my elective course choice.]			0,698		
[5. The "similarity to courses I have taken and was successful at until now" affects my elective course choice.]			0,400		
[6. The "previous elective course(s)" affects my elective course choice.]			0,442		
[7. "Whether the course is applied or not" affects my elective course choice.]			0,517		
[8. "Whether the course is up-to-date" affects my elective course choice.]			0,685		
[9. "Whether the course encourages to conduct research" affects my elective course choice.]			0,595		
[10. "The view of the instructor towards			0,448		

absenteeism" affects my elective course choice.]					
[11. "My views about the instructor of the course" affect my elective course choice.]			0,544		
[12. "The academic career of the lecturer of the course" affects my elective course choice. (Prof. Dr. – Assoc. Prof. Dr. – Dr. Lecturer)".]			0,647		
[13. "The lecturers I consult" affect my elective course choice.]			0,725		
[14. The "examination type (written, oral, test, etc.)" affects my elective course choice.]			0,672		
[15. The "possible contribution of the course to my professional life" affects my elective course choice.]	0,793				
[16. The "possible contribution of the course to my academic development" affects my elective course choice.]	0,813				
[17. The "possible contribution of the course to my personal development" affects my elective course choice.]	0,829				
[18. The "possible contribution of the course to my knowledge of general culture" affects my elective course choice.]	0,720				
[19. The "possible contribution of the course to my theoretical knowledge" affects my elective course choice.]	0,783				
[20. The "possible contribution of the course to my practical knowledge" affects my elective course choice.]	0,708				
[21. "My personal interests" affect my elective course choice.]		0,558			
[22. "My personal skills" affect my elective course choice.]		0,547			
[23. "My expectations" affect my elective course choice.]		0,655			
[24. "My expectations about academic life" affect my elective course choice.]		0,717			
[25. The "possibility that the course will raise my grade point average" affects my elective course choice.]		0,650			
[26. The "students who have taken that course before" affect my elective course choice.]				0,809	
[27. The "courses my friends will select" affect my elective course choice.]				0,742	
[28. The "opinions of the people from the later years even if they did not take the course" affect my elective course choice.]				0,738	

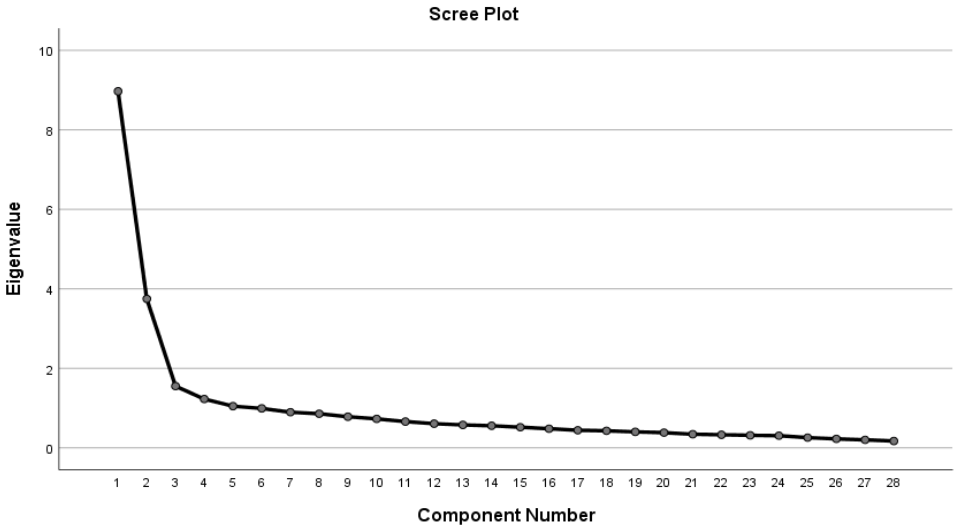

Figure 1. Elective course preferences selection attitude items

DISCUSSION and CONCLUSION

In terms of reliability analysis, the reliability coefficient as an analysis method is calculated determines the extent to which the items that make up the measurement tool are related to the entirety of the measurement tool and is used frequently in item selection (Bland and Altman 1997, Allen and Yen 2002, Bonett 2002, 2003, 2010, Cronbach and Shavelson 2004). For the construct validity of the scales, total item score analysis is used for validity as well as reliability. The item-total correlation coefficient explains the relationship between the scores obtained from the test items and the total score of the test. That the item-total correlation is positive and high indicates that the items exemplified similar behaviors and the level of internal consistency of the test is high. In a test using Likert-type rating scales, the item-total correlation is calculated by the correlation coefficient (Cronbach and Shavelson 2004). The high correlation obtained for each item indicates that the level of the relation of that item with the theoretical structure is high. In other words, it shows that the item is effective and sufficient to measure the intended behavior (Tezbaşaran 1997). Although not specified, the acceptable selection coefficient is recommended to be greater than 0.20 or even 0.25. It is stated that if deemed necessary, items with a coefficient value between 0.20-0.30 can be included or should be corrected, and items with a value of less than 0.20 should not be included (Bonett 2002). The studies in the literature state that items with a factor load value of below 0.20 should be removed from the scale (Tezbaşaran 1997). Since the items' total correlation value in the study scale was not found to be lower than 0.20, it was determined that the 28 items in the scale were of high reliability and therefore no item was removed from the prepared data form (Table 2). That none of the 28 items of the scale was removed after the item analysis can be regarded as a very positive development for the study.

Ways to calculate the reliability coefficient differ depending on the type, source, and the number of applications of the variables. The changes in the methods of calculation also change the interpretive meaning of the reliability coefficient. The reliability coefficient is the degree of the nonexistence of random errors and gives information about the amount of error in the measurement results. The reliability obtains values ranging from 0 to +1, but it is expected to be close to +1. A coefficient of reliability value of more than 0.70 is a desired result (Cronbach and Meehl 1955). Among the methods recommended in the examination of Likert scales is the Cronbach's alpha (α) technique (Cronbach and Shavelson 2004). In the study, Cronbach's alpha (α) reliability coefficient was found to be 0.906 (Table 3). Considering that this coefficient is above 0.80, it can be said that the study data form is very functional.

The construct validity measured by the factor analysis method is defined as showing the degree of accuracy of the indicators of the theoretical structure to be measured (Balcı 1995, Dempsey and Dempsey 2000). If the KMO value obtained before factor analysis is below 0.50, it means that the sample size is insufficient, and if the value is between 0.60-0.69 it is deemed to be sufficient (Kaiser 1974, Cerny and Kaiser 1977). However, in order for the sample size to sufficient, the results of Bartlett's sphericity test should be statistically significant as well (Kaiser 1958, 1974). The chi-square value of Bartlett's sphericity test measures the suitability of the data for factor analysis. The higher this value, the more suitable the dataset is for factor analysis (Barlett and Fowler 1937). In this study, the KMO value before the factor analysis was found to be 0.909 and the chi-square value of Bartlett's sphericity test was found to be $\chi 2$ = 4921,612. These results were found to be

statistically significant ($p<0.01$), which shows that the values obtained in the study were suitable for factor analysis ($p<0.05$). It can also be said that the results of the factor analysis show that the structural validity of the scale was achieved.

In order to obtain a total scale score by the addition of the item scores, Tukey's range test is applied (Tukey 1949). It is seen after the test that the range value of the study was $p>0.05$ (Table 5), and it can be said that the scale is suitable for obtaining a total scale score by the addition of the item scores.

While the eigenvalues of the variables are used to determine the number of factors to be created in the development of a scale, and so is the enthalpy–entropy chart proposed by Cattell and Raymond (1966). It is also stated that the enthalpy-entropy graph is more successful than other methods in creating factors. Due to this feature, the enthalpy-entropy graph was used to determine the number of factors in the study (Figure 1). However, it can be said that since the study aimed to explain a larger part of the total variability, a 5th main component was included.

In a study conducted by Örs Özdil and Kınay (2015) titled "*Scaling 5th Grade Elective Course Preferences with Rank-Order Judgments*", a 15-item elective course list offered to the 5th-grade students was given to the 4th-grade students of private and public schools in Ankara affiliated with the Ministry of National Education, and they were asked to score these courses. It is seen that scaling was made according to the scoring performed in the study. Yaşar (2014) conducted a study titled "*Developing an Attitude Scale Related to Scientific Research Methods Course*" with students of Pamukkale University, Faculty of Education in the academic year 2011-2012. In the study, 20 questions and 4 sub-factors were determined. One of the factors includes the dimensions of "*Daily Life and Occupational Relations*". Kılınç and Salman (2007) developed a 20-item School Experience Lessons Scale of Attitude (ODDTÖ) in the study titled "*Developing an Attitude Scale towards the Lessons of School Experience*" conducted with students in the departments of Mathematics, Physics, Chemistry, and Biology. In the study, a scale consisting of 28 questions and 5 factors was developed (Table 6). It is seen in the literature that no scale studies were conducted on the attitude of veterinary medicine students in specific and university students in general towards elective course preferences. The data obtained with the study shows that the developed scale can be used to investigate the elective course preferences of veterinary medicine students, and it can also be used to evaluate elective course preferences of students in other departments.

In conclusion, the research findings suggest that the "*Elective Course Preference Attitude Scale*" is reliable and valid to be used with veterinary medicine students and can be used to evaluate the reasons for students' elective course preferences.

Ethics Committee Approval: Selcuk University, Faculty of Veterinary Medicine, Ethic Committee, 27.02.2020 dated, 2020/24 numbered of decision.

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REFERENCES

Allen MJ, Yen WM. Introduction to Measurement Theory. IL: Waveland Press, Long Grove. 2002.

Balcı A. Sosyal Bilimlerde Araştırma Yöntem, Teknik ve İlkeler. Ankara Üniversitesi Eğitim Bilimleri Fakültesi, Ankara. 1995.

Bartlett MS, Fowler RH. Properties of sufficiency and statistical tests. Proc. R. Soc. Lond. 1937; A160:268–282.

Bland JM, Altman DG. Statistics notes: Cronbach's alpha. BMJ. 1997; 314(7080):572.

Bonett DG. Sample size requirements for testing and estimating coefficient alpha. Journal of Educational and Behavioral Statistics.2002; 27:335–340.

Bonett DG. Sample size requirements for comparing two alpha reliability coefficients. Applied Psychological Measurement. 2003; 27:72–74.

Bonett DG. Varying coefficient meta-analytic methods for alpha reliability. Psychological Methods. 2010; 15:368–385.

Cattell RB, Raymond B. The scree test for the number of factors. Multivariate Behavioral Research. 1966; 1(2):245–276.

Cerny CA, Kaiser HF. A study of a measure of sampling adequacy for factor-analytic correlation matrices. Multivariate Behavioral Research. 1977; 12(1):43-47.

Cronbach LJ, Meehl PE. Construct validity in psychological tests. Psychological Bulletin. 1955; 52(4):281–302.

Cronbach LJ, Shavelson RJ. My current thoughts on coefficient alpha and successor procedures. Educational and Psychological Measurement. 2004; 64(3):391–418.

Dempsey PA, Dempsey AD. Using nursing research process, critical evaluation and utilization. 5th Edition, Lippincott, Philadelphia-New York, 2000.

Durmuşçelebi M, Mertoğlu B. Ortaokul öğrencilerinin eğitiminde seçmeli derslerin yeri. Uluslararası Toplum Araştırmaları Dergisi Gençlik Araştırmaları Özel Sayısı. 2018; 8:170-211.

Dündar S. Ders seçiminde analitik hiyerarşi proses uygulaması. Süleyman Demirel Üniversitesi İİBF Dergisi. 2008; 13(2):217-226.

EARGED (2008): Seçmeli derslerin seçim kriterlerinin değerlendirilmesi araştırması. Milli Eğitim Bakanlığı. Available at https://www.meb.gov.tr/earged/earged/secmeli_dersler_arastirmasi.pdf Accessien date: 01.05.2020

Ersöz F, Kabak M, Yılmaz Z. Lisansüstü öğrenimde ders seçimine yönelik bir model önerisi. Afyon Kocatepe Üniversitesi, İİBF Dergisi. 2011; 8(2):227-249.

Kaiser HF. The varimax criterion for analytic rotation in factor analysis. Psychometrika. 1958; 23(3):187-200.

Kaiser HF. An index of factor simplicity. Psychometrika. 1974; 39(1):31–36.

Kılınç A, Salman S. Okul deneyimi derslerine yönelik tutum ölçeği geliştirilmesi. GÜ Gazi Eğitim Fakültesi Dergisi. 2007; 27(1): 23-35.

Kutlu BS, Abalı YA, Eren T. Çok ölçütlü karar verme yöntemleri ile seçmeli ders seçimi. Sosyal Bilimler. 2012; 2(2):5-25.

Örs Özdil S, Kınay E. 5. Sınıf ders tercihlerinin sıralama yargıları kanunuyla ölçeklenmesi. Eğitimde ve Psikolojide Ölçme ve Değerlendirme Dergisi. 2015; 6(2):268-278.

Tezbaşaran A. Likert tipi ölçek geliştirme klavuzu. 2. Baskı. Türk Psikologlar Derneği Yayınları, Ankara, 1997.

Tezcan H, Gümüş Y. Üniversite öğrencilerinin seçmeli ders tercihlerine etki eden faktörlerin araştırılması. GÜ, Gazi Eğitim Fakültesi Dergisi, 2008; 28(1): 1-17.

Tukey JW. One degree of freedom for non-additivity. Biometrics. 1949; 5(3):232–242.

Yaşar M. Bilimsel araştırma yöntemleri dersine yönelik tutum ölçeği geliştirme çalışması: Geçerlik ve güvenirlik. EBAD-JESR. 2014; 4(2): 109-129.

APPENDIX 1: Elective Course Preference Attitude Scale (Turkish)

Seçmeli Ders Tutum Ölçeği

1. Seçmeli ders tercihimde "dersin içeriği" etkili oluyor.
2. Seçmeli ders tercihimde "ders seçme sisteminin" etkisi oluyor.
3. Seçmeli ders tercihimde "dersin işlenme biçimi (geleneksel anlatım, öğrenci araştırması, öğrenci sunumu vb)" etkili oluyor
4. Seçmeli ders tercihimde "ders saatleri (sabah veya öğleden sonra olması)" etkili oluyor.
5. Seçmeli ders tercihimde "şimdiye kadar almış olduğum ve başarılı olduğum derslere yakın olması" etkili oluyor.
6. Seçmeli ders tercihimde "daha önce almış olduğum seçmeli ders/ler" etkili oluyor.
7. Seçmeli ders tercihimde "dersin uygulamalı olup olmaması" etkili oluyor.
8. Seçmeli ders tercihimde "alacağım dersin güncel olup olmaması" etkili oluyor.
9. Seçmeli ders tercihimde "dersin araştırmaya teşvik edici olup olmaması" etkili oluyor.
10. Seçmeli ders tercihimde "devamsızlık sorunu olmaması" etkili oluyor.
11. Seçmeli ders tercihimde "dersi veren öğretim üyesi hakkındaki görüşlerim" etkili oluyor.
12. Seçmeli ders tercihimde "dersi veren öğretim üyesinin akademik kariyeri (Prof.Dr.–Doç.Dr.–Dr.Öğr.Üyesi)" etkili oluyor.
13. Seçmeli ders tercihimde "danıştığım öğretim üyeleri" etkili oluyor.
14. Seçmeli ders tercihimde "dersi veren öğretim üyelerinin sınav sistemi (yazılı, sözlü, test vb)" etkili oluyor.
15. Seçmeli ders tercihimde "meslek hayatıma katkı sağlayabilecek olması" etkili oluyor.
16. Seçmeli ders tercihimde "akademik gelişimime katkı sağlayabilecek olması" etkili oluyor.
17. Seçmeli ders tercihimde "dersin kişisel gelişimime katkı sağlayabilecek olması" etkili oluyor.
18. Seçmeli ders tercihimde "dersin genel kültürüme katkı sağlayabilecek olması" etkili oluyor.
19. Seçmeli ders tercihimde "teorik bilgilerimi artırabilecek olması" etkili oluyor.
20. Seçmeli ders tercihimde "pratik becerilerimi artırabilecek olması" etkili oluyor.
21. Seçmeli ders tercihimde "kişisel ilgilerim" etkili oluyor.
22. Seçmeli ders tercihinde "kişisel yeteneklerim" etkili oluyor.
23. Seçmeli ders tercihimde "kişisel beklentilerim" etkili oluyor.
24. Seçmeli ders tercihimde "akademik hayatla ilgili beklentilerim" etkili oluyor.
25. Seçmeli ders tercihimde "not ortalamamın artabilecek olması" etkili oluyor.
26. Seçmeli ders tercihimde "daha önce o dersi almış olan öğrenciler" etkili oluyor.
27. Seçmeli ders tercihimde "arkadaş çevremin seçeceği dersler" etkili oluyor.
28. Seçmeli ders tercihimde "o dersi almasa da üst sınıf öğrencilerin görüşleri" etkili oluyor.

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RESEARCH ARTICLE

A Method Validation Procedure for Some Quality Parameters in Goat Milk

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ABSTRACT

Goat milk has great importance in human health and nutrition. It may be used to manufacture wide variety of products due to its chemical characteristics. In this study, an easy and quick method for the analysis of fat, fat-free dry matter (FFDM), lactose, protein percentage, electrical conductivity, freezing point and density in milk was validated. The repeatability values determined by the operators were 4.65 and 4.68 for lactose; 3.10 for protein, 4.38 and 4.33 for fat; 8.52 and 8.55 for FFDM. The same values for reproducibility were 4.78; 3.17 and 3.18; 4.38 and 4.39; 8.73 and 8.75, respectively. There was no significant difference between the data obtained by the operators in all parameters subject to the study (P>0.05). Horwitz ratio (HorRat) was used as comparison for reproducibility. HorRat values are required to be less than 2. HorRat values determined in all parameters measured in this study were between 0.25 and 0.94. Finally, the expanded uncertainty and the combined standard uncertainty were calculated. By the way, the present study provided a fast, and reliable protocol for these analysis. Further research is needed to gain knowledge on the suitability and advantages of the usage of validated method approach for different goat milk components.

Keywords: Goat, Milk composition, Method validation

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Keçi Sütünde Bazı Kalite Parametreleri İçin Metot Validasyonu

ÖZ

Keçi sütü insan sağlığı ve beslenmesinde büyük öneme sahiptir. Kimyasal özelliklerinden dolayı, keçi sütü, çok çeşitli ürünlerin üretiminde kullanılabilir. Bu çalışmada, Şam keçisinin sütündeki yağ, yağsız kuru madde (YKM), laktoz, protein oranı, elektriksel iletkenlik, donma noktası ve yoğunluk analizi için kolay ve hızlı bir yöntem geçerli kılınmıştır. Operatörler tarafından tespit edilen tekrarlanabilirlik değerleri, laktoz için 4.65 ve 4.68; protein için 3.10; yağ için 4.38 ve 4.33; YKM için 8.52 ve 8.55 olmuştur. Aynı değerler tekrarüretilebilirlik için sırasıyla, 4.78; 3.17 ve 3.18; 4.38 ve 4.39; 8.73 ve 8.75 olarak tespit edilmiştir. Çalışmaya konu olan tüm parametrelerde, operatörler tarafından elde edilen veriler arasındaki farklılık önemli olmamıştır (P>0.05). Horwitz oranı (HorRat) tekrar üretilebilirlik için karşılaştırma yapılmasında kullanılmıştır. HorRat değerlerinin 2'den küçük olması istenir. Bu çalışmada ölçülen tüm parametrelerde belirlenen HorRat değerleri 0.25 ile 0.94 arasında olmuştur. Son olarak, her bir parametre için birleşik belirsizlik ve genişletilmiş belirsizlik hesaplanmıştır. Böylelikle, bu çalışma, bazı süt kalite parametrelerinin analizleri için hızlı ve güvenilir bir protokol sağlamıştır. Geçerli kılınmış yöntem yaklaşımının kullanımının uygunluğu ve avantajları hakkında bilgi edinmek amacıyla, farklı keçi sütü bileşenleri için daha fazla araştırmaya ihtiyaç vardır.

Anahtar Kelimeler: Keçi, Süt kompozisyonu, Metot validasyonu

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INTRODUCTION

Thousands of tests and analysis are needed every day in different laboratories around the world. The cost of these analytical methods is too high and they are mostly time consuming (Magnusson 2014). Therefore, for an analytical method, it is very important to be suitable for its intended use (Hopfgartner 2020). On the other hand, since the decision taken based on the result must be sufficiently reliable, the method must ensure that every result of the measurement is close enough to the unknown correct result. (González and Herrador 2007). If method performance is validated and the uncertainty on the result, at a given level of confidence, estimated, an analytical result is accompanied by indication of the correct results and data quality (Magnusson 2014).

Method validation is an essential component of the analytical measurements for the laboratories to produce analytical data of high quality with confidence (Mohamed et al., 2020). Validation procedure demonstrates that an analytical method is appropriate for purpose and evaluates risks of measurements (Anonymous 2005). It provides precision knowledge and experience of performing the method. The analytical requirements, critical steps in the process are clearly defined and method capabilities are confirmed by validation (Magnusson 2014). Thus the steps necessary, the specific matrices, the reference standard and the reagents will be used should be described in details to perform each analytical test (Mohamed et al., 2020).

Milk and milk products are essential food sources dependent on all of the basic nutrients they have (Niero et al., 2017; Lu et al., 2020). People with cow's milk allergy consume goat milk because of its known beneficial and therapeutic effects on them (Ribeiro et al., 2010). Goat milk's digestibility is higher than cow milk (Luna et al., 2008; Schettino et al., 2017; Mazzaglia et al., 2020). These nutritional, healthy and therapeutic benefits increase goat milk's and its products's importance for the human health and also for markets (Silanikove et al., 2010; Serhan et al., 2016).

Due to growing commercial interest in production and characterization of goat milk, the accuracy of its composition has great importance (Costa et al., 2015). Besides their effects on health, milk composition influence technological traits of milk. Extensive milk products attributes milk composition variability (Franzoi et al., 2018). For goat milk, its tolerance for technological processes, its properties (being healthy, secure, hygienic) and its nutritional value, sensory attributes may define its quality (Ribeiro et al., 2010). Non-fat solids in goat milk, provide satisfactory curd tension (Martín-Diana et al., 2003). Fat and proteins are one of the most important components of goat milk in terms nutritional quality. Goat dairy products's color and flavor are affected from its lipid ratio (Niero et al., 2017). In addition, carbohydrates could be an excellent substitute for human milk carbohydrates (Slačanac et al., 2010). Since milk quality is significant for manufacturing dairy products in high quality, a successful strategy for measuring parameters are needed.

Investigation of these components separately is difficult, because it implies time consuming and more expensive methodologies. Moreover, it would be more difficult to attribute to analysis. Since, cow milk is mainly used for manufacturing traditional milk products (Mazzaglia et al., 2020), there are lots of research in which cow milk is a matrice used and the methods used for the measurements of its characteristics are validated. However, although, goat milk production is a growing industry, their validation for milk quality parameters are very limited. Therefore, the aim of this study was to validate a simple, robust, fast and cost-effective method for the determination of fat, fat-free dry matter (FFDM), lactose, protein percentage, electrical conductivity, freezing point and density of goat milk.

MATERIALS and METHODS

Sample Collection and Parameter Measurement

This study was conducted in the laboratory of Genetic Department, Faculty of Veterinary Medicine, Hatay Mustafa Kemal University. In the study, a total of 4.5 liters of milk sample were collected from 10 Damascus goats. Animals were 2-4 years old. Generally, health conditions of the goats were good and they did not have mastitis. Milk sample was taken during routine milking procedure in 4-5th months of the lactation. The goats spent the day on the pasture and the night on the pen, and they always had access to fresh water in both the pasture and the pen. During morning milking, samples were collected, brought to the laboratory by a cold chain of +4 °C in approximately 15 minutes. Before studied, collected milk was divided into 30 subsamples of 150 ml and stored at +4 °C during validation process. For each measurement, only the subsamples were used were taken and then analyzed for fat, fat-free dry matter (FFDM), lactose, protein percentage, electrical conductivity, freezing point, density (Milkotester Master Classic LM2 P1 - Bulgaria).

Method Validation Process

EURACHEM guidelines was used for method validation parameters (Magnusson 2014). In this study, repeatability and reproducibility were considered for the method precision. Repeatability was conducted as soon as milk samples are brought to the laboratory. 16 measurements on 16 subsamples, were separately processed within the

same day by 2 different operators who handled 8 subsamples each. Since the device manual declares a measurement accuracy of 48 hours, reproducibility study was carried out within a period of 48 hours. For reproducibility, 14 measurements on 14 subsamples, were separately processed after 0, 8, 16, 24, 32, 40, and 48 hours by 2 different operators who handled 7 subsamples each.

Estimation of measurement uncertainty

The uncertainty U (P) was obtained by identifying, quantifying and combining all individual contributions to uncertainty (Eq. 1). The relative expanded uncertainty, uncertainty expressed as a relative standard deviation, was calculated by using the covarage factors (k), repeatability and reproducibility, of 2 at 95 % confidence level.

$$U(P) = \sqrt{(\text{repeatability})^2 + (\text{reproducibility})^2} \quad (1)$$

Statistical analysis

Repeatability was calculated as the relative standard deviation (RSD_r) of measurements within the same day. Similarly, reproducibility was calculated as the relative standard deviation (RSD_R) of measurements obtained across the different times of analyses, as proposed in EURACHEM guidelines (Magnusson 2014). Also, the Horwitz ratio (HorRat) was calculated for comparison of reproducibility (Eq. 2).

$$\text{HorRat} = \frac{RSD_R}{PRSD_R} \quad (2)$$

The Horwitz ratio (HorRat) is a normalized performance parameter indicating the acceptability of methods of analysis with respect to among-laboratory precision (reproducibility) (Horwitz and Albert 2006). Student t-test was used in SPSS 22.0 package program to test the significance of differences between operators in term of milk quality parameters. $P<0.05$ was accepted as the level of significance.

RESULTS AND DISCUSSION

In order to confirm that a method is suitable for certain applications and that it provides reliable outcomes, method validation performance parameters must be evaluated and complied with certain legal requirements (Leite et al., 2020). In this study, several guidance documents on the performance of analytical methods with different concepts used to confirm the validation parameters.

The precision of the method for the determination of fat, FFDM, lactose, protein percentage and electrical conductivity, freezing point, density of milk were assessed through repeatability relative standard deviation (RSD_r) and reproducibility relative standard deviation (RSD_R). RSD is the ratio between the standard deviation and mean.

All of the quality parameters measured for the purpose of repeatability of milk in the study were within the values of goat reported in the literature (Yakan et al., 2019; Slačanac et al., 2010) (Table 1). In addition, there was no statistically significant difference between operator results. The good precision both within and between days reached in the present study could be partly due to the success of the operators in their own data as a result of paying attention the steps of the measurements, like clearness of equipments, time needed for the measurement etc.

Values of RSD_r and RSD_R showed good precision both within and between days (Table 1). When the RSD_r values for 1st and 2nd operator's results were calculated, it was determined that the RSD_r values were 0.42 and 0.43; 1.18 and 0.87; 0.92 and 1.02; 0.93 and 1.88; 0.48 and 0.64; 0.43 and 0.48 for freezing point, lactose, protein, fat, FFDM and density, respectively. On the other hand, freezing point RSD_R were 0.96 and 1.08; electrical conductivity RSD_R were 0.83 and 0.84; lactose RSD_R were 1.10 and 1.08; protein RSD_R were 1.29 and 1.32; fat RSD_R were 0.93 and 0.46; FFDM RSD_R were 0.79 and 1.02; density RSD_R were 0.83 and 1.18 as a result of 1st and 2nd operator's reproducibility measurements respectively. Overall, the results of the present study were acceptable according to the IUPAC Technical Report (Thompson et al., 2006).

In the study, HorRat was used as comparison for reproducibility of the experimental and the expected RSDR (Franzoi et al., 2018). HorRat values should be in the desirable range (<2) (Horwitz and Albert 2006). All values obtained for milk quality parameters in this study were below the desired range of <2. This confirmed the accuracy of measurements of validation.

During the validation study of the analytical procedure, the precision uncertainty sources had been thoroughly investigated. Both uncertainties were combined to obtain a representative or single estimation of precision uncertainty. Precision uncertainty U (P) values were as 0.0046, 0.0055, 0.0065, 0.0069, 0.0074, 0.0045, and 0.0047 for freezing point, electrical conductivity, lactose, protein, fat, FFDM, and density of milk, respectively. Finally,

the expanded uncertainty was calculated by multiplying the combined standard uncertainty with a coverage factor of 2 with a confidence level of 95 % and the values are summarized in Table 2.

The uncertainty values are so important for the exact results of the parameters. When, a result of lactose is 4.67% for example, it should be evaluated that, it is 4.67%±0.0130 actually. Another example, protein percentage, should be considered as 3.15%±0.0139 while the measurement value is 3.15. This is very important while reporting the analysis.

Table 1: Precision Data of Measured Parameters (Means±%RSD)

Parameter	Repeatability		P Values	Reproducibility		P Values	HorRat
	1st Operator	2nd Operator		1st Operator	2nd Operator		
Freezing Point (°C)	0.58±0.42	0.58±0.43	0.06	0,59±0.96	0.59±1.08	0.85	0.55
E. Conductivity (mS/cm)	4.98±0.82	4.97±1.04	0.14	4.92±0.83	4.88±0.84	0.18	0.81
Lactose (%)	4.65±1.18	4.68±0.87	0.06	4.78±1.10	4.78±1.08	0.78	0.82
Protein (%)	3.10±0.92	3.10±1.02	0.84	3.17±1.29	3.18±1.32	0.90	0.81
Fat (%)	4.38±0.93	4.33±1.88	0.21	4.38±0.93	4.39±0.46	0.34	0.25
FFDM (%)	8.52±0.48	8.55±0.64	0.53	8.73±0.79	8.75±1.02	0.59	0.83
Density (kg /m^3)	28.27±0.43	28.45±0.48	0.06	29.04±0.83	29.04±1.18	0.91	0.94

RSD: Relative Standard Deviation; FFDM: Fat-free Dry Matter; E. Conductivity: Electrical Conductivity

Table 2: Precision Uncertainties, Combined Standard Uncertainty and Expanded Uncertainty of Measured Parameters

Parameter	Repeatability Uncertainty	Reproducibility Uncertainty	Combined Standard Uncertainty	Expanded Uncertainty
Freezing Point (°C)	0.0019	0.0042	0.0046	0.0092
E. Conductivity (mS/cm)	0.0043	0.0034	0.0055	0.0109
Lactose (%)	0.0047	0.0044	0.0065	0.0130
Protein (%)	0.0044	0.0053	0.0069	0.0139
Fat (%)	0.0068	0.0030	0.0074	0.0148
FFDM (%)	0.0026	0.0037	0.0045	0.0091
Density (kg /m^3)	0.0021	0.0042	0.0047	0.0093

FFDM: Fat-free Dry Matter; E. Conductivity: Electrical Conductivity

CONCLUSION

In conclusion, the present study provided a fast, reliable and succesfully validated protocol for analysing fat, FFDM, lactose, protein percentage, electrical conductivity, freezing point and density of goat milk. The method showed good precision which acceptable under the validation criteria of EURACHEM guidelines and IUPAC Technical Report. The proposed method can effectively apply for the routine analysis of the parameters studied in this study. Further research is needed to gain knowledge on the suitability and advantages of the usage of validated method approach for different goat milk components.

REFERENCES

Anonymous. ICH Harmonised Tripartite Guide: Validation of analytical procedures: text and methodology. Q2 (R1) 2005; 1:1-15.

Costa MP, Frasao BS, Silva ACO, Freitas MQ, Franco RM, Conte-Junior CA. Cupuassu (Theobroma grandiflorum) pulp, probiotic, and prebiotic: Influence on color, apparent viscosity, and texture of goat milk yogurts. Int. J. Dairy Sci. 2015; 98(9):5995-6003.

Franzoi M, Niero G, Penasa M, Cassandro M, De Marchi M. Development and validation of a new method for the quantification of soluble and micellar calcium, magnesium, and potassium in milk. Int. J. Dairy Sci. 2018; 101(3):1883-1888.

González AG, Herrador MÁ. A practical guide to analytical method validation, including measurement uncertainty and accuracy profiles. Trac-Trend Anal Chem 2007; 26(3):227-238.

Hopfgartner G. Bioanalytical method validation: How much should we do and how should we document? Anal. Bioanal. Chem. 2020; 412:531-532

Horwitz W, Albert R. The Horwitz ratio (HorRat): A useful index of method performance with respect to precision. J AOAC Int. 2006;89(4):1095-109.

Leite M, Freitas A, Silva AS, Barbosa J, Ramos F. Maize (Zea mays L.) and mycotoxins: A review on optimization and validation of analytical methods by liquid chromatography coupled to mass spectrometry. Trends Food Sci Tech 2020; 99:542-565

Lu J, Zhang Y, Song B, Zhang S, Pang X, Sari RN, Lv J. Comparative analysis of oligosaccharides in Guanzhong and Saanen goat milk by using LC–MS/MS. Carbohydr. Polym. 2020; 235:115965.

Luna P, Bach A, Juárez M, De La Fuente MA. Effect of a diet enriched in whole linseed and sunflower oil on goat milk fatty acid composition and conjugated linoleic acid isomer profile. Int. J. Dairy Sci. 2008; 91(1):20-28.

Magnusson B, Örnemark U. Eurachem Guide: The fitness for purpose of analytical methods – A laboratory guide to method validation and related topics, 2nd Ed., Eurachem. 2014.

Martín-Diana AB, Janer C, Peláez C, Requena T. Development of a fermented goat's milk containing probiotic bacteria. Int. Dairy J. 2003; 13(10):827-833.

Mazzaglia A, Legarov V, Giaquinta R, Lanza CM, Restuccia C. The influence of almond flour, inulin and whey protein on the sensory and microbiological quality of goat milk yogurt. LWT 2020; 124:109138.

Mohamed R, Zainudin BH, Yaakob AS. Method validation and determination of heavy metals in cocoa beans and cocoa products by microwave assisted digestion technique with inductively coupled plasma mass spectrometry. Food Chem 2020; 303:125392.

Niero G, Penasa M, Currò S, Masi A, Trentin AR, Cassandro M, De Marchi M. Development and validation of a near infrared spectrophotometric method to determine total antioxidant activity of milk. Food Chem 2017; 220:371-376.

Ribeiro AC, Ribeiro SDA. Specialty products made from goat milk. Small Ruminant Res 2010; 89(2-3):225-233.

Schettino B, Vega S, Gutiérrez R, Escobar A, Romero J, Domínguez González-Ronquillo M. Fatty acid profile of goat milk in diets supplemented with chia seed (Salvia hispanica L.). Int. J. Dairy Sci. 2017; 100(8):6256-6265.

Serhan M, Mattar J, Debs L. Concentrated yogurt (Labneh) made of a mixture of goats' and cows' milk: Physicochemical, microbiological and sensory analysis. Small Ruminant Res 2016; 138:46-52.

Silanikove N, Leitner G, Merin U, Prosser CG. Recent advances in exploiting goat's milk: quality, safety and production aspects. Small Ruminant Res 2010; 89(2-3):110-124.

Slačanac V, Božanić R, Hardi J, Rezessyné Szabó JUDIT, Lučan M, Krstanović V. Nutritional and therapeutic value of fermented caprine milk. Int. J. Dairy Technol. 2010; 63(2):171-189.

Thompson M, Ellison SL, Wood R. The International Harmonized Protocol for the proficiency testing of analytical chemistry laboratories (IUPAC Technical Report). Pure Appl. Chem. 2006; 78(1):145-196.

Yakan A, Özkan H, Şakar AE, Ateş CT, Ünal N, Koçak Ö, Özbeyaz C. Milk yield and quality traits in different lactation stages of Damascus goats: Concentrate and pasture based feeding systems. Ankara Üniv Vet Fak Derg 2019; 66: 117-29.

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RESEARCH ARTICLE

Influence of Proanthocyanidin on Motility and Osmotic Resistance Parameters of Merino Ram Sperm During Short Term Storage

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ABSTRACT

The activity of *proanthocyanidin* oligomers is approximately 50 times greater than that of vitamin C and vitamin E in terms of antioxidant action. The aim of this study is to investigate the effects of different doses proanthocyanidin adding to extender on ram sperm motility, membran integrity and viability test, during liquid storage of ram semen. Ejaculates were collected from four Merino rams using an electroajaculator once a week and this process was repeated six times in non-breeding season. Ejaculates were split into four aliquots and diluted to a final concentration of $150x10^6$ spermatozoa/ml with the base extender containing proanthocyanidin (10, 50 and 100 µg/ml) and no additive (control). Diluted semen samples were transferred and stored at +4°C and sperm motility, membrane integrity and viability was analysed in determined intervals during three days storage period. Motility was evaluated subjectively by a phase contrast microscopy at 37°C. Membrane integrity and viability was analysed in hypo-osmotic resistance test with eosin staining test. After 24, 48 and 72 h, 10 µg / ml *proanthocyanidin* treatment sperm samples presented higher motility and HOST/E test results than control group ($P < 0.05$) in ram semen stored at +4°C. In conclusion, it was determined that the doses of 10 µg / ml of *proanthocyanidin* added to extender in +4 ^{0}C storage of ram sperm showed a protective effect compared to the control group.

Key Words: Ram semen, Proanthocyanidin, Motility, Short term storage, Osmotic resistance

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Kısa Süreli Saklanan Merinos Koç Spermasında Proantosiyanidin Motilite ve Ozmotik Direnç Parametreleri Üzerine Etkisi

ÖZ

Proantosiyanidin oligomerlerinin aktivitesi, antioksidan etki açısından C vitamini ve E vitaminine göre yaklaşık 50 kat daha fazladır. Bu çalışmanın amacı, koç spermasının kısa süreli saklanması sırasında, farklı dozlardaki sulandırıcıya eklenen proantosiyanidin, koç spermatozoon motilitesi, membran bütünlüğü ve canlılığı üzerine etkilerini araştırmaktır. Ejakülatlar, sezon dışında dört Merinos koçtan elektroajakülatör yardımıyla haftada bir kez toplandı ve bu işlem altı kez tekrarlandı. Ejakülatlar dört eşit parçaya bölünerek proantosiyanidin (10, 50 ve 100 ug / ml) içeren ve hiçbir katkı maddesi içermeyen (kontrol) sulandırıcılar ile nihai ml'de $150x10^6$/ml spermatozoa olacak şekilde sulandırıldı. Sulandırılan sperma örnekleri + 4 ° C'de 72 saat süresince saklandı ve spermatozoa motilitesi, membran bütünlüğü ve canlılığı üç günlük saklama süresi boyunca değerlendirildi. Motilite, 37 ° C'de faz kontrast mikroskop ile sübjektif olarak değerlendirildi. Membran bütünlüğü ve canlılığı, eozin boyama testi ile hipoozmotik direnç testinde (HOST/E) değerlendirildi. 24, 48 ve 72 saat sonra, + 4 ° C'de saklanan koç spermasında 10 µg / ml proantosiyanidin ile muamele edilmiş spermatozoon örnekleri kontrol grubuna göre daha yüksek motilite ve HOST / Eosin test sonuçları göstermiştir ($p < 0,05$). Sonuç olarak koç sperminin +4 ^{o}C'de saklanmasında sulandırıcıya eklenen 10 µg / ml proantosiyanidin kontrol ve diğer antioksidan gruplarına göre daha iyi bir koruyucu etki gösterdiği belirlendi.

Anahtar Kelimeler: Koç sperması, Proantosiyanidin, Motilite, Kısa Süreli Saklama, Ozmotik Direnç

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GİRİŞ

Spermanın kısa süreli (likit saklama) saklanması sperma ısısının belirli seviyeye kadar düşürülmesi (0-5°C veya 10-15°C) ve bu düzeylerde spermatozoanın geri dönüşümlü olarak inaktive edilmesi esasına dayanır. Bazı bilim insanları likit saklama için en uygun sıcaklıkların 10-15°C olduğunu, diğer bir grup ise, koç ve boğa spermalarının canlılıklarını devam ettirebilmeleri için 0-5°C arasında değerlerin daha iyi saklama aralıkları olduğunu iddia etmişlerdir (Saloman ve Maxwell 2000, Johnson ve ark. 2000, Huo ve ark. 2002). Koçlarda sperma kalitesindeki düşüşün en önemli nedenlerinden biri oksidatif hasardır. Spermanın saklanması sırasında serbest radikaller ve reaktif oksijen türleri (ROS) üretilir, ortaya çıkan ROS lipid peroksidasyona ve spermatozoonun normal fizyolojik fonksiyonunun bozulmasına yol açar (Aitken 2017). Koç spermasında çoklu doymamış yağ asitlerinin yoğunluğu diğer türlerdekinden daha fazladır, dolayısıyla koç spermatozoonu oksidatif hasara karşı duyarlıdır (Gündoğan ve ark. 2010). Yapılan çalışmalarda, sperma sulandırıcılarına antioksidanların eklenmesinin, serbest radikallerin ve ROS'un etkili bir şekilde atılmasına neden olduğu, böylece spermatozoonu oksidatif hasardan koruyarak spermatozoon kalitesini artırdığı ve spermanın saklama süresini uzattığı bildirilmektedir (Kasimanickam ve ark. 2011, Zakošek ve ark. 2017).

Oligomerik proantosiyanidin (OPC'ler) olarak da bilinen proantosiyanidinler, flavonoidler denen geniş ailenin bir parçasıdırlar ve bunlar üzüm çekirdeği ekstraktı ve çam kabuğu ekstraktında bulunmaktadır. OPC iki ila beş polimere sahiptir ve suda oldukça iyi çözünürler (Brillouet ve ark. 2017). OPC'ler güçlü antioksidanlardır. Etkili hidrojen bağı ve eşleşmemiş bir elektronun yer değiştirmesi, OPC'lere güçlü bir antioksidan özelliği sağlamaktadır (Han ve ark. 2016). Bu proantosiyanidinlerin serbest radikalleri inhibe ederek askorbik asit ve E vitamininden daha etkin antioksidan özelliğe sahip olduğu (Nandakumar ve ark. 2008) ve serbest radikallerin neden olduğu hücre lipidleri, proteinler ve DNA hasarını engellediği belirtilmektedir (Bagchi ve ark. 1997). LDL seviyesini düşürüp birçok hastalığın gelişimini yavaşlattığı, (Garavaglia ve ark. 2016) oligomerik bileşikleri, serbest radikallere ve oksidatif strese karşı geniş bir biyolojik, farmakolojik ve terapötik aktivite spektrumuna sahip olduğu söylenmektedir (Bagchi ve ark. 2000). Üzüm zarı, antosiyaninler ve flavonolları içerirken çekirdek ise; flavan-3-ol monomerleri ve gallik asit türevleri içerir. Flavan-3-ol basit monomerleri (kateşin ve izomeri epikateşin); oligomer ve polimer moleküllerini oluşturur (Nunes ve ark. 2016). Alkhedaide ve ark. (2016). OPC'lerin güçlü bir antioksidan olduklarını ve ratları kadminyum kaynaklı testis disfonksiyonlarına karşı etkili bir şekilde koruduğunu ortaya koymuşlardır. Day ve ark. (1997). üzüm özlerinin, A, C ve E vitaminleri gibi diğer antioksidanların etkinliğini artırdığını bildirdi. Serbest radikal temizleyici olarak kat kat daha güçlü oldukları için, bu vitaminleri diğer işlevlerini yerine getirmek üzere serbest bırakırlar. Koç spermasının likit olarak ya da dondurularak saklanmasında sulandırıcıya antioksidan özelliğinden yararlanmak amacıyla ilave edilen proantosiyanidin ile ilgili herhangi bir çalışmaya rastlanmamış olması sunulan çalışmanın özgünlüğünü ortaya koymaktadır.

Bu çalışma farklı yoğunlukta sulandırıcısına katılan proantosiyanidinin koç spermasının kısa süreli saklanmasında (0., 24., 48. ve 72. saat) spermatozooa motilite ve ozmotik direnç parametreleri üzerine olan etkileri belirlemek amacıyla yapılmıştır.

MATERYAL VE METOT

Araştırmamızın materyalini A.K.Ü Hayvancılık Uygulama ve Araştırma Merkezi bünyesindeki 2-3 yaşlı 4 adet Merinos koçlar oluşturdu. Damızlık hayvanlar arasından, fenotipik olarak ırk özelliklerini yansıtanlar çalışma için belirlendi. Seçilen koçlar androlojik açıdan muayene edildi. Genital organlara ait herhangi bir patolojik lezyon olmadığı kontrol edildi. Koçlar yarı açık besi şartlarında tane-kaba yem karışık olarak beslendi. Koçlardan aşım sezonu dışında haftada bir kez altı hafta boyunca sperma elektroejakülatör yardımıyla alındı (Hafez 1987). Temel sulandırıcı Tris içine değişik yoğunluklarda proanthocyanidin (10, 50 ve 100 μg/ml) ve antioksidansız (kontrol) 4 grup oluşturuldu. Spermalar koçlardan alındıktan sonra bir tüpte birleştirilerek spermatolojik muayeneleri yapıldıktan sonra 4'e ayrıldı. Sperma örnekleri önceden hazırlanmış sulandırıcı grupları ile ml'de $150x10^6$ olacak şekilde sulandırıldı. Spermalar 5ºC'de 2-2,5 saat ekilibrasyondan sonra 5 ºC'de 0. saat motilite ve membran bütünlüğü değerlendirmeleri yapıldıktan sonra 5ºC'de muhafaza edilip 24., 48. ve 72. saat motilite ve membran bütünlüğü değerlendirmeleri yapılıp kaydedildi. Çalışma süresi boyunca bu işlem 6 kere yapıldı.

Spermatolojik Muayeneler

Motilite muayeneleri sıcaklığı ayarlanmış ısıtma tablalı faz kontrast mikroskop yardımıyla subjektif olarak değerlendirildi (Demirci 2002).

Hipo-ozmotik şişme testinin Eosin ile birlikte uygulandığı HOS/E test, 37°C'taki 100 mOsm'luk HOST solüsyonundan 1 ml alınıp üzerine sperma numunesinden 10 μl eklenerek eosin boyası ilave edilip karışım 37°C'lik su banyosunda 30 dk. inkübasyona bırakılması şeklinde yapıldı. Spermatozoon baş kısmının tamamın ya da bir bölümünün boya alıp almamasına ve kuyruktaki çeşitli şekillerdeki kıvrılma veya şişmeye olup olmamasına göre değerlendirildi.

I. Kuyruk şişmiş, baş boya almamış HOS+/E-
II. Kuyruk şişmemiş, baş boya almamış HOS-/E-
III. Kuyruk şişmiş, baş boya almış HOS+/E+
IV. Kuyruk şişmemiş, baş boya almış HOS-/E+ (Gündoğan ve ark. 2010)

İstatistiksel Analiz

Shapiro–Wilk testi sonucu verilerin normal olarak dağıldı görüldü ve elde ettiğimiz bulguların istatistiksel analizinde tek yönlü varyans analizi (ANOVA) ile yapıldı. Post-hoc Duncan testi gruplar arası farkın önemini belirlemek için uygulandı. Analizler SPSS (13.0) paket programında gerçekleştirildi.

BULGULAR

0. Saat Spermatozoon Motilite ve Membran Bütünlüğü Değerleri

0. saatte motilite ve HOS/E test değerleri Tablo 1' de sunuldu. Motilite oranlarında gruplar arasında istatistiksel olarak bir fark olmadığı gözlemlenmiş, H+E- oranlarında ise 10 µg / ml ve 100 µg / ml gruplardaki artış kontrol grubuna göre istatistiki olarak önemli bulunmuştur ($p<0,05$).

24. Saat Spermatozoon Motilite ve Membran Bütünlüğü Değerleri

Motilite oranlarında gruplar arasında istatistiksel olarak bir fark olmadığı gözlemlenmiş (Tablo 2), H+E- oranlarında ise 10 µg / ml ve 100 µg / ml gruplardaki artış kontrol grubuna göre istatistiki olarak önemli bulunmuştur ($p<0,05$).

48. Saat Spermatozoon Motilite ve Membran Bütünlüğü Değerleri

Motilite ve HOS/E test oranları Tablo 3' de sunuldu. Motilite oranlarında 10 µg / ml'lik gruptaki artış kontrol ve diğer gruplara göre istatistiki açıdan önemli bulundu ($p<0,05$). H+E- oranları açısından kontrole göre tüm antioksidan içeren gruplardaki artış istatistiki açıdan önemli bulundu ($p<0,05$).

72. Saat Spermatozoon Motilite ve Membran Bütünlüğü Değerleri

Tablo 4' de motilite ve HOS/E test oranları verilmiştir. Motilite oranlarında 10 µg / ml'lik gruptaki artış kontrol ve diğer gruplara göre istatistiki açıdan önemli bulundu ($p<0,05$). H+E- oranları açısından kontrole göre 10 µg / ml ve 50 µg / ml gruplardaki artış istatistiki açıdan önemli bulundu ($p<0,05$).

Tablo 1. Çalışmada 0. saatte elde edilen ortalama motilite ve HE test oranları ($\overline{X} \pm$ SEM, n:6).

Table 1. Mean motility and HE test obtained at 0. h in the study ($\overline{X} \pm$ SEM, n:6).

Gruplar	Motilite (%)	H+/E- (%)	H-/E- (%)	H+/E+ (%)	H-/E+ (%)
Kontrol	83,3±2,10	68,2±0,80^{b}	10,5±0,56^{ab}	11,8±0,79^{a}	9,1±0,70^{b}
10 µg/ml	86,6±2,10	73,5±0,76^{a}	10,0±0,36^{b}	10,0±0,85^{ab}	6,5±0,42^{c}
50 µg/ml	83,3±2,58	67,3±0,66^{b}	12,8±1,35^{a}	8,3±0,84^{b}	11,5±0,61^{a}
100 µg/ml	81,6±1,66	72,5±1,66^{a}	10,3±0,55^{b}	8,3±0,49^{b}	8,8±1,07^{b}

a-b: Her bir sütun içerisinde farklı harf taşıyan değerler arasındaki farklar istatistiki açıdan önemlidir ($P < 0.05$)

Tablo 2. Çalışmada 24. saatte elde edilen ortalama motilite ve HE test oranları ($\overline{X} \pm$ SEM, n:6).

Table 2. Mean motility and HE test obtained at 24. h in the study ($\overline{X} \pm$ SEM, n:6).

Gruplar	Motilite (%)	H+/E- (%)	H-/E- (%)	H+/E+ (%)	H-/E+ (%)
Kontrol	75,0±2,23^{ab}	62,0±1,52^{c}	16,0±0,96^{b}	11,6±1,33^{a}	10,3±1,56
10 µg/ml	78,3±1,66^{a}	67,8±0,47^{ab}	11,8±0,79^{c}	11,1±1,07^{a}	9,1±0,94
50 µg/ml	73,3±2,10^{ab}	64,8±0,79^{bc}	19,5±0,42^{a}	7,3±0,55^{b}	8,3±0,84
100 µg/ml	71,6±1,67^{b}	68,8±1,60^{a}	14,0±0,44^{b}	7,0±1,00^{b}	10,1±1,22

a-c: Her bir sütun içerisinde farklı harf taşıyan değerler arasındaki farklar istatistiki açıdan önemlidir ($P < 0.05$)

Tablo 3. Çalışmada 48. saatte elde edilen ortalama motilite ve HE test oranları ($\overline{X}$ ± SEM, n:6).

Table 3. Mean motility and HE test obtained at 48. h in the study ($\overline{X}$ ± SEM, n:6).

Gruplar	Motilite (%)	H+/E- (%)	H-/E- (%)	H+/E+ (%)	H-/E+ (%)
Kontrol	65,0±2,23^{b}	55,6±0,66^{b}	15,3±1,11^{b}	16,5±1,17^{a}	12,5±1,23^{a}
10 µg/ml	76,6±2,10^{a}	63,0±0,96^{a}	20,6±0,55^{a}	7,5±0,76^{b}	9,3±0,80^{b}
50 µg/ml	66,6±2,11^{b}	61,3±1,02^{a}	19,3±1,11^{a}	9,6±0,42^{b}	9,6±0,21^{b}
100 µg/ml	63,3±2,10^{b}	60,5±0,76^{a}	16,1±0,94^{b}	9,8±0,79^{b}	13,5±1,08^{a}

a-b: Her bir sütun içerisinde farklı harf taşıyan değerler arasındaki farklar istatistiki açıdan önemlidir (P < 0.05)

Tablo 4. Çalışmada 72. saatte elde edilen ortalama motilite ve HE test oranları ($\overline{X}$ ± SEM, n:6).

Table 4. Mean motility and HE test obtained at 72. h in the study ($\overline{X}$ ± SEM, n:6).

Gruplar	Motilite (%)	H+/E- (%)	H-/E- (%)	H+/E+ (%)	H-/E+ (%)
Kontrol	55,0±2,23^{b}	53,5±0,76^{c}	19,1±0,70^{ab}	13,5±0,22^{a}	14,1±1,01^{a}
10 µg/ml	68,3±1,66^{a}	59,8±0,70^{a}	21,5±0,76^{a}	8,3±1,11^{b}	10,3±0,98^{b}
50 µg/ml	58,3±3,07^{b}	57,3±1,62^{ab}	21,4±0,56^{a}	8,5±0,56^{b}	11,0±1,23^{ab}
100 µg/ml	55,0±2,23^{b}	55,5±0,76^{bc}	18,0±1,31^{b}	12,0±1,57^{a}	14,5±1,33^{a}

a-c: Her bir sütun içerisinde farklı harf taşıyan değerler arasındaki farklar istatistiki açıdan önemlidir (P < 0.05)

TARTIŞMA

Bu çalışmada, sperma saklama süresi arttıkça, koç spermasının kalitesi azalma eğiliminde olmuştur. Bu azalma, spermatozoa oksidatif hasarıyla açıklanabilir. Diğer türlere oranla koç spermatozoasının, düşük kolesterol-fosfolipid membranına oranına sahip olması spermatozoayı oksidatif hasara daha duyarlı hale getirmektedir (Gündoğan ve ark. 2010). Spermanın kısa süreli saklanmasında en önemli sperma kalite kriterlerinden biri de spermatozoon motilitesidir. Oksidatif stres ise spermatozoon motilitesinin değerlendirilmesindeki en önemli belirleyicidir. Koç sperma sulandırıcısına antioksidan ilave etmek spermatozoonların oksidayona maruz kalmasını önemli ölçüde engellerken aynı zamanda da spermanın saklanması sırasında spermatozoonların motilitesinin artmasına yardımcı olmaktadır. Bu çalışmada, OPC'nin eklenmesi, diğer antioksidanların değerlendirildiği önceki çalışmaların sonuçlarına benzer spermatozoon motilitesini etkili bir şekilde arttırmıştır (Bucak ve Tekin 2006, Tuncer ve ark. 2010, Avdatek ve Gündoğan M. 2018, Güngör ve ark., 2019). Membran bütünlüğü spermatozoon metabolizmasının yanı sıra kapasitasyon, akrozom reaksiyonu olayının oluşmasında gerekli olan bir unsurdur. Ozmotik toleransın ve ölü-canlı spermatozoon oranının birlikte ele alındığı HOS/E test, fertilitenin belirlenmesinde kullanılan önemli sperma kalite testlerinden biri olarak değerlendirilmektedir (Avdatek ve ark. 2018). Çalışmamızda sulandırıcıya OPC'nin eklenmesi, diğer antioksidanların değerlendirildiği önceki çalışmaların sonuçlarına uygun olarak spermatozoon membran bütünlüğünü önemli bir şekilde koruduğunu gözlemledik. Saklama sürecinin uzaması spermada serbest radikaller ve ROS'un artışına bu artışın da eşik değeri aşması sonucu oksidatif strese neden olduğu, bu durumunda spermatozoa plazma membranının yapısındaki doymamış yağ asitlerinin peroksidasyonuna neden olduğu bilinmektedir.

Çalışmamızın 0., 24., 48. ve 72. saat bulgularına bakıldığında farklı konsantrasyonlar değişik zaman dilimlerinde motilite ve membran bütünlüğü üzerine olumlu etki yaptı ancak özellikle 10 µg/ml proantosiyanidin ilave edilen grup hem motilite hem de membran bütünlüğü yönünden değerlendirildiğinde kontrol ve diğer gruplara göre spermanın kısa süreli saklanmasında etkili olduğu belirlendi.

Sunulan çalışmamızı destekler nitelikte, Wen ve ark. (2019). teke spermasının kısa süreli saklanmasında sulandırıcıya farklı dozlarda (10, 30, 50 ve 70 mg/L) üzüm çekirdeği ekstresi proantosiyanidin ilave ederek 120 saate kadar spermatolojik parametreler ve diğer antioksidan parametreleri değerlendirdikleri çalışmalarında 30 mg/L ilave edilen grubun gerek kontrol gerekse diğer gruplara göre spermatozoon motilitesini, akrozom ve membran bütünlüğü artırdığını bildirmektedirler. Li ve ark. (2018) Guanzhong-Black domuz sperma sulandırıcısına farklı konsantrasyonlarda oligomerik proantosiyanidin ilave ettikleri kısa süreli saklama çalışmalarında 50

µg/mL ilave edilen proantosiyanidinin spermatozoon motilitesi ve membran bütünlüğünü daha iyi koruduğunu bildirmektedirler.

Sperma sulandırıcısına proantosiyanidin ilave edilmesi sonucu spermatozoon motilitesinde ve membran bütünlüğündeki artışın en önemli nedeni proantosiyanidinlerin çok zengin antioksidan içeriğinden kaynaklandığı, üzümden elde edilen proantosiyanidin gibi flavonoidler zararlı serbest radikalleri oldukça etkili bir şekilde temizlediği, bu bileşiklerin hücre zarlarına gömülü olan yağlar ve LDL kaynaklı lipid peroksidasyon hasarının azaltmasında yararlı olduğu vurgulanmaktadır (Evans ve ark. 1996, Puiggròs ve ark. 2005). Bagchi ve ark. (1997) proantosiyadimlerin vücuda saniyeler içinde ve çok hızlı bir şekilde nüfuz ettiğini serbest radikal temizleyici olarak çok daha güçlü olduklarını böylece A, C ve E vitaminleri gibi diğer antioksidanların etkinliğini arttırarak bu vitaminlerin diğer işlevlerini yerine getirmeleri için onları serbest bıraktığını da bildirmektedir.

Çalışmamızla aynı yönde farklı türlerde yapılan çalışmalarda, Al-Daraji (2012) kısa süreli saklamak için horoz sperma sulandırıcılarına ilave ettiği farklı üzüm bileşenleri içeren çalışmasında proantosiyanidin ilavesinin spermatozoon motilitesini artırdığını bildirmektedir. Zhao ve ark. (2014) ratlarda intraperitonal olarak verdikleri cisplatin (DDP) ile indüklenmiş toksisitede gavaj yoluyla koruyucu madde olarak 200 ve 400 mg proantosiyanidin uyguladıkları çalışmalarında özellikle 400 mg proantosiyanidin verilen gurubun cisplatin kaynaklı testis toksisitesini azalttığı ayrıca azalan spermatozoon motilitesini ve yoğunluğunu artırdığı bununla beraber artan anormal spermatazoon sayısını da azalttığını bildirmektedirler. Long ve ark. (2017). erkek farelere 10 gün boyunca günlük 75 ile 150 mg/kg arasında değişen miktarlarda mide içi proantosiyanidin verildikten sonraki 11. günde 40 mg/kg Zearalenone verdikleri çalışmalarında proantosiyanidinin spermatozoon kalitesini artırdığını ve testiste Zearalenone sebep olduğu toksisiteyi azalttığını bildirmektedirler. Su ve ark. (2011). üzüm çekirdeği proantosiyanidinin ekstresi'nin Nikel kaynaklı apoptoz ve oksidatif stresi dengeleyerek ratlarda spermatozoon motilitesini artırdığını belirtmektedir. Attia ve ark. (2010) farelerde yaptıkları çalışmalarında proantosiyanidinlerin doksorubisin ile indüklenen mutagenezin azaltılmasında ve en azından kısmen radikal süpürücü aktivitelerinde bulunan farelerin germinal hücrelerinde hücre proliferasyon değişikliklerinin azaltılmasında koruyucu bir role sahip olduğunu bildirmektedirler. Farklı türlerde farklı uygulama yöntemlerine göre alınan sonuçların elde edilen bulgularımızla uyumlu olması proantosiyanidinin seçkin antioksidan özelliğini değişik ortamlarda dahi gösterebildiği yönünde yorumlanmıştır.

SONUÇ

Araştırmamızda sperma sulandırıcısına ilave ettiğimiz farklı yoğunluktaki proantosiyanidinin 10 µg/ml dozunda spermatozoon motilitesi ve membran bütünlüğü üzerine iyileştirici etkisi olduğu düşünülmektedir.

TEŞEKKÜR

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Çıkar Çatışması: Yazarlar, çıkar çatışması olmadığını beyan eder.

KAYNAKLAR

Aitken R.J. Reactive oxygen species as mediators of sperm capacitation and pathological damage. Mol. Reprod. Dev., 2017, 84: 1039–1052.

Al-Daraji H. The Use of Certain Grape Constituents for Improve Semen Quality and Storage Ability of Diluted Roosters' Semen. Am. J. PharmTech Res., 2012, 2(5): 308-322.

Attia S.M., Bakheet A.S., Al-Rasheed N.M. Proanthocyanidins produce significant attenuation of doxorubicin-induced mutagenicity via suppression of oxidative stress. Oxidative Medicine and Cellular Longevity, 2010, 3(6): 404-413.

Avdatek F., Gündoğan M. Effects of Some Antioxidant Additives on Spermatological Parameters, Oxidative Stress and DNA Damage After Freezing-Thawing Process in Ram Semen. F.U. Vet. J. Health Sci., 2018, 32 (2): 135–142.

Avdatek F., Yeni D., Gündoğan M. Merinos Koçlarda Spermaya Katılan Antioksidanların Kısa süreli Saklama Sırasında Spermatolojik Parametreler ve DNA Hasarı Üzerine Etkileri. Kocatepe Vet J., 2018, 11(2): 126-133.

Bagchi D., Bagchi M., Stohs S.J., Das D.K., Ray S.D., Kuszynski C.A., Joshi S.S., Pruess H.G. Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention. Toxicol, 2000, 148: 187–197.

Bagchi D., Garg A., Krohn R.L., Bagchi M., Tran M.X., Stohs S.J. Oxygen free radical scavenging abilities of vitamins C and E, and a grape seed proanthocyanidin extract *in vitro*. Research Communications in Molecular Pathology and Pharmacology, 1997, 95(2): 179-189.

Brillouet J., Fulcrand H., Carrillo S., Roumeas L., Romieu C. Isolation of native proanthocyanidins from grapevine (Vitis vinifera) and other fruits in aqueous buffer. J. Agric. Food Chem., 2017, 65: 2895–2901.

Bucak M.N., Tekin N. Protective effect of taurine, glutathione and trehalose on the liquid storage of ram semen. Small Ruminant Res, 2006, 73: 103-108.

Day A.P., Kemp H.J., Bolton C., Hartog M., Stansbie D. Effect of concentrated red grape juice consumption on

serum antioxidant capacity and low – density lipoprotein oxidation. Ann Nutr Metab, 1997, 41: 353-357.

Demirci E. Evcil hayvanlarda reprodüksiyon, Suni tohumlama ve androloji ders notları (1nd ed). F.Ü.Vet.Fak., 2002, Ders Teksiri No:53 Elazığ.

Evans R., Miller N.J., Paganga G. Structure –antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Bio Med, 1996, 20: 933-956.

Garavaglia J., Markoski M.J., Oliveira A., Marcadenti A. Grape Seed Oil Compounds: Biological and Chemical Actions for Health. Nutrition and Metabolic Insights, 2016, 9: 59-64.

Gundogan M., Yeni D., Avdatek F., Fidan A.F. Influence of sperm concentration on the motility, morphology, membrane and DNA integrity along with oxidative stress parameters of ram sperm during liquid storage. Anim Reprod Sci., 2010, 122: 200-207.

Güngör, Ş., İnanç M.E., Ata A. Effect of gallic acid on ram semen spermatological parameters at +4 °C storage. Eurasian Journal of Veterinary Sciences 2019, 35 (2), 87-92.

Hafez, E.S.E. Semen Evaluation (5 Ed) In: Hafez, E. S. E. (Ed) Reproduction in farm animals. Philadelphia. Lea and Fabiger. 1987.

Han M., Song P., Chang H., Rezaei A., Farrar S., Brown, M.A., Xi, M. Dietary grape seed proanthocyanidins (GSPs) improve weaned intestinal microbiota and mucosal barrier using a piglet model. Oncotarget, 2016, 7: 80313-80236.

Huo L.J., Yue K.Z., Yang Z.M. Characterization of viability, mitochondrial activity, acrosomal integrity and capacitation status in boar sperm during in vitro storage at different ambient temperatures. Reprod Fert Develop., 2002, **14:** 509–514.

Johnson L.A., Weıtze K.F., Fıser P., Maxwell W.M.V. Storage of boar semen. Anim Reprod Sci., 2000; **62:** 143–172.

Kasimanickam R., Kasimanickam V., Tibary A., Pelzer K. Effect of semen 344 extenders on sperm parameters of ram semen during liquid storage at 4°C. 345 *Small Ruminant Res*, 2011, 99: 208-213.

Li Q., Shaoyong W., Li Y., Chen M., Hu Y., Liu B., Yang G., Hu J. Effects of oligomeric proanthocyanidins on quality of boar semen during liquid preservation at 17 °C. Animal Reproduction Science, 2018, 98: 47–56.

Long M., Yang S., Zhang Y., Li P., Han J., Dong S., Chen X., He J. Proanthocyanidin protects against acute zearalenone-induced testicular oxidative damage in male mice. Environ Sci Pollut Res., 2017, 24: 938–946.

Nandakumar V., Singh T., Katiyar S.K. Multi-targeted prevention and therapy of cancer by proanthocyanidins. Cancer Letters, 2008, 269.

Nunes M.A., Pimentel F., Costa A.S.G., Alvers R.C., Oliveria M.B.P.P. Cardioprotective properties of grape seed proanthocyanidins: An update. Trends in Food Science & Technology, 2016, 57: 31-39.

Puiggròs F., Llópiz N., Ardévol A., Bladé C., Arola L., Salvadó M.J. Grape seed procyanidins prevent oxidative injury by modulating the expression of antioxidant enzyme systems. J. Agric. Food Chem., 2005, 53: 6080–6086.

Saloman S., Maxwell W.M.C. Storage of ram semen. Anim. Reprod. Sci., 2000; **62 (1-3):** 77-111.

Su L., Deng Y., Zhang Y., Li C., Zhang R., Sun Y. Protective e_ects of grape seed procyanidin extract against nickel sulfate-induced apoptosis and oxidative stress in rat testes. Toxicol. Mech. Methods, 2011, 21: 487–494.

Tuncer P.B., Bucak M.N., Büyükleblebici S., Sarıözkan S., Yeni D., Eken A., Akalın P.P., Kinet H., Avdatek F., Fida A.F., Gündoğan M. The effect of cysteine and glutathione on sperm and oxidative stress parameters of post-thawed bull semen. Cryobiol, 2010, 61: 303-307.

Wen F., Li Y., Feng T., Du Y., Ren F., Zhang L., Han N., Ma S., Li F., Wang P., Hu J. Grape Seed Procyanidin Extract (GSPE) Improves Goat Sperm Quality When Preserved at 4 °C. Animals, 2019, 9: 810; doi:10.3390/ani9100810

Zakošek P.M., Mrkun J., Nemec S.A., Zrimšek P. Improvement of liquid stored boar semen quality by removing low molecular weight proteins and supplementation with α-tocopherol. Anim. Reprod. Sci., 2017, 186: 52–61.

Zhao Y., Gao L., Zhang H., Guo J., Guo P. Grape seed proanthocyanidin extract prevents DDP-induced testicular toxicity in rats. Food Funct., 2014, 5: 605–611.

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RESEARCH ARTICLE

Morphohistometric Evaluation of Embryonic Development of Spleen in Chicken

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ABSTRACT

The aim of this study was to evaluate the morphohistometric development of chick spleen by considering specific embryonic periods. For the study, spleens obtained from 18 Babcock White Leghorn chick embryos on the 13th, 16th and 21st days of incubation were used. The sections were stained with Crossmon's trichrome stain and Pappenheim's panoptic stain and differential leukocyte counts were made in the blood smears. In the measurements, an increase in the spleen volume, embryo weight and vitellus sac weight were determined. There was an increase between the 13th and 16th–21st days in spleen volume. The highest heterophil granulocytes (74.83%) and lowest lymphocyte ratio (23%) were found on the 21st day. On the 13th day, there were very few lymphocytes around the vessels. On the 16th day, arteria centralis were frequently encountered and periarteriolar lymphoid tissue formation with lymphocyte accumulations around them started to develop in the spleen parenchyma. The red and white pulp areas could be easily distinguished in splenic parenchyma on the 21st day. It was concluded that the structures characterised by lymphocyte infiltrations in the spleen parenchyma were formed and caused changes in the number of lymphocytes in peripheral blood during the embryonal period.
Key Words: Chicken, Embryonic Development, Spleen Morphohistometry, Spleen Volume

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Civcivlerde Dalağın Embriyonik Gelişiminin Morfohistometrik Değerlendirmesi

ÖZ

Bu çalışmada, civcin dalağının belirli embriyonik dönemler göz önüne alınarak morfohistometrik gelişiminin değerlendirilmesi amaçlanmaktadır. Çalışmada, kuluçkanın 13., 16. ve 21. günlerinde 18 Babcock White Leghorn civciv embriyosundan elde edilen dalaklar kullanıldı. Rutin histolojik incelemeler ve hacim hesaplamaları için doku kesitleri Crossmon trikrom boyası ve Pappenheim'ın panoptik boyası ile boyandı ve civciv embriyolarından alınan kan örneklerinden de formül lokösitleri çıkarıldı. Ölçümler sonucunda dalak hacminde, embriyo ağırlığında ve vitellus kese ağırlığında bir artış tespit edildi. 13. ve 16.-21. günler arasında dalak hacminde fark tespit edildi. 21. gün kanındaki en yüksek lökosit oranı % 74.83 ile heterofil granülositlerde gözlenirken; en düşük lenfosit oranı % 23 olarak bulundu. Kuluçka 13. günündeki dalak kesitlerindeki damarların etrafında çok az sayıda lenfosit vardı. 16. gün kesitlerinin dalak paranşimasında sıklıkla arteryal centralis ile karşılaşıldı ve etraflarında lenfosit birikimli periarteriolar lenfoid doku oluşumunun gelişmeye başladığı görüldü. 21. günün dalak parankiminde kırmızı ve beyaz pulpa bölgelerinin kolayca ayırt edilmekteydi. Embriyonik dönemdeki dalağın parankiminde lenfosit infiltrasyonları ile karakterize edilen yapıların şekillendiği ve periferik kandaki lenfosit sayısında değişimlerin olduğu sonucuna varıldı.
Anahtar Kelimeler: Civciv, Embriyonik Gelişim, Dalak Morfohistometrisi, Dalak Hacmi

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INTRODUCTION

The spleen, which has between-species morphological differences, is known as the largest peripheral lymphoid organ due to its function such as role in immunity, blood production, filtration and storage (Song et al. 2012). In chickens, the globular shaped spleen has an important role in the interaction of lymphoid and non-lymphoid cells in the incubation period of the poultry without lymph node due to their haematopoietic functions in the fetal period (Rajput et al. 2013). The development of embryonic spleen, which starts with mesenchymal cell accumulation in the first 48 hours of incubation, continues with the formation of sinusoiuds in mesenchymal tissue on the 5th day of incubation. Erythropoiesis begins on the 7th day of incubation and granulopoiesis starts on the 11th day. Splenic development is completed in the first 10 weeks after incubation following antigenic stimulation (Olah and Vervelde 2008, Liman and Bayram 2011).

The spleen is surrounded by a capsule consisting of fibroelastic tissue, smooth muscle cells and has a closed circulation, but no trabecular structure is developed and there is also no marginal zone (Kannan et al. 2015, Khenenou et al. 2018, Aka and Eren 2019). Lienal artery continues as central artery in the pulp and branches to the penicillary arterioles into capillaries associated with venous sinuses (Olah and Vervelde 2008). The spleen is morphologically and functionally composed of white and red pulp regions. The white pulp is material made up of T and B lymphocyte deposits around arterioles and the red pulp is composed of spleen sinuses and spleen cords which are the area of blood filtering (Steiniger 2005, Cesta 2006). In the white pulp of the chicken spleen, periarterial lymphoid tissue (PAL) or periarterial lymphoid sheath (PALS) is found around a. centralis, where T lymphocytes are located (Olah and Vervelde 2008, Liman and Bayram 2011). In addition, perivenous lymphoid tissue (PVL) around the vena, lymphoid tissue around the ellipsoid with dense B lymphocytes (PEL/PELT), subcapsular lymphoid tissue (SCL) under capsule and germinal centres (GC) with B lymphocyte regions are other structures seen in white pulp (Aka and Eren 2019). The germinal centres of the chicken spleen are seen only after antigenic stimulation and are located only as secondary follicles (Yasuda et al. 2003). The immune response to blood-borne antigen reactions starts with the white pulp area located around a. centralis (Cesta 2006). Lymphoid and non-lymphoid cells are found in the red pulp (Olah et al. 2012).

The aim of this study was to evaluate the morphohistometric development of the chick spleen by considering specific embryonic periods. It might be important in terms of creating a model for embryological studies in humans by using chick embryos during incubation.

MATERIALS and METHODS

For the study, spleens obtained from 18 Babcock White Leghorn chick embryos on the 13th, 16th and 21st days of incubation were used. The research was approved by The Ethical Committee of Faculty of Health Sciences of Karamanoglu Mehmetbey University (2019/10-04).

First, the weights and pre-hatch weights of the eggs were recorded (Table 1). Then six embryos randomly selected in each group were fixed in buffered 10% formal saline (pH 7.4) for a week, dehydrated in alcohol, cleared in xylene and paraffin blocks of spleens were prepared.

Table 1: Spleen volumes obtained using Cavalieri method and morphometric data (mean±SD; n=6)

	13th day	16th day	21st day
Spleen volume (mm³)	3.91±0.29[b]	11.17±2.18[a]	12.02±2.60[a]
Embryo weight (g)	8.91±0.46[a]	22.74±2.71[b]	41.41±2.33[c]
Egg weight (g)	59.85±3.89[a]	58.03±5.50[a]	56.07±3.74[a]
Pre-hatching egg weight (g)	53.06±5.18[a]	50.79±3.85[a]	49.02±2.22[a]
Vitellus sac (g)	1.23±0.09[b]	2.13±0.44[a]	3.39±1.60[a]
Capsule thickness (µm)	8.93±3.17[b]	17.74±5.29[a]	21.43±12.39[a]

Different letters in the same row ([a, b, c]) indicate statically significant differences ($p<0.001$).

Serial tissue sections of 5 µm were taken at regular intervals from paraffin blocks. Sampling was performed systematically and randomly at a ratio of 1/20 by starting from a random one among the first 10 cross-sections and taking following every 20th cross-section. As a result of systematic random sampling between 13 and 19 cross-sections were obtained from chick embryos spleens. For routine histological examinations and volume calculations, the sections were stained with Crossmon's trichrome stain (Selçuk and Tıpırdamaz 2019) and Pappenheim's panoptic stain (Konuk 1981). The histological preparations were examined with a light microscope (Leica DM-2500 attached to a DFC-320 digital camera). Blood samples were also taken from the chick embryos on the 13th, 16th and 21st days of the incubation and the prepared blood smears were stained with May Grünwald-Giemsa stain (Konuk 1981). Granulocyte production in poultry starts on the 11th day of incubation and its development continues throughout incubation. Therefore, leukocyte separation could not be made on the 13th

and 16th days. Blood samples were taken on the 21st day to determine the peripheral blood leukocyte (PBL) ratio. To determine this ratio, 100 leukocyte cells were counted under a light microscope using a 100x objective lens and the leukocyte formula was determined.

Spleen volumes were calculated using a point area measurement scale (d=0.1 mm) on the cross-sectional images taken at 4× lens magnification. ImageJ programme was utilised for the calculations. In addition to the program, Cavalieri's principle was applied as calculation method (Figure 1).

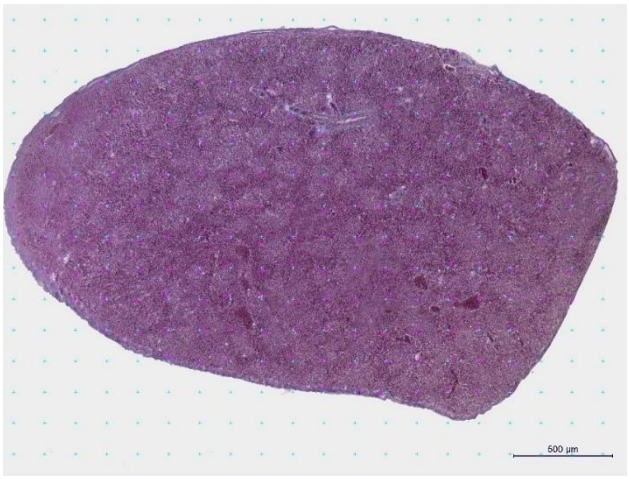

Figure 1. Application of point counting grid on histological sections of the spleen. Crossmon's trichrome stain (bar=500 µm).

The volumes of the structures of interest in the sections were calculated using the formula V= (a/p) × ∑P × t (Mayhew and Gundersen 1996). In this formula, V = the volume of the structure of the sample of interest, a/p = the area of a point in the point area measuring scale, ∑P = the total number of points on the structure of interest, t = average cross-sectional thickness (Gundersen et al. 1999, Chen et al. 2012, Selcuk and Bahar 2014). Several methods are used for coefficient of error (CE) calculation stereological research. In this study, CE formula of Gundersen et al. (1999) was used.

Egg weight and pre-hatching egg weights were compared using Wilcoxon test. The difference between the groups was compared using Kruskal Wallis test. Statistical analysis was conducted with SPSS software version 21.0. P<0.05 was accepted statistically significant. Data are expressed as means ± standard deviation (SD).

RESULTS

The spleen volumes obtained using Cavalieri's method and some morphometric data of the chicks are given in Table 1. As a result of the measurements, an increase in the spleen volume, embryo weight and vitellus sac weight were determined as the hatching time of the chicks approached and a decrease in egg weights was detected. There was a significantly statistical difference (p <0.001) between 13th and 16th–21st days in spleen volume increase, whereas no difference between 16th and 21st days was noted . The mean CE values for 13th, 16th and 21st day were determined as 0.017, 0.007 and 0.012, respectively.

When the egg weight and pre-hatching egg weight measurements were compared, it was found that weight decreased towards the 21st day (p<0.05). Spleen capsule thickness was found to be increased, and it was found to be statistically different between 13th and 16th–21st days.

As a result of microscopic blood examination, it was observed that granulocytes were predominantly lymphocytes on the 13th day of the incubation, whereas the number of heterophil granulocytes was higher than that of lymphocytes on the 16th day. On the 13th and 16th days, there were erythrocytes in various stages of development. According to the PBL counts on the 21st day of incubation, the highest heterophil granulocytes and lowest lymphocyte ratio were found as 74.83% and 23%, respectively (Fig. 2; Table 2).

On the 13th day of incubation, there were very few lymphocytes around the vessels (Fig. 3). On the 16th day of incubation, spleens from embryos were surrounded by a developing thin capsule. Smooth muscle cells and vascularisation in the capsule could not be seen clearly. A trabecular structure extending from the capsule to the splenic parenchyma but not developed in the parenchyma was detected. Trabecular vien and artery was found in connective tissue. In the parenchyma, central artery was frequently encountered and it was seen that PAL formation with lymphocyte accumulations around them started to develop. It was also observed that PEL formations (lymphoid deposits around ellipsoid structures and ellipsoid capillaries,) were developing in white pulp areas. The presence of venous sinuses showed that the red pulp was also developing vessels

(Fig. 4). The embryonal development of the spleens was completed on the 21st day of incubation. The spleen capsule consisting of fibrous connective tissue increased significantly compared to the 16th day. The smooth muscle cells in the capsule were easily visible and denser. Vascularisation was more advanced than on day 16th and connective tissue from the capsule into the splenic parenchyma contained an undeveloped trabecular structure as well as trabecular vessels. Lienalis ramus and lineal vena were found in the connective tissue outside the capsule. The most significant change in the splenic parenchyma was that the red and white pulp areas could be easily distinguished. PEL formation around the ellipsoid capillaries along with the dense lymphocyte-containing PAL regions around the central artery were clearly seen in the white pulp. The ellipsoid structures in the parenchyma could be observed easily. In addition, SCL formed by lymphocyte accumulations under the capsule and PVL around the vena were also found out (Fig. 5).

Table 2: Proportions of peripheral blood leukocyte on 21st day of the incubation (%). Data are presented as mean±SD (n=6)

Leukocyte types	
Lymphocyte	23.00±1.39
Heterophil	74.83±1.99
Monocyte	1.17±0.40
Eosinophil	1.67±0.21
Basophil	0.67±0.21

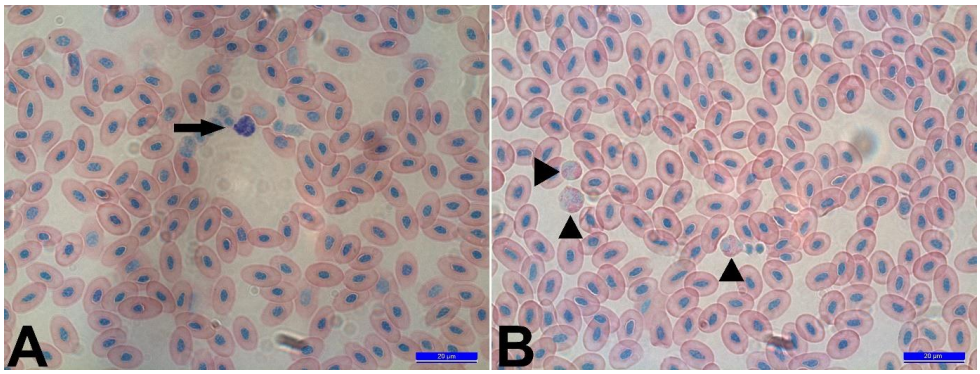

Figure 2. Peripheral blood smear of an embryo on the 21st day of incubation. A. Lymphocyte (→); B. Heterophil leukocytes (▶). May Grünwald-Giemsa stain (bar=20 μm).

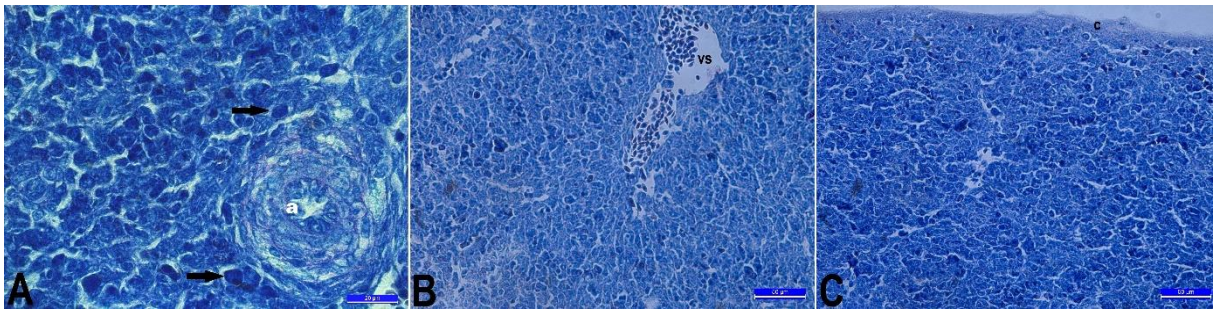

Figure 3. Spleen section of an embryo on the 13th day of incubation. Pappenheim's panoptic stain. A: Central artery (a), lymphocytes (→) (bar=20 μm). B: venous sinus (vs); C: capsule (c) (bar=50 μm).

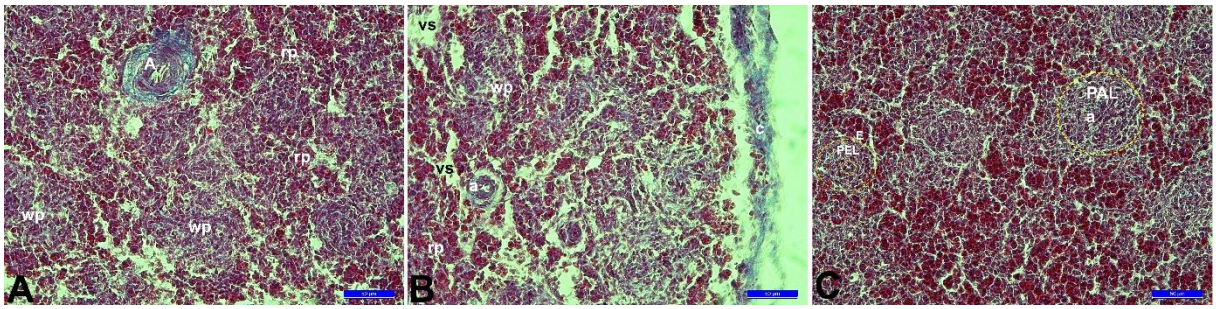

Figure 4. Spleen section of an embryo on the 16th day of incubation. Crossmon's trichrome stain. **A:** Trabecular artery (A), red pulp (rp), white pulp (wp). **B:** Venous sinus (vs), red pulp (rp), white pulp (wp), central artery (a), capsule (c). **C:** central artery (a), ellipsoid (E), PAL and PEL regions (bar = 50 μm).

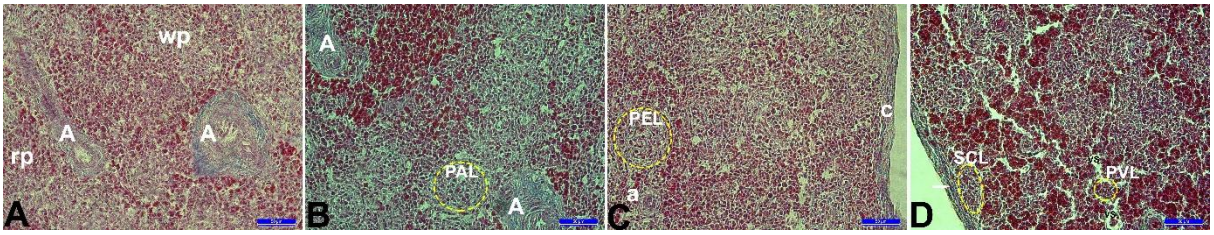

Figure 5. Spleen section of an embryo on the 21st day of incubation. Crossmon's trichrome stain. A: Trabecular artery (A), red pulp (rp), white pulp (wp); B: Trabecular artery (A), PAL; C: Central artery (a), capsule (c), PEL region; D: SCL and PVL regions, venous sinus (vs) (bar=50 μm).

DISCUSSION

Spleen, the largest lymphoid organ of poultry without lymph nodes, is known as a secondary lymphoid organ in which T and B lymphocytes are located (Aka and Eren 2019). Immune response is accepted as a production site (Steiniger 2005). Morphological and histological structures show differences depending on age, genetics and species. The spleen is oval in ostriches, triangular in ducks and globular in chickens (Cesta 2006, Song et al. 2012). Song et al. (2012) reported that the ostrich spleen was dark red, elliptical, and was located tightly to the posterior of the proventriculus, vena caudalis and the right kidney. Khenenou et al. (2018) reported that the spleen reached maximum size in the first six weeks after incubation. Some studies have reported that the spleen grows rapidly in the first six weeks after incubation and reaches maximum size at 10 weeks (Olah and Vervelde 2008, Liman and Bayram, 2011). In this study, globular-shaped chick embryonal spleens were located between the proventriculus and the posterior of the vena caudalis.

As a result of the measurements, an increase in the spleen volume, embryo weight and vitellus sac weight was determined as the hatch time of the chicks approached. Increased spleen volume and embryo weight could be explained by the development and growth of the chick. The increase in the weight of the vitellus sac was thought to be due to the fact that the vitellus sac consisting of endoderm was initially covered with splanchnic mesoderm and the development of blood vessels on the vitellus. It was determined that egg weights decreased as the hatching time of the chicks approached. The reason for this was thought to be that the nutrients contained in the egg were burned with oxygen and converted into carbon dioxide and water and thrown out of the egg.

The foetal spleen begins to form as an accumulation of primitive reticular cells in the dorsal mesogastrium (Cesta 2006). Seymour et al. (2006) found the first splenic tissue in mice on 12.5 days of pregnancy and the first haematopoietic cells on 15.5 days of pregnancy. In this study, it was found that in the spleen parenchyma small number of lymphocyte began to infiltrated the spleen on the 13th day of incubation. On the 13th day, peripheral blood was predominantly lymphocytic. On the 16th day, the number of lymphocytes in the circulating blood decreased; however, lymphocyte infiltrations in the spleen parenchyma and white pulp started to develop. On the 21st day of the incubation, where the two pulp separations were made clearly and the structures characterised by lymphocyte accumulation were easily seen, the percentage of lymphocytes in the peripheral blood was found to be low. This was probably due to the migration of lymphocytes into the spleen parenchyma.

There is no real trabecular structure in the winged spleen wrapped with a thin capsule (Olah and Vervelde 2008, Liman and Bayram 2011, Bingöl et al.

2014, Khenenou et al. 2018). Song et al. (2012) reported that the spleen membrane thickness in ostriches was 25.0–41.5 μm and undeveloped spleen trabeculae were encountered. In this study, it was seen that the thickness of the capsule covering the spleen from the 13th day to the 21st day of the incubation increased. The spleen membrane, which was 8.93±3.17 μm thick on 13th day, reached 17.74±5.29 μm on 16th day and 21.43±12.39 μm on 21st day. The density of smooth muscle cells in the capsule was also proportional to the thickness of the spleen membrane.

The parenchyma of the spleen consists of white and red pulp areas with unclear borders (Song et al. 2012, Khenenou et al. 2018, Aka and Eren, 2019). In this study, it was observed that lymphocyte accumulation gradually increased in the spleen on the 13th day of incubation. From the 16th day of incubation, it was seen that the separation of white and red pulp was clear. It was observed that PAL gradually expanded with lymphocyte infiltration and this ratio was found to be larger in the spleens on the 21st day compared to the other days.

The first cells in the foetal spleen are haematopoietic cells (Cesta 2006). Spleen migration of T and B lymphocytes start on the 52nd day of pregnancy in dogs, 120th day on horses, and 13th–14th days of incubation on chickens (HoganEsch and Hahn 2001). The first lymphocytes seen are T lymphocytes that accumulate in the PALS region (Van Rees et al. 1996). Sur and Çelik (2004) reported that they were encountered in a large number of haemopoietic foci in the spleen on the 12th day of incubation and a small number of lymphocytes located in the tunica adventitia floor of the small arteries and that white pulp development became more prominent with lymphocyte infiltration on the 13th day of incubation. On the 15th day of incubation, more advanced lymph follicles were observed; on the 18th day, it was stated that there was a rich stroma of reticular network with prominent white and red pulp areas. It is reported that the histological development of the spleens on the first day of hatching was complete and the germinal centres are formed. The data obtained from Sur and Çelik (2004) were similar to those found in our study. Lymphocyte infiltration and the development of white pulp was detected in the spleens on the 16th day. Arterial, arteriole, capillary, venous sinuses and ellipsoid structures were encountered and developing PAL were clearly identified. At the time of incubation, spleens completed embryonic development and the presence of white and red pulp areas was easily seen in all sections.

Penicillary capillaries with endothelial cells and reticular cells form ellipsoid and this structure acts as a filter apparatus. The ellipsoid-associated cells (EACs), which are phagocytic cells, are of blood origin and are located in the antigen-spanning region of the chicken spleen (Igyarto et al. 2007, Song et al. 2012, Aka and Eren 2019). Kannan et al. (2015) reported that there were a few macrophages in EACs. White pulp in chicken spleen consists of five different parts. These are PAL, PVL, PEL, SCL and GC (Liman and Bayram 2011, Aka and Eren 2019). GCs of chicken spleen are seen only after antigenic situmulation and are considered as secondary follicles (Yasuda et al. 2003). In this study, it was seen that PAL, PEL, PVL and SCL regions started to take shape in spleen sections taken from 16th day embryos and these structures were more advanced on the 21st day. Since there was no antigenic stimulation, GCs were not formed in the primary follicles.

The formation of structures characterised by lymphocyte infiltrations in the parenchyma of the spleen in the embryonal period also led to changes in the lymphocyte counts in the peripheral blood. Depending on the embryonic development, parts such as PAL/PALS, PEL/PELT, PVL, and SCL were found to form the white pulp, but no GCs were detected because the spleen did not encounter any antigenic stimulation. Therefore, it was concluded that the spleen completed its embryonic development but its immunological development was not fully completed.

Conflict of Interest: The authors declare that they have no conflict of interest

REFERENCES

Aka E, Eren Ü. Kuluçka sonrası ilk iki haftada lipopolisakkarit uygulanan ve uygulanmayan broyler civcivlerde dalağın histolojik gelişimi. Erciyes Üniv Vet Fak Derg. 2019; 16(1):8-15.

Bingöl SA, Gülmez NY, Deprem T, Taşci SK, Aslan Ş. Histologic and histometric examination of spleen in geese (Anser anser). Atatürk University J Vet. 2014; 9(3):157-162.

Chen X, Zhao H, Yao J. A fully automated framework for renal cortex segmentation, In: Abdominal Imaging, ed; Yoshida H, Hawkes D, Vannier MW, Springer, Berlin. 2012; pp. 208–217.

Cesta MF. Normal structure, function, and histology of the spleen. Toxicol Pathol. 206; 34:455–465.

HoganEsch H, Hahn FF. The Lymphoid organs: Anatomy, development, and age-related changes, In: Pathobiology of the Aging Dog, ed; Mohr U, Carlton WW, Dungworth DL, Benjamin SA, 1st Ed., Iowa State University Press, Ames. 2001; pp. 127–135.

Gundersen HJ, Jensen EB, Kieu K, Nielsen J. The efficiency of systematic sampling in stereology reconsidered. J Microsc. 1999; 193:199–211.

Igyarto BZ, Magyar A, Olah I. Origin of follicular dendritic cell in the chicken spleen. Cell Tissue Res. 2007; 327:83–92.

Kannan TA, Ramesh G, Ushakumari S, Dhinakarraj G, Vairamuthu S. Electron microscopic studies of spleen in chicken (Gallus domesticus). IJAVST. 2015; 4(1):160-165.

Khenenou T, Berghiche A, Rahmoun DE, Berberis A. Morpho histological study of the spleen of broiler chickens during post-haching age. IJVSAH. 2018; 3:22–23.

Liman N, Bayram GK. Structure of the quail (Coturnix coturnix japonica) spleen during pre- and post-hatching periods. Rev Med Vet. 2011; 162:25–33.

Mayhew T, Gundersen H. If you assume, you can make an ass out of u and me: a decade of the disector for stereological counting of particles in 3D space. J Anat. 1996; 188:1–15.

Olah I, Vervelde L. Structure of the avian lymphoid system, In: Avian Immunology, Ed; Davison F, Kaspers B, Schat K, Academic Press, London. 2008; pp. 13–50.

Olah I, Nagy N, Vervelde L. Structure of the avian lymphoid system, In: Avian Immunology, Ed; Schat KA, Kaspers B, Kaiser P, 2_{nd} eEd., Academic Press, London. 2014; pp. 11–43.

Selcuk ML, Bahar S. The morphometric properties of the lumbar spinal cord segments in horses. J Anim Vet Adv. 2014; 13:653–659.

Selçuk ML, Tıpırdamaz S. A morphological and stereological study on brain, cerebral hemispheres and cerebellum of New Zealand rabbits. Anat Histol Embryol. 2020; 49:90–96.

Seymour R, Sundberg JP, Hogenesch H. Abnormal lymphoid organ development in immunodeficient mutant mice. Vet Pathol. 2006; 43:401–423.

Song H, Peng K, Li S, Wang Y, Wei L, Tang L. Morrphological characterization of the immune organs in ostrich chicks. Turk J Vet Anim Sci. 2012; 36:89–100.

Steiniger, B., 2005. Spleen. In: Encyclopedia of Life Sciences, John Wiley & Sons, pp. 1–9.

Sur E, Çelik İ. Yumurtaya verilen aflatoksin B1'in tavuk dalağının embriyonik gelişimi üzerindeki etkileri: histolojik bulgular. Vet Bil Derg. 2004; 20:103–110.

Rajput IR, Wu BB, Li LY, Xu X. Establishment of optimal culturing method and biological activity analysis of chicken bone marrow dendritic cells using Chi-rGM-CSF. IJAB. 2013; 15:401–409.

Van Rees EP, Sminia T, Dijkstra CD. Structure and development of the lymphod organs, In: Pathobiology of the Aging Mouse, Ed; Mohr U, Dungworth CC, Capen CC, CarltonWW, 1_{st} Ed., ILSI Press, Washington. 1996; pp. 173–187.

Yasuda M, Kajiwara E, Ekino S, Taura Y, Hirota Y, Horiuchi H, Matsuda H, Furusawa S. Immunobiology of chicken germinal center: I. Changes in surface Ig class expression in the chicken splenic germinal center after antigenic stimulation. Dev Comp Immunol. 2003; 27:159–166.

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RESEARCH ARTICLE

Evaluation of C-Reactive Protein, Albumin, Neopterin, Urokinase Type Plasminogen Activator Receptor and Leukocyte Levels as Prognostic Parameters in Dogs with Parvoviral Enteritis

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ABSTRACT

The aim of this study was to determine the changes in C-reactive protein (CRP), albumin, neopterin (Np), urokinase type plasminogen activator receptor (uPAR) and leukocyte levels in dogs with parvoviral enteritis and to show the prognostic importance of these. In the study, a total of 48 dogs, 40 with parvoviral enteritis and 8 were healthy, were used. The dogs with parvoviral enteritis were divided into two subgroups, non-surviving (n=12) and surviving (n=28). The non-surviving dogs with parvoviral enteritis in the study had significantly ($p<0.05$) lower leukocyte levels than the control group and the surviving dogs with parvoviral enteritis. Serum albumin concentrations of non-surviving dogs with parvoviral enteritis were also significantly ($p<0.05$) lower than the control group. On the contrary, the CRP levels of the non-surviving and surviving dogs with parvoviral enteritis were significantly ($p<0.05$) higher than the control group. There was also no statistically significant difference between the groups in terms of Np and uPAR levels. The cut-off values of leukocyte, CRP and albumin were 4.5×10^9L, 120.50 mg/L and 2.28 g/dL, respectively. As a result, it can be stated that decreased leukocyte and albumin levels and increased CRP levels in dogs with parvoviral enteritis may be an indicator of poor prognosis. It was also determined that serum Np and uPAR levels in dogs with parvoviral enteritis do not have any prognostic importance.

Keywords: Parvoviral enteritis, C-reactive protein, neopterin, urokinase type plasminogen activator receptor, dog

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Parvoviral Enteritisli Köpeklerde Prognostik Parametreler Olarak C-Reaktif Protein, Albümin, Neopterin, Ürokinaz Tipi Plazminojen Aktivatör Reseptörü ve Lökosit Seviyelerinin Değerlendirilmesi

ÖZ

Bu çalışmanın amacı, parvoviral enteritisli köpeklerde C-reaktif protein (CRP), albumin, neopterin (Np), ürokinaz tipi plazminojen aktivatör reseptörü (uPAR) ve lökosit seviyelerindeki değişiklikleri belirlemek ve bunların prognostik önemini göstermekti. Çalışmada 40'ı parvoviral enteritisli ve 8'i sağlıklı olmak üzere toplam 48 köpek kullanıldı. Parvoviral enteritisli köpekler ölen (n=12) ve hayatta kalanlar (n=28) olarak iki gruba ayrıldı. Çalışmada ölen parvoviral enteritisli köpeklerin lökosit seviyesi kontrol grubu ve yaşayan parvoviral enteritisli köpeklere göre önemli düzeyde ($p<0.05$) düşük bulundu. Ölen parvoviral enteritisli köpeklerin serum albümin konsantrasyonları da kontrol grubundan önemli düzeyde ($p<0.05$) düşük bulundu. Aksine, ölen ve yaşayan parvoviral enteritisli köpeklerin CRP düzeyi kontrol grubuna göre önemli düzeyde ($p<0.05$) yüksek olarak tespit edildi. Np ve uPAR düzeyleri açısından ise gruplar arasında istatistiksel olarak anlamlı fark yoktu. Lökosit, CRP ve albüminin cut-off değerleri sırasıyla 4.5×10^9L, 120.50 mg/L ve 2.28 g/dL olarak tespit edildi. Sonuç olarak, parvoviral enteritisli köpeklerde azalmış lökosit ve albumin seviyeleri ile artmış CRP seviyelerinin kötü prognozun bir göstergesi olabileceği ifade edilebilir. Ayrıca serum Np ve uPAR düzeylerinin parvoviral enteritisli köpeklerde prognostik bir öneme sahip olmadığı belirlenmiştir.

Anahtar Kelimeler: Parvoviral enteritis, C-reactive protein, neopterin, ürokinaz plazminojen aktivatör reseptör, köpek

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INTRODUCTION

Canine parvoviral enteritis is an infectious viral disease which is caused by the canine parvovirus (CPV) type-2 from the Parvoviridae family. It is characterized by severe vomiting, hemorrhagic gastroenteritis and leukopenia (Kalli et al. 2010, Decaro and Buonavoglia 2012, Castro et al. 2013, Mylonakis et al. 2016). The disease is one of the common infections in puppies and morbidity and mortality rates are high in the disease (Kocaturk et al. 2010). The disease has two forms, enteritis and myocarditis (Nandi and Kumar 2010, Ford et al. 2017).

Most of the acute phase proteins formed during infectious diseases exhibit a non-specific immunological (inflammatory) response. Most of these proteins are glycoprotein-structured and are of liver origin. Increased acute phase proteins (for example, C-reactive protein and serum amyloid A) are called positive acute phase proteins while decreased acute phase proteins (for example albumin and transferrin) are called negative acute phase proteins (Cerón et al. 2005). CRP is the most important acute phase protein in dogs; it increases rapidly in infectious diseases and reaches its peak in 24-48 hours (Cerón et al. 2005, Schmidt and Eckersall 2015). When CRP binds to bacteria, it promotes complement binding which facilitates bacterial uptake of phagocytes, inhibits chemotaxis and regulates neutrophil function, and also induces anti-inflammatory cytokine production (Schmidt and Eckersall 2015). Increased CRP levels in patients with sepsis in human medicine were found to be associated with mortality (Koozi et al. 2020). Studies conducted in veterinary medicine have indicated that serum CRP, as one of the species-specific acute phase proteins, may provide information about diseases such as pancreatitis, neoplasias, sepsis, parvoviral enteritis, pyometra, systemic inflammatory response syndrome (SIRS), leishmaniosis, ehrlichiosis and babesiosis in dogs (Christensen et al. 2014, Ok et al. 2015, Daza González et al. 2019).

During the course of infectious diseases, Np as the end product of pteridine metabolism is released from monocytes and macrophages by interferon-gamma stimuli which are released from T lymphocytes (Berdowska and Zwirska-Korczala 2001). Increased Np concentrations have been reported in malignancies, infectious and autoimmune diseases where cellular immunological mechanisms are activated, and it has been suggested that these concentrations be used to evaluate the clinical course of diseases (Hoffmann et al. 2003, Pourakbari et al. 2010, Bastan et al. 2013, Ünüvar and Aslanhan 2019). In addition, significant increases in neopterin levels were detected in dogs with SIRS (Basbug et al. 2020) and trypanosomiasis (Rokos et al. 1992), cattle with Lumpy Skin Disease (Başbuğ et al. 2016), and calves with septicaemia (Ercan et al. 2016). Szczubiał et al. (2014) has been reported that in dogs with primary mammary cancer, the neopterin concentration is lower than in healthy animals.

uPAR is released mainly from neutrophils, endothelial and peripheral mononuclear blood cells (Donadello et al. 2012). It is involved in various immunological functions such as cell adhesion, migration, differentiation and proliferation. It has been stated that uPAR can be a potential biomarker for diseases in human medicine (Donadello et al. 2012, Çekmez et al. 2014). Although uPAR has been reported as a potential prognostic marker in sepsis, pulmonary and malignant diseases in human individuals and it can be used in intensive care units in human medicine (Stephens et al. 1997, Donadello et al. 2012, Wu et al. 2013), no literature on its use in veterinary medicine has been found in the literature.

This study aimed to determine the prognostic significance of CRP, albumin, neopterin, uPAR and leukocyte levels in dogs with parvoviral enteritis.

MATERIAL and METHODS

This study was approved by the Local Ethics Committee for Animal Experiments, Sivas Cumhuriyet University (approval number: 2016-81). The animal material of the study consisted of 40 dogs aged 6-18 weeks (7.07±1.2 weeks) of both genders (22 female, 18 male) and different breeds (25 Anatolian shepherd dogs, 5 Golden retriever, 5 Rottweiler, 3 Pointer, 2 German shepherd dogs) which had parvoviral enteritis, and which had been brought to the Internal Medicine Clinic at the Veterinary Faculty, Sivas Cumhuriyet University for examination and treatment, and 8 healthy dogs (control group) without any symptoms of disease. The dogs in the control group were of different breeds (4 Anatolian shepherd dogs, 1 Rottweiler, 1 Rottweiler, 1 Pointer, 1 German shepherd dogs), genders (5 female, 3 male) and were different ages 6-18 weeks old (6.92±0.4 weeks). Also, the dogs between 2.5-15 kg (parvoviral enteritis 5.03±0.4 kg, control 5.25±0.9 kg) were included in the study. Parvoviral enteritis in dogs was diagnosed by clinical symptoms, fecal antigen test and hematological findings.

The dogs with parvoviral enteritis and the healthy were examined for giardia and coccidiosis, and those with negative results were included in the study. The dogs were divided into two groups, surviving and non-surviving, after a follow-up of the health statuses of the dogs subsequent to the treatment.

After all clinical examinations, 5 ml of blood samples were taken from the vena cephalica antebrachi of the dogs to the tubes with anticoagulant and without anticoagulant once before treatment. In the blood samples with EDTA the levels of leukocyte,

erythrocyte, hematocrits, hemoglobin and platelets were determined by a hematological analyzer (BC-2800 Vet hematology analyzer, Mindray Bio-Medical Electronics Co. Ltd., Nanshan, Shenzhen). The blood samples with no anticoagulant were centrifuged at 3000 rpm for 10 min, and the serum samples were collected by centrifugation. These samples were stored at -80°C until biochemical analyses were performed. Serum albumin concentrations were measured on an automated analyzer (BS 200 chemistry analyzer, Mindray Bio-Medical Electronics Co. Ltd, Nanshan, Shenzhen) using commercial test kits. The levels of Canine CRP (Tri-Delta Phase CRP, Tri-Delta Diagnostic, Boonton Township, NJ), Np (Canine Neopterin ELISA Kit, Yehua Biological Technology Co. Ltd, Shanghai) and uPAR (Canine uPAR ELISA Kit, Sunred Biological Technology Co. Ltd, Shanghai) were determined using species specific ELISA kits according to the manufacturer's instructions. Absorbances were measured using a microplate reader (Thermo Multiskan GO Microplate Spectrophotometer, Waltham, Massachusetts).

In all the dogs included in the study, stool examination was performed with an antigen test kit (SNAP Parvo Test, Idexx, Westbrook, ME) without cross-reaction to modified live vaccines.

Treatment

All dogs with parvoviral enteritis were kept under observation for seven days in the infectious disease unit of the clinic. The sick dogs were given intravenous fluid containing balanced electrolyte solution to correct dehydration via intravenous catheter, which was placed in the vena cephalica antebrachi. Fluid therapy was continued until vomiting disappeared and food intake began. All dogs with parvoviral enteritis were administered with 50 mg/kg ceftriaxone (Desefin 1 gr IV, Deva, Istanbul) once a day. In addition, 2 mg/kg ranitidine (Ulcuran, Abfar, Istanbul), 0.2 mg/kg (Metpamid, Recordati, Istanbul) and 250 mg transaminic acid (Transamine 10%, Fako Istanbul) were administered twice a day, and 500 mg ascorbic acid (Injacom C, Ceva Animal Science, Istanbul) and B-complex vitamin (Bemiks, Zentiva, Istanbul) were administered once a day.

Statistical Methods

The data were shown with mean and standard error. The ANOVA test was used to determine the difference between the groups. $p<0.05$ was accepted as statistically significant. The Receiver Operating Characteristic (ROC) curve was used to determine a cut-off value for non-surviving and surviving dogs with parvoviral enteritis in terms of CRP, albumin and leukocyte measurements. Likelihood Ratio (LR) was calculated for each cut-off threshold and the highest LR was considered as the optimal cut-off point. The Pearson correlation coefficient was used to quantify the relationship between CRP, neopterin, uPAR, albumin and leukocyte. For analysis of the data, the SPSS software program (Version 15.0, SPSS Inc. Ltd. Chicago USA) was used.

RESULTS

The dogs with parvoviral enteritis were observed to have loss of appetite, decreased interest in the environment, depressed appearance, vomiting, bloody diarrhea and dehyration. Despite intensive care, 12 of dogs with parvoviral enteritis died within the first 48 hours. Also, all animals were followed for 1 week and information was obtained from the owners. Necropsy was performed on dogs that non-survivors and the diagnosis of parvoviral enteritis was confirmed in the necropsy.

The results of hematological analysis of the dogs with parvoviral enteritis and healthy are given in Table 1. The leukocyte levels of non-surviving dogs with parvoviral enteritis were found to be significantly ($p<0.05$) lower than those of the control group and surviving dogs with parvoviral enteritis.

The changes in CRP, Np, uPAR and albumin levels of the dogs with parvoviral enteritis and healthy are shown in Table 2. The CRP levels of the non-surviving and surviving dogs with parvoviral enteritis were significantly ($p<0.05$) higher than the control group. On the contrary, the serum albumin concentration of non-surviving dogs with parvoviral enteritis was found to be significantly ($p<0.05$) lower than that of the control group. Albumin showed a negative correlation with CRP, while it showed a positive correlation with leukocyte (Table 3).

The cut-off values, sensitivity, specificity, and area under the curve of CRP, albumin and leukocyte levels of surviving and non-surviving dogs with parvoviral enteritis are given in Table 4. The cut-off values for CRP, albumin and leukocyte levels were determined as 120.5 (mg/L), 2.28 (g/dL) and 4.5 ($\times 10^9$L), respectively.

Table 1: Clinical finding and hematological parameters in the dogs with parvoviral enteritis and healthy

Parameters	Healthy group	Survivors	Non- Survivors
Leukocyte ($\times10^9$L)	9.35 ± 0.77[a]	9.86 ± 1.10[a]	3.69 ± 1.17[b]
Erythrocyte ($\times10^{12}$L)	5.32 ± 0.38	5.24 ± 0.19	5.99 ± 0.23
HCT (%)	36.85 ± 2.81	33.31 ± 2.08	41.68 ± 2.90
Hg (g/dL)	10.31 ± 1.00	8.73 ± 0.60	11.45 ± 0.66
PLT ($\times10^9$L)	383.75 ± 37.92	398.52 ± 60.66	396.92 ± 37.44
Temperature (°C)	38.41 ± 0.11	38.72 0.19	37.97 ± 0.27

HCT; hematocrit, Hg; hemoglobin, PLT; platelet. a, b: the difference between the average values with different letters in the same row is significant ($p<0.05$).

Table 2: CRP, neopterin, uPAR and albumin levels in the dogs with parvoviral enteritis and healthy

Parameters	Healthy group	Survivors	Non- Survivors
CRP (mg/L)	9.20 ± 2.64[b]	111.30 ± 6.12[a]	133.04 ± 3.48[a]
Neopterin (nmol/mL)	13.28 ± 3.70	8.22 ± 0.49	10.44 ± 1.83
uPAR (ng/mL)	2.59 ± 0.73	1.64 ± 0.10	2.02 ± 0.29
Albumin (g/dL)	2.47 ± 0.29[a]	2.44 ± 0.60[ab]	2.21 ± 0.65[b]

CRP; C-reactive protein, uPAR; urokinase type plasminogen activator receptor. a, b: the difference between the average values with different letters in the same row is significant ($p<0.05$).

Table 3. Pearson correlation coefficient between CRP, neopterin, uPAR, albumin and leukocyte in the dogs with parvoviral enteritis and healthy

Parameters	Neopterin	uPAR	Albumin	Leukocyte
CRP	-,242	-,232	-,358*	-,251
Neopterin		,909**	,046	-,043
uPAR			,040	-,090
Albumin				,445**

CRP; C-reactive protein, uPAR; urokinase type plasminogen activator receptor. *Correlation is significant at the 0.05 level (2-tailed), ** Correlation is significant at the 0.01 level (2-tailed)

Table 4: Cut-off, sensitivity, specificity and area under the curve values of CRP, albumin and leukocyte in the dogs with parvoviral enteritis and healthy

Parameters	CRP (mg/L)	Albumin (g/dL)	Leukocyte ($\times10^9$L)
AUC	0.68	0.72	0.90
Cut off	120.50	2.28	4.5
Sensitivity (%)	92.3	69.2	84.6
Specificity (%)	54.0	71.4	92.6
p	0.073	0.029	< 0.001
SEM	0.082	0.087	0.069

AUC; Area under the curve, SEM; standard error of mean, CRP; C-reactive protein

DISCUSSION

Myocarditis, sepsis, systemic inflammatory response syndrome (SIRS) and endotoxemia that develop as a result of CPV infection may be the cause of death (Turk et al. 1990, Otto et al. 1997, Prittie 2004). In this study, changes in serum CRP, uPAR, Np, albumin and leukocyte levels and the prognostic

significance of these in dogs with parvoviral enteritis were evaluated.

CRP is a sensitive marker of inflammation, tissue damage and infection. An increased CRP level has been reported as a potential indicative marker of poor prognosis which is related to the inflammatory response (Kocaturk et al. 2010, Kocaturk et al. 2015). CRP is the major acute phase protein used in the evaluation of inflammation in dogs and is synthesized in the liver by stimulation of cytokines secreted mainly from the inflamed tissue. The CRP level has been reported to reach peak values after 48 hours and to return to normal levels within 1-2 weeks (Cerón et al. 2005). Healthy dogs have a very low level of serum CRP (Schmidt and Eckersall 2015). In human and veterinary medicine, CRP measurement is a test which shows inflammation and it has also been reported as a potential prognostic marker in some diseases (Cerón et al. 2005, Kocaturk et al. 2010, Nandi and Kumar 2010). In dogs with parvoviral enteritis, serum levels of CRP may be 10 times higher than in healthy subjects; it may be a biomarker that shows the severity of the disease. McClure et al. (2013) have been reported that although serum CRP concentration was associated with outcome in puppies with parvoviral enteritis, it did not prove to be a good predictor of outcome when used alone. Kocaturk et al. (2010) reported that mortality rate was 91% in dogs with parvoviral enteritis which had CRP levels above 92.4 mg/L. In our study, serum CRP levels were found to be significantly ($p<0.05$) higher in the surviving and non-surviving dogs with parvoviral enteritis compared to the control group. When the cut-off value for CRP was evaluated as 120.5 mg/L to differentiate survivors from non-survivors, the sensitivity and specificity were determined as 92.3% and 54.0% respectively. While significant increases in CRP level are mainly observed in bacterial infections in dogs, the increase in viral infections is at smaller levels (Gruys et al. 2005). In studies conducted (Kocaturk et al. 2010, Kocaturk et al. 2015), it has been reported that secondary bacterial infections and sepsis may develop in dogs with parvoviral enteritis. This explains this increase in CRP level in dogs with parvoviral enteritis. In addition, this suggests that increased CRP may be evaluated as a sign of poor prognosis in the dogs with parvoviral enteritis in consistent with other studies.

In medical practice, inflammatory mediators such as serum Np and uPAR are analyzed to identify the extent of inflammation in different infectious diseases and to provide information about clinical prognosis (Berdowska and Zwirska-Korczala 2001, Donadello et al. 2012, Grove et al. 2014). Neopterin is released by macrophages in response to stimuli of cytokines such as interferon-γ in infectious patients (Hoffmann et al. 2003). It has been stated that Np levels may be a prognostic factor in patients with sepsis (Tasdelen Fisgin et al. 2010). Nevertheless, viral infections have been reported to increase Np levels in blood before the appearance of clinical symptoms (Chan et al. 2006, Başbuğ et al. 2016). Kaufmann et al. (1998) reported that Np may provide more valuable information than CRP in the determination of the severity of pancreatitis in human medicine. Başbuğ et al. (2016) reported a positive correlation between the clinical appearance of lumpy skin disease and blood Np level in cattle. Rokos et al. (1992) has been reported that an increase in serum neopterin levels in dogs after Trypanosoma infection and this supports the activation of the cellular immune system. Basbug et al. (2020) has been stated that serum neopterin levels significantly increased in dogs with SIRS compared to healthy dogs. In contrast, Szczubiał et al. (2014) has been reported that in dogs with primary mammary cancer, the neopterin concentration is lower than in healthy animals, and this low neopterin level may be associated with impaired cell-mediated immunity. In another study (Strasser et al. 2003), they reported that a significant reduction in neopterin level was observed in dogs following polyvalent vaccination. In this study, it was found that serum Np levels were low in dogs with parvoviral enteritis compared to the control group, but there was no statistical difference. Decreased neopterin levels in dogs with parvoviral enteritis may be associated with impaired cell-mediated immunity.

It is reported that uPAR can be evaluated as one of the indicators of inflammation in human medicine (Wu et al. 2013, Genua et al. 2015). uPAR, which is released from cells such as monocytes, macrophages, neutrophil, T cells, and endothelial, is considered as a marker for fibrinolysis and inflammation (Plesner et al. 1997, Genua et al. 2015). Increased uPAR levels have been reported as a marker for immune system activation in conditions such as inflammation and infection (Mondino and Blasi 2004, Genua et al. 2015). Florquin et al. (2001) reported that uPAR was significantly increased in experimental endotoxemia and urosepsis models. In this study, it was found that serum uPAR levels were low in dogs with parvoviral enteritis compared to the control group, but there was no statistical difference. The decrease in uPAR levels of dogs with parvoviral enteritis may be associated with leukopenia due to bone marrow and lymphoid tissue damage. Because uPAR is secreted by cells such as monocytes, macrophages, neutrophil and T cells.

Protein losses and hypoalbuminemia due to enteropathies are common signs (Willard 2015). Plasma protein and albumin levels were decreased in the dogs with parvoviral enteritis (Kocaturk et al. 2010, Bastan et al. 2013). It has been reported that the cause of this decrease is enteritis and/or haemorrhagic diarrhea, anorexia and malabsorption (Wingfield and Raffe 2002). Many studies have found a positive correlation between low albumin levels and morbidity and mortality (Mazzaferro et al. 2002, Kalli et al. 2010, Kocaturk et al. 2010). Albumin is also a negative acute phase protein and its concentration is

reduced by 25% during the inflammatory response (Cerón et al. 2005, Eckersall 2008). Albumin has a low clinical value in the diagnosis and monitoring of inflammation, although its measurement is easier. Decreased albumin level is a marker for the acute phase reaction in dogs and cats with infection and inflammation. However, their sensitivity and specificity rates are not as high as CRP for clinical or subclinical diseases (Christensen et al. 2014, Torrente et al. 2015). In this study, serum albumin levels decreased in the non-surviving and surviving dogs with parvoviral enteritis compared to the control group. However, only the decrease in serum albumin levels of the non-surviving dogs with parvoviral enteritis was statistically significant ($p<0.05$). In this study, the cut off value for albumin was found to be 2.28 g/dL; its sensitivity and specificity were 69.2% and 71.4%, respectively. According to the results of the study, the serum albumin level in the dogs with parvoviral enteritis is useful in the evaluating of the prognosis of the disease and low albumin levels may be a marker of poor prognosis.

The predominant hematological abnormality in dogs with parvoviral enteritis is leukopenia, because bone marrow precursors and the lymphoid tissues are destroyed (Turk et al. 1990). There was a relationship between leukopenia and death in dogs with parvoviral enteritis. Furthermore, leukopenia may also be an important tool for determining the prognosis (Willard 2015). Macartney et al. (1984) reported that panleukopenia, which is characterized with lymphopenia and granulocytopenia, is a significant laboratory finding in the first 72 hours when the clinical symptoms of dogs with parvoviral enteritis are observed. It has been stated that the severity of leukopenia is positively correlated with the clinical pattern of the disease in dogs with parvoviral enteritis, that the total leukocyte counts increases with recovery and that the leukocyte counts may be used in the determination of the prognosis of the disease (Macartney et al. 1984, Kuffer et al. 1997). In this study, a significant ($p<0.05$) decrease in leukocyte level was found in the non-surviving dogs with parvoviral enteritis compared to the surviving dogs with parvoviral enteritis and the control group. In addition, the sensitivity and specificity of leukocyte counts were determined as 84.6% and 92.6% respectively when the cut-off value used was $4.5x10^9$L. These results may provide important knowledge on the prognosis of the disease in dogs with parvoviral enteritis. Furthermore, a low leukocyte count may be a marker of poor prognosis.

In conclusion, it was evaluated that decrease in albumin and leukocyte levels and increase in CRP levels in dogs with parvoviral enteritis may be predictors of poor prognosis. In addition, serum Np and uPAR levels in dogs with parvoviral enteritis were found to have no prognostic significance.

Conflict of Interest: The authors declare that there is no conflict of interest.

REFERENCES

Basbug O, Aydogdu U, Agaoglu ZT. Neopterin and soluble urokinase type plasminogen activator receptor as biomarkers in dogs with systemic inflammatory response syndrome. J Hellenic Vet Med Soc. 2020; 71(1): 1945-1952.

Başbuğ O, Ağaoğlu ZT, Tuzcu N, Coşkun A, Aydoğdu U, Yığın A. Tumour necrosis factor-alpha, haptoglobin, serum amyloid A and neopterin levels in cattle with lumpy skin disease. Kafkas Üniv Vet Fak Derg. 2016; 22(3): 417-424.

Bastan I, Kurtdede A, Özen D. Prognostic usefulness of some parameters in dogs with canine parvovirus. Ankara Üniv Vet Fak Derg. 2013; 60: 53-58.

Berdowska A, Zwirska-Korczala K. Neopterin measurement in clinical diagnosis. J Clin Pharm Ther. 2001; 26(5): 319-329.

Castro TX, Cubel Garcia Rde C, Gonçalves LP, Costa EM, Marcello GC, Labarthe NV, Mendes-de-Almeida F. Clinical, hematological, and biochemical findings in puppies with coronavirus and parvovirus enteritis. Can Vet J. 2013; 54(9): 885-888.

Çekmez F, Aydemir G, Yildirim S, Bulut Ö, Tunç T, Kul M, İnce EZ, Çoban A. Diagnostic value of 25-hydroxyvitamin D level and new cytokines in neonatal sepsis. Eur J Inflamm. 2014; 12(2): 297-304.

Cerón JJ, Eckersall PD, Martínez-Subiela S. Acute phase proteins in dogs and cats: current knowledge and future perspectives. Vet Clin Pathol. 2005; 34(2): 85-99.

Chan CP, Choi JW, Cao KY, Wang M, Gao Y, Zhou DH, Di B, Xu HF, Leung MF, Bergmann A, Lehmann M, Nie YM, Cautherley GW, Fuchs D, Renneberg R, Zheng BJ. Detection of serum neopterin for early assessment of dengue virus infection. J Infect. 2006; 53(3): 152-158.

Christensen MB, Langhorn R, Goddard A, Andreasen EB, Moldal E, Tvarijonaviciute A, Kirpensteijn J, Jakobsen S, Persson F, Kjelgaard-Hansen M. Comparison of serum amyloid A and C-reactive protein as diagnostic markers of systemic inflammation in dogs. Can Vet J. 2014; 55(2): 161-168.

Daza González MA, Fragío Arnold C, Fermín Rodríguez M, Checa R, Montoya A, Portero Fuentes M, Rupérez Noguer C, Martínez Subiela S, Cerón JJ, Miró G. Effect of two treatments on changes in serum acute phase protein concentrations in dogs with clinical leishmaniosis. Vet J. 2019; 245: 22-28.

Decaro N, Buonavoglia C. Canine parvovirus-a review of epidemiological and diagnostic aspects, with emphasis on type 2c. Vet Microbiol. 2012; 155(1): 1-12.

Donadello K, Scolletta S, Covajes C, Vincent JL. suPAR as a prognostic biomarker in sepsis. BMC Med. 2012; 10:2.

Eckersall PD. Proteins, proteomics, and the dysproteinemias. In: Clinical Biochemistry of Domestic Animals, Ed; Kaneko JJ, Harvey JW, Bruss ML, 6th Ed., Academic Press, San Diego, USA. 2008; pp. 117-155.

Ercan N, Tuzcu N, Başbug O, Tuzcu M, Alim A. Diagnostic value of serum procalcitonin, neopterin, and gamma interferon in neonatal calves with septicemic colibacillosis. J Vet Diagn Invest. 2016; 28(2): 180-183.

Florquin S, van den Berg JG, Olszyna DP, Claessen N, Opal SM, Weening JJ, van der Poll T. Release of urokinase plasminogen activator receptor during urosepsis and endotoxemia. Kidney Int. 2001; 59(6): 2054-2061.

Ford J, McEndaffer L, Renshaw R, Molesan A, Kelly K. Parvovirus infection is associated with myocarditis and myocardial fibrosis in young dogs. Vet Pathol. 2017; 54(6): 964-971.

Genua M, D'Alessio S, Cibella J, Gandelli A, Sala E, Correale C, Spinelli A, Arena V, Malesci A, Rutella S, Ploplis VA, Vetrano S, Danese S. The urokinase plasminogen activator receptor (uPAR) controls macrophage phagocytosis in intestinal inflammation. Gut. 2015; 64(4):589-600.

Grove LM, Southern BD, Jin TH, White KE, Paruchuri S, Harel E, Wei Y, Rahaman SO, Gladson CL, Ding Q, Craik CS, Chapman HA, Olman MA. Urokinase-type plasminogen activator receptor (uPAR) ligation induces a raft-localized integrin signaling switch that mediates the hypermotile phenotype of fibrotic fibroblasts. J Biol Chem. 2014; 289(18): 12791-12804.

Gruys E, Toussaint MJM, Niewold TA, Koopmans SJ. Acute phase reaction and acute phase proteins. J Zhejiang Univ Sci B. 2005; 6(11): 1045-1056.

Hoffmann G, Wirleitner B, Fuchs D. Potential role of immune system activation-associated production of neopterin derivatives in humans. Inflamm Res. 2003; 52(8): 313-321.

Kalli I, Leontides LS, Mylonakis ME, Adamama-Moraitou K, Rallis T, Koutinas AF. Factors affecting the occurrence, duration of hospitalization and final outcome in canine parvovirus infection. Res Vet Sci. 2010; 89(2), 174-178.

Kaufmann P, Tilz GP, Demel U, Wachter H, Kreijs GJ, Fuchs D. Neopterin plasma concentrations predict the course of severe acute pancreatitis. Clin Chem Lab Med. 1998; 36(1): 29-34.

Kocaturk M, Martinez S, Eralp O, Tvarijonaviciute A, Ceron J, Yilmaz Z. Prognostic value of serum acute-phase proteins in dogs with parvoviral enteritis. J Small Anim Pract. 2010; 51(9): 478-483.

Kocaturk M, Tvarijonaviciute A, Martinez-Subiela S, Tecles F, Eralp O, Yilmaz Z, Ceron JJ. Inflammatory and oxidative biomarkers of disease severity in dogs with parvoviral enteritis. J Small Anim Pract. 2015; 56(2):119-124.

Koozi H, Lengquist M, Frigyesi A. C-reactive protein as a prognostic factor in intensive care admissions for sepsis: A Swedish multicenter study. J Crit Care. 2020; 56:73-79.

Kuffer M, Hartmann K, Kraft W. Canine parvovirose: Aspekte zu epidemiologie, klinik, laborbefunden. Therapie und impfung. Tierärztl Prax. 1997; 25: 518-524.

Macartney L, McCandlish IA, Thompson H, Cornwell HJ. Canine parvovirus enteritis 1: Clinical, haematological and pathological features of experimental infection. Vet Rec. 1984; 115(9):201-210.

Mazzaferro EM, Rudloff E, Kirby R. The role of albumin replacement in the critically ill veterinary patient. J Vet Emerg Crit Care (San Antonio). 2002; 12(2): 113-124.

McClure V, van Schoor M, Thompson PN, Kjelgaard-Hansen M, Goddard A. Evaluation of the use of serum C-reactive protein concentration to predict outcome in puppies infected with canine parvovirus. J Am Vet Med Assoc. 2013; 243(3): 361-366.

Mondino A, Blasi F. uPA and uPAR in fibrinolysis, immunity and pathology. Trends Immunol. 2004; 25(8), 450-455.

Mylonakis ME, Kalli I, Rallis TS. Canine parvoviral enteritis: an update on the clinical diagnosis, treatment, and prevention. Vet Med (Auckl). 2016; 7:91-100.

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

Ok M, Er C, Yıldız R, Çöl R, Aydoğdu U, Şen İ, Güzelbekteş H. Evaluation of acute phase proteins, some cytokines and hemostatic parameters in dogs with sepsis. Kafkas Üniv Vet Fak Derg. 2015; 21(5):761-766.

Otto CM, Drobatz KJ, Soter C. Endotoxemia and tumor necrosis factor activity in dogs with naturally occurring parvoviral enteritis. J Vet Intern Med. 1997; 11(2): 65-70.

Plesner T, Behrendt N, Ploug M. Structure, function and expression on blood and bone marrow cells of the urokinase-type plasminogen activator receptor, uPAR. Stem Cells. 1997; 15(6):398-408.

Pourakbari B, Mamishi S, Zafari J, Khairkhah H, Ashtiani MH, Abedini M, Afsharpaiman S, Rad SS. Evaluation of procalcitonin and neopterin level in serum of patients with acute bacterial infection. Braz J Infect Dis. 2010; 14(3): 252-255.

Prittie J. Canine parvoviral enteritis: a review of diagnosis, management, and prevention. J Vet Emerg Crit Care (San Antonio). 2004;14(3):167-176.

Rokos H, Wiegers P, Leonhard M, Ahmed JS. Biopterin and Neopterin Serum Levels in Trypanosoma-infected dogs. Pteridines. 1992; 3(1-2):79-81.

Schmidt EMS, Eckersall PD. Acute phase proteins as markers of infectious diseases in small animals. Acta Vet-Beograd. 2015; 65(2):149-161.

Stephens RW, Pedersen AN, Nielsen HJ, Hamers MJ, Høyer-Hansen G, Rønne E, Dybkjaer E, Danø K, Brünner N. ELISA determination of soluble urokinase receptor in blood from healthy donors and cancer patients. Clin Chem. 1997; 43(10): 1868-1876.

Strasser A, May B, Teltscher A, Wistrela E, Niedermüller H. Immune modulation following immunization with polyvalent vaccines in dogs. Vet Immunol Immunopathol. 2003; 94(3-4):113-121.

Szczubiał M, Dąbrowski R, Łopuszyński W. Serum neopterin levels in female dogs with malignant mammary tumours. Vet Comp Oncol. 2014; 12(2):143-148.

Tasdelen Fisgin N, Aliyazicioglu Y, Tanyel E, Coban AY, Ulger F, Zivaliogl M, Esen S, Leblebicioglu H. The value of neopterin and procalcitonin in patients with sepsis. South Med J. 2010; 103(3):216-219.

Torrente C, Manzanilla EG, Bosch L, Fresno L, Rivera Del Alamo M, Andaluz A, Saco Y, Ruiz de Gopegui R. Plasma iron, C-reactive protein, albumin, and plasma fibrinogen concentrations in dogs with systemic inflammatory response syndrome. J Vet Emerg Crit Care (San Antonio). 2015; 25(5):611-619.

Turk J, Miller M, Brown T, Fales W, Fischer J, Gosser H, Nelson S, Shaw D, Solorzano R. Coliform septicemia and pulmonary disease associated with canine parvoviral enteritis: 88 cases (1987-1988). J Am Vet Med Assoc. 1990; 196(5):771-773.

Ünüvar S, Aslanhan H. Clinical significance of increased serum neopterin in chronic kidney failure as a biomarker of cell-mediated immunity. J Med Biochem.2019;38(1):1-5.

Willard M. Canine protein losing enteropathies. Isr J Vet Med 2015; 70:17-20.

Wingfield W, Raffe M. The veterinary ICU book. Teton NewMedia. 2002.

Wu XL, Long D, Yu L, Yang JH, Zhang YC, Geng F. Urokinase-type plasminogen activator receptor as a predictor of poor outcome in patients with systemic inflammatory response syndrome. World J Emerg Med. 2013; 4(3): 190-195.

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RESEARCH ARTICLE

Effects of Different Parthenogenetic Activation Periods on Mouse Embryo Development and Quality

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ABSTRACT

In the present study we investigate the effects of parthenogenetic activation on *in vitro* embryo development and quality in different activation periods. oocytes were obtained 14 hours after human chorionic gonadotropin (hCG) injection from superovulated B6D2F1 female mice then parthenogenetic activation started 18 hours after hCG injection. The oocytes were activated at different activation periods for 3, 4, 5 or 6 hours in 10 mM strontium chloride ($SrCl_2$) + 5 µg/mL^{-1} Cytohalasine B (CB) + 5 nM Trichostatin A (TSA) containing a Ca^{2+} free Chatot Ziomek Brinster (CZB) activation medium, followed by further incubation for two hours at 37°C and 5% CO_2 in embryo culturing medium + TSA. The results in the present study suggested that the parthenogenetic activation of the 6 hour activation period was found to be higher than at 3, 4 and 5 hours.

Keywords: Mouse, Oocyte, Parthenogenetic, Activation period

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Farklı Partenogenetik Aktivasyon Sürelerinin Fare Embriyo Gelişimi ve Kalitesi Üzerine Etkileri

ÖZ

Çalışmamızın amacı, partenogenetik aktivasyonda farklı aktivasyon sürelerinin *in vitro* embriyo gelişimi ve kalitesi üzerindeki etkilerinin araştırılmasıdır. Superovule B6D2F1 ırkı dişi farelere uygulanan insan koryonik gonadotropin (hCG) enjeksiyonundan 14 saat sonra oositler elde edildi ve 18 saat sonra partenogenetik aktivasyona başlandı. Oositler, 10 mM stronsiyum klorür ($SrCl_2$) + 5 µg/mL^{-1} sitokalazin B (CB) + 5 nM trikostatin A (TSA) Ca^{2+} içermeyen Chatot Ziomek Brinster (CZB) medyumu içerisinde 3, 4, 5 ve 6 saat bekletildi. Aktivasyon sonrası, embriyo kültür medyumu + TSA'da inkübatörde 37°C ve %5 CO_2 ortamında 2 saat bekletildi. Son olarak, tüm embriyolar 120 saat süre ile kültüre edildi. Bu çalışmadan elde edilen sonuçlar göre, 6 saatlik partenogenetik aktivasyon başarısının, 3, 4 ve 5 saatlik sürelere göre daha yüksek olduğu saptandı.

Anahtar Kelimeler: Fare, Oosit, Partenogenetik, Aktivasyon süresi

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INTRODUCTION

Studies of reproductive biotechnology tend to focus primarily on the obtaining of more embryos, increased storage capacity (cryopreservation), embryo culturing or developmental mechanisms. Parthenogenetic activation, which is used in reproductive biotechnology, allows for *in vitro* embryo development without presence of sperm (Cuthbertson et al. 1981, Kline 1996).

Parthenogenetic activation is used in such activities as fertilization modeling, somatic cell nuclear transfer (SCNT) (cloning) and stem cell research. In cloning, the most important stage in *in vitro* development is the activation of the oocyte following the somatic cell transfer (Kishikawa et al. 1999, Campbell 1999, De Sousa et al. 2002, Ma et al. 2005, Demir et al. 2014). Electrical and chemical methods are widely utilized in mouse oocytes to achieve parthenogenetic activation, with the $SrCl_2$ chemical activation approach being the most common (Versieren et al. 2010, Han and Gao 2013, Bai et al. 2016).

Due to ethical concerns, human embryo studies in embryonic stem cell research in particular make use of parthenogenetic embryos as an alternative. Parthenogenetic embryonic stem cells serve as a suitable research model for regenerative medicine studies into, for example, cell therapy and tissue repair (Fulka et al. 2011, Didié et al. 2013, Daughtry and Mitalipov 2014).

In vitro parthenogenic embryo development rates may differ, depending on the activation period (Ma et al. 2005, Versiersen et al. 2010, Han and Gao 2013, Heytens et al. 2008, Sung et al. 2010, Gao et al. 2019), and the cloning success ratios. A review of literature identified no studies comparing the effects of the most common activation periods on the rate and quality of parthenogenetic embryo development. To address this situation, the present study evaluates the *in vitro* development rates and development qualities of parthenogenetic activations at different activation periods.

MATERIALS and METHODS

All mice experiments and animal care protocols were approved by the Koç University Local Ethics Committee for Animal Experiments (approval number: 2014 - 05). The animals were kept in the Koç University Animal Research Facility of Centre for Translational Medicine (KUTTAM) in individually ventilated cages with individual HEPA-filtered ventilation and in a 12 hours light–12 hours dark cycle. The mice were fed a diet of commercial pellet food *ad libitum,* and automatic water containers were provided.

Superovulation and oocyte collection

B6D2F1 female mice aged 6–8 weeks were obtained for the study (n=12), selected from among unmated fertile adults. The mice were given 10 IU pregnant mare serum gonadotropin hormone (SIGMA G4877-PMSG) at 5:00 pm via intraperitoneal injection for superovulation, and 48 hours later, 10 IU hCG (SIGMA C8554-hCG) was given intraperitoneally, again at 5:00 pm. The superovulated mice were then sacrificed and a small incision was made in the ampulla region of each oviduct with the help of sterile-toothed forceps. The oocytes with cumulus cells were collected via the rupture of the oviductal ampulla, and were washed with Human Tubal Fluid + HEPES buffered (HTF, global total w/ HEPES) medium + 80 IU/mL hyaluronidase (SIGMA H-3506) + 4 mg/mL Bovine Serum Albumin (BSA, SIGMA A-3311), and the isolated oocytes were washed three times in different 500 µl HTF media. The oocytes were again washed three times in different HTF media with HEPES, and selected high quality oocytes were kept in a four-well plate (Mallol et al. 2014, Taskin et al. 2019a).

Parthenogenetic oocyte activation and embryo culture

Eighteen hours after the hCG injection, the oocytes were incubated for 3, 4, 5 or 6 hours in a 10 strontium chloride ($SrCl_2$) + 5 $\mu g/mL^{-1}$ Cytohalasine B (CB) + 5 nM Trichostatin A (TSA) containing a Ca^{2+} free Chatot Ziomek Brinster (CZB) medium, and then incubated for 2 hours in an incubator at 37°C and 5% CO_2 in an embryo culturing medium (LifeGlobal Media, LGGG-020) + TSA. For the assessment of embryo development, all embryos were transferred into embryo culture drops (10 µl each) and covered completely with mineral oil (LifeGlobal® Oils, LGOL-500) to prevent contamination and evaporation, and to preserve integrity. The embryo culture media were incubated at 5% CO_2 and 37°C temperature and high humidity in an incubator for equilibration for at least 2 hours before embryo culturing. The oocytes were then cultured in an embryo culture medium supplemented with 4 mg/mL BSA (Fraction V. A3311) for 120 hours (Sung et al. 2010, Mallol et al. 2014).

Determination of cell numbers

Blastocysts were incubated in a solution of 100 µg/mL propidumiode (PI) + HTF medium + 1% TritonX100 for 10–12 seconds, and then transferred to a 100 µg/mL 100% ethanol (EMPROVE) + 25 µg/mL Hoechst 33258 (H1398, Molecular Probes, Inc.) solution for overnight incubation at 4°C. On the following day, the blastocysts were transferred to the glycerol droplet after washing in 5 µl glycerol droplet on each glass slide, and covered with a coverslip for blastocyst stabilization. The blastocyst preparations were observed using an inverted microscope with a red and blue fluorescence attachment for the determination of trophectoderm (TE) and inner cell mass (ICM) cell numbers (Mallol et al. 2013, Taşkın et al. 2019b).

Statistical analyses

All experiments were replicated three times. The SPSS Statistics 22.0 program was used for the statistical evaluation of the results. A One-Way ANOVA with a Berferroni post hoc test was used to identify between-group differences.

RESULTS

Results of *In Vitro* Culture

The development evaluations carried out after the *in vitro* culture revealed blastocyst development rates of 66.09%, 70.00%, 73.87% and 87.73% in the 3, 4, 5 and 6 hour activation groups, respectively, and the development rate of the 6 hour parthenogenetic activation was found to significantly higher than that of the 3, 4, 5 ($P < 0.05$) hour groups (Table 1).

Total Cell Numbers

Differential staining of blastocysts revealed the total cell numbers in the 3, 4, 5 and 6 hour groups to be 44.33 ± 4.19, 37.33 ± 6.60, 46 ± 1.63 and 51.33 ± 1.89, respectively. The numbers of cell in the 6 hour group was found to be significantly greater than in the 3, 4 and 5 ($P<0.05$) hour groups (Table 2).

Table 1: *In vitro* development rates.

Parthenogenetic Activation Period	Number of Oocytes (n)	Number of Blastocysts	*In Vitro* Development Rate (%) ± St. dev
3 h	42	28	66.09 ± 6.13^{b}
4 h	40	28	70.00 ± 8.16^{b}
5 h	41	30	73.87 ± 17.53^{b}
6 h	39	34	87.73 ± 5.47^{a}

Differences between the same columns with different symbols ($^{a, b}$) were found to be significant ($P < 0.05$).

Table 2: Cell numbers of activated blastocysts according to differential fluorescence labeling.

Parthenogenetic Activation Period	Mean of Inner Cell Mass Number ± St. Dev.	Mean Trophectoderm Cell Number ± St. Dev.	Mean of Total Cell Number ± St. Dev.
3 h	34.67 ± 4.64	10.67 ± 0.94	44.33 ± 4.19^{b}
4 h	24 ± 5.89	12.67 ± 0.94	37.33 ± 6.60^{b}
5 h	38.33 ± 6.34	11 ± 0.82	46.00 ± 1.63^{b}
6 h	37 ± 2.16	14.33 ± 3.68	51.33 ± 1.89^{a}

Differences between the same columns with different symbols ($^{a, b}$) were found to be significant ($P < 0.05$).

DISCUSSION

Fertilization occurs *in vivo* when an egg is covered by spermatozoa, and when one spermatozoon manages to penetrate the egg through membrane fusion. Oocyte activation is a serial cell mechanism that occurs during fertilization. Parthenogenetic embryo development mimics this condition without spermatozoa, and has been used as a model in human embryonic stem cell research due to ethical concerns, especially in the cloning studies of many species over the last 20 years. A low embryonic development rate is a fundamental problem in cloning studies in particular. In their Honolulu method, Wakayama et al. (1998) injected nuclei from cumulus cells into enucleated oocytes, thus producing the first cloned mouse, who they named "Cumulina". Cloning research now focuses on increasing the birth rates of cloned embryos transferred into surrogate mothers. The success rates from cloning are 0–20%, while the birth rates of cloned mice are 1–2%. The $SrCl_2$ chemical activation method is widely used in cloning. Studies have been performed on such $SrCl_2$ activation parameters as activation time, concentration rate and manipulation media, and have shown that embryo culturing success in particular varies according to the mouse oocyte activation period (Dandekar and Glass 1987, Heytens et al. 2008).

Ma et al. (2005) achieved 50.8% blastocyst development ratio 18 hours after hCG injection in Kunming-strain mouse oocytes at 2.5 hours of activation with 10 mM $SrCl_2$ + Ca^{2+}- free + CB 5 µg/mL. Han et al. (2013), on the other hand, identified a 27.62% blastocyst development ratio in CD1-strain mouse oocytes at 30 minutes following activation with 10 mM $SrCl_2$ + Ca^{2+}- free + CB 5 µg/mL, 18 hours after hCG injection. In the present study, blastocyst development rates as high as 80–90% were observed in the 5 and 6 hour activation groups. Our results suggest that prolonged activation (5 or 6 hours) can have a positive effect on *in vitro* development rates, and the longer the duration of strontium treatment, the greater the calcium oscillations in mouse meiotic oocytes. Moreover, mouse oocyte activation was found to increase in aged oocytes more as the oocyte ages, and also as mitogen-activated protein kinase (MAPK) activity decreases (Alberio et al. 2001).

Heytens et al. (2008) activated B6D2F1-strain mice oocytes for 3 hours at 10 mM $SrCl_2$ + Ca^{2+}- free + Cytohalasine D, 17 hours after hCG injection, and observed a 65% blastocyst development rate. We recorded a similar result 3 hours following activation in B6D2F1-strain mice, although our activation period was shorter, and Cytohalasine B was used in the activation medium instead of Cytohalasine D.

B6D2F1 mice oocytes were activated for 6 hours with $SrCl_2$ + Ca^{2+}free + CB 5 µg/mL by Sung et al. (2010), and a blastocyst development rate of 97.3% was recorded. A similar B6D2F1-strain of mice and the same activation period were used in the present study, and the best activation results were recorded at 6 hours.

Gao et al. (2019) applied 4 hours of chemical activation to the oocytes of C57BL/6j-strain mice and obtained a blastocyst development rate of 84.61%. We also used a 4 hour activation period in the present study, but recorded a lower blastocyst development rate (70.00%). The different strains of mice used in the studies could be one of the reasons for the differences in activation results.

In conclusion, the present study has identified the ideal protocol for chemical activation through a comparison of the *in vitro* development rates and total cell numbers of parthenogenetic mouse blastocysts obtained after different activation periods. Further studies into the molecular mechanisms of parthenogenetic development will support such research areas as cloning, intracytoplasmic sperm injection and stem cell studies.

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REFERENCES

Alberio R, Zakhartchenko V, Motlik J, Wolf E. Mammalian oocyte activation: lessons from the sperm and implications for nuclear transfer. Int J. Dev. Biol. 2001;45(7): 797-809.

Bai GY, Song SH, Wang ZD, Shan ZY, Sun RZ et al. Embryos aggregation improves development and imprinting gene expression in mouse parthenogenesis. Dev Growth Differ. 2016;58 (3): 270-279.

Campbell KHS. Nuclear equivalence, nuclear transfer, and the cell cycle. Cloning. 1999; 1(1): 3–15.

Cuthbertson KS, Whittingham DG, Cobbold PH. Free Ca2+ increases in exponential phases during mouse oocyte activation. Nature. 1981; 294(5843): 754-757.

Dandekar PV, Glass RH. Development of mouse embryos *in vitro* is affected by strain and culture medium. Gamete Res. 1987;17(4): 279–285.

Daughtry B, Mitalipov S. Concise review: parthenote stem cells for regenerative medicine: genetic, epigenetic, and developmental features. Stem. Cells. Transl. Med. 2014;3(3): 290-298.

De Sousa PA, Dobrinsky JR, Zhu J, Archibald AL, Ainslie A et al. Somatic cell nuclear transfer in the pig: control of pronuclear formation and integration with improved methods for activation and maintenance of pregnancy. Biol. Reprod. 2002;66(3): 642–650.

Demir K, Can A, Ertürk E, Özdemirci S, Karacam, H et al. Effect of different activation techniques on immature and in vitro matured cat oocytes. Kafkas Univ. Vet. Fak. Derg. 2014;20: 565-570.

Didié M, Christalla P, Rubart M, Muppala V, Döker S et al. Parthenogenetic stem cells for tissue-engineered heart repair. J. Clin. Invest. 2013;123(3): 1285-1298.

Fulka H, Hirose M, Inoue K, Ogonuki N, Wakisaka N et al. Production of mouse embryonic stem cell lines from maturing oocytes by direct conversion of meiosis into mitosis. Stem Cells. 2011;29(3): 517-527.

Gao W, Yu X, Hao J, Wang L, Qi M et al. Ascorbic acid improves parthenogenetic embryo development through TET proteins in mice. Biosci. Rep. 2019;39 (1): 1-8.

Han BS, Gao JL. Effects of chemical combinations on the parthenogenetic activation of mouse oocytes. Exp. Ther. Med. 2013;5(5): 1281-1288.

Heytens E, Soleimani R, Lierman S, De Meester S, Gerris J et al. Effect of ionomycin on oocyte activation and embryo development in mouse. Reprod. Biomed. Online. 2008;17 (6): 764-771.

Kishikawa H, Wakayama T, Yanagimachi R. Comparison of oocyte-activating agents for mouse cloning. Cloning. 1999;1(3): 153–159.

Kline D. Activation of the mouse egg. Theriogenology. 1996; 45(1): 81–90.

Ma SF, Liu XY, Miao DQ, Han ZB, Zhang X et al. Parthenogenetic activation of mouse oocytes by strontium chloride: A search for the best conditions. Theriogenology. 2005;64(5): 1142–1157.

Mallol A, Santaló J, Ibáñez E. Comparison of three differential mouse blastocyst staining methods. Syst. Biol. Reprod. Med. 2013;59 (2): 117-122.

Mallol A, Santaló J, Ibáñez E. Psammaplin a improves development and quality of somatic cell nuclear transfer mouse embryos. Cell Reprogram. 2014;16 (5): 392-406.

Sung LY, Chang CC, Amano T, Lin CJ, Amano M et al. Efficient derivation of embryonic stem cells from nuclear transfer and parthenogenetic embryos derived from cryopreserved oocytes. Cell Reprogram. 2010;12 (2): 203-211.

Taşkın AC, Kocabay A, Ebrahimi A, Karahüseyinoğlu S, Şahin GN et al. Leptin treatment of *in vitro* cultured embryos increases outgrowth rate of inner cell mass during embryonic stem cell derivation. In Vitro Cell Dev. Biol. Anim. 2019a;55 (7): 473-481.

Taşkın AC, Kocabay A. Leptin supplementation in embryo culture medium increases in vivo implantation rates in mice. Turk J Vet Anim Sci.2019b;43 (3): 359-363.

Versieren K, Heindryckx B, Lierman S, Gerris J, De Sutter P. Developmental competence of parthenogenetic mouse and human embryos after chemical or electrical activation. Reprod Biomed Online.2010;21(6): 769-775.

Wakayama T, Perry AC, Zuccotti M, Johnson KR, Yanagimachi R. Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. Nature. 1998;394 (6691): 369-374.

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RESEARCH ARTICLE

Animal Welfare Attitudes of Pet Owners: An Investigation in Central and Western Parts of Turkey

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ABSTRACT

Today with the start of global pandemics, the concept of "One health-One welfare" is becoming a fact of life as never before. Responsible ownership and good care duty do affect the health and welfare of pet animals, one of the stakeholders of social life. In this study, pet owners' attitudes towards animal welfare were examined. The research was carried out in İzmir, Ankara and Afyonkarahisar that are located in the Central and Western parts of Turkey. Animal Welfare Attitude Scale (AWAS) was applied to pet owners who voluntarily participated in the study. According to the results obtained in the cognitive dimension of the AWAS, pet owners think that animal welfare is affected mostly by housing, feeding and health conditions, and less by slaughter, sacrifice or naming of animals. Findings regarding the behavioral dimension demonstrated that pet owners treat their pets and street animals well but they do not support non-governmental organizations (NGO) working for animal rights and animal protection and they are not willing to purchase and pay more for foods produced under animal friendly standartds. Also, it has been observed that pet owners believe animals are sensitive beings and have rights like humans, but they agree little with the judgment that animals are created for humans. It was concluded that pet owners can support strategies to increase animal welfare and, increasing pet owners' knowledge and awareness relationships between animals and humans, values and norms as well as NGOs can provide valuable opportunities for increasing animal welfare in Turkey.

Key Words: Animal welfare, Attitudes, Owner, Pet animal

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Pet Hayvanı Sahiplerinin Hayvan Refahı Tutumu: Türkiye'nin Orta ve Batısında Bir Araştırma

ÖZ

Günümüzde küresel pandemilerin başlamasıyla birlikte "Tek sağlık-Tek refah" kavramı hiç olmadığı kadar hayatın bir olgusu haline gelmektedir. Sorumlu sahiplik ve iyi bakım toplumsal yaşamın paydaşlarından birisi olan pet hayvanlarının sağlığı ve refahını etkilemektedir. Bu araştırmada pet hayvan sahiplerinin hayvan refahına yönelik tutumları incelenmiştir. Araştırma Türkiye'nin orta ve batısında yer alan İzmir, Ankara ve Afyonkarahisar'da yürütülmüştür. Araştırmaya gönüllü olarak katılan pet sahiplerine Hayvan Refahı Tutum Ölçeği (AWAS) uygulanmıştır. Hayvan refahı tutumunun bilişsel boyutunda elde edilen sonuçlara göre pet sahipleri hayvan refahının en çok barınma, besleme ve sağlık koşulları, en az hayvanların kesilmesi, kurban edilmesi veya onlara isim verilmesi ile etkilendiğini düşünmektedir. Davranışsal boyuta ilişkin bulgular pet sahiplerinin kendi hayvanlarına ve sokak hayvanlarına iyi davrandıklarını ancak hayvan hakları ve hayvan koruma konusunda faaliyet gösteren sivil toplum örgütleri (STK)'ni desteklemediklerini ve yüksek hayvan refahı standartlarında üretilen hayvansal gıdaları satın alma ve daha fazla ödemeye istekli olmadıklarını ortaya koymuştur. Ayrıca evcil hayvan sahiplerinin hayvanların hassas varlıklar olduğu ve insanlar gibi haklara sahip olduklarına inandıkları ancak hayvanların insanlar için yaratıldığı yargısına daha az katıldıkları görülmüştür. Pet hayvan sahiplerinin, hayvan refahını arttırmaya yönelik stratejileri destekleyebilecekleri ve pet hayvan sahiplerinin hayvanlar ve insanlar arasındaki ilişkiler, değerler ve normlar ile STK'larla ilgili bilgi ve farkındalığının artırılmasının Türkiye'de hayvan refahının artırılması için değerli fırsatlar sağlayabileceği sonucuna varılmıştır.

Anahtar Kelimler: Hayvan refahı, Tutum, Sahip, Pet hayvanı

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INTRODUCTION

In the last decades, environmental and animal protection awareness has increased due to climate change and public health crises (Scott 2004, McMichael 2013). Animal protection activist's efforts and global lobbying activities that were aimed to informing people about animal welfare and rights have led to changes in legislation around the world and specifically in the EU on companion, farm and laboratory animals (Odendaal 1988, Boissy et al. 2007, Broom 2017, De la Fuente et al. 2017). In 2013, the European Union initiated studies on welfare of cats and dogs in commercial practices within the scope of animal welfare strategy of 2012-2015. Although there is no specific EU regulation on pet animal welfare yet, there are EU regulations that cover identification (Regulation (EC) No 998/2003, Regulation (EU) No 576/2013, Commission Implementing Regulation (EU) No 577/2013, Council Directive 92/65/EEC), movements (Council Regulation (EC) No 1/2005, Commission Decision 2003/803/EC, Council Directive 92/65/EEC), protection in animal breeding (Council Directive 98/58/EC) and health (Commission Decision 2004/824/EC) of pet animals. Turkey signed the European Convention on Protection of Pet Animals in 1999, and accepted European Union approaches in this area. Except for Law No 5199 on Animal Protection and implementing regulation thereof (dated 12.05.2006 and No 26166), there is no specific regulation on pet animal welfare in Turkey. However, within Turkey's accession negotiations for full membership to EU, several studies for aligning national legislation with the EU legislation on welfare of pet animals continued since 2011.

Legislative sanctions aside, the first step to encourage a worldwide change in people's attitudes concerning animal protection is understanding people's perceptions and attitudes in relation to this issue (De la Fuente et al. 2017, Bozkurt and Sarıal Kubilay 2019). Although there are many factors that have potential to affect pet animal welfare, it is essential to determine the effects of general and specific factors on the human being that are the basis of owner or caregiver behavior (Kirk 2019). Pet owner's duty of care includes social, ethical and public health concepts (Glanville et al. 2020). Besides being a moral duty of protection of animals whose responsibility is assumed, the pet owners' attitudes and behaviors directly affect the welfare of animals. Because pet owners are the people who are providing care, housing and social needs of pet animals (Odendaal 1994, Sanderson et al. 2005, Michel et al. 2008, Slater et al. 2012). Animal welfare perceptions and attitudes are influenced by personal characteristics such as sociodemographic traits (Paul and Serpell 1993, Reevy and Delgado 2015) and values and beliefs (Glanville et al. 2020). Taylor and Signal (2005) reported that those who live with a pet animal have more positive animal welfare attitudes.

Pet owners know the behavioral characteristics of their pets best and they can best understand whether their pets are stressed or not. Therefore, participation of the pet owners in the surveys provides valuable findings in the domain of pet animal welfare (Mariti et al. 2012, Stamps and Groothuis 2010). Mariti et al. (2012) reported that awareness of owners on stress level and well-being of pets play a key role. A good understanding of pet owner's perceptions and attitudes on animal welfare can help increase the welfare of their pets by leading to an increase in the quality of pet care and management (Michel et al. 2008). Paul and Serpell (1993) reported that the results to be obtained by analyzing pet owner's attitudes towards animal welfare can contribute to the development of effective humane education interventions and programs.

The need for the protection of pet animals have increased further with the economic growth of the pet sector. The pet sector is attracting the attention of global markets more than ever before because of pet owners' purchasing behavior for their fur-babies, including health, plastic surgery, spa treatments, foods, accessory and designer clothes (Holbrook and Woodside 2008, Guzman 2017, Haldeman 2018, Kirk 2019). The global size of the pet products and services industry is billions of dollars (American Pet Products Association 2018). There are 66 and 61 million cat and dog owners in the European Union (European Dog&Cat Alliance 2019). It is estimated that there are at least one pet animal in 1.5 million households and a pet sector with a transaction volume of more than 2 billion dollars per year in Turkey (Anonymous 2019). The objective of this study was to examine the attitudes of Turkish pet owners towards animal welfare living in Central and Westesn parts of Turkey.

MATERIALS and METHODS

Survey implementation and data collection

The study was conducted in Izmir, Ankara and Afyonkarahisar that were located in Central and Western of Turkey. The pet owners volunteered to participate in the survey after the scientific objectives of the study were explained to them. The sample size for the cities were determined according to the method applied by Kılıç and Bozkurt 2020. Consideringly the minimum sample size for each region a total of 940 pet owners who has at least one cat or dog were participated in the survey. Animal Welfare Attitude Scale (AWAS), developed by Kılıç and Bozkurt (2020) was applied to examine pet owner's attitudes regarding animal welfare. The questionnaires that were determined to be a contradiction in the answers given to similar questions, or to be imperfect or incorrect data were

excluded, and then the statistical analyzes were done with the 916 questionnaires. In the first part of the AWAS there are the questions about the age, gender and educational background of the animal owners. The questions measuring attitudes towards animal welfare in terms of cognitive (20 items), behavioral (11 items) and affective (10 items) dimensions are in the second part of the scale. Animal welfare attitude scale was applied to pet owners individually and via face to face interviews. The research was summarized from the first author's master's thesis numbered 2019-008 and approved by AKÜHADYEK (Reference no: AKUHADYEK-245-17).

Statistical analysis

The items in cognitive, behavioural and affective dimensions within AWAS scale were applied to pet owners to evaluate their level of agreement according to the 5-point Likert Type rating (1: Strongly Disagree, 2: Disagree, 3: Neutral, 4: Agree, 5: Strongly Agree). The attitudes regarding animal welfare of pet owners were described by calculating the frequency and percentages, means and standard deviations for each dimension and items. All data were analyzed with SPSS 18.0 for Windows (SPSS, Inc., Chicago).

RESULTS

The findings regarding the demographic characteristics of pet owners participating in the study are presented in Table 1. The rates of female and male pet owners were 63.31 and 36.69 %. The percentage of participants that were aged 18 and younger, 19-25, 26-32, 33-39 and 40-50 years old and 51 and older were 5.42, 51.09, 22.38, 9.61, 7.31 and 4.37% respectively. The majority of the participants were university graduate (69.65%) and high-school graduate (19.65%) while 3.38% and 7.32% of them were primary and secondary school graduate.

Descriptive statistics regarding the cognitive dimension of the AWAS scale are given in Table 2. The pet owners were in more positive agreement with the arguments "C3. Animal health conditions affect animal welfare" ($\bar{X}$=4.59), "C2. Animal feeding requirements affect animal welfare" ($\bar{X}$=4.58) and "C1. Conditions of shelter affect animal welfare" ($\bar{X}$=4.52) under cognitive dimention of AWAS scale. The participants showed the lowest agreement for the items "C15. Religious sacrificing of the animals affect animal welfare" ($\bar{X}$=3.39), "C12. Slaughtering of livestock affect animal welfare" ($\bar{X}$=3.62), and "C13. Naming animals affect animal welfare" ($\bar{X}$=3.62).

The results regarding the behavioral dimension of the AWAS scale are presented in Table 3. Under affective dimension of the scale, the respondents approved the arguments with the highest rate "B8. I always behave animals well" ($\bar{X}$=4.52), "B5. I treat to street animals with compassion" ($\bar{X}$=4.39), and "B4. I encourage people to treat animals well" ($\bar{X}$=4.38) as well as the lowest rates were given to the items "B11. I buy food products produced under high animal welfare standards even if they are expensive" ($\bar{X}$=3.80), "B6. I support the civil society organizations involved in animal protection" ($\bar{X}$=3.87), "B10. I buy food products that have been produced in compliance with high animal welfare standards" ($\bar{X}$=3.96), and "B2. Animal welfare issue affect my choices when purchasing animal food products"($\bar{X}$=3.96).

In Table 4, the descriptive statistics concerning affective dimension of the animal welfare attitude scale were presented. The results obtained in this dimension showed that, the highest mean values were calculated for the items "A6. Behave cruel to animals is atrocious" ($\bar{X}$=4.65), "A4. I believe animals are sentient beeings" ($\bar{X}$=4.53), "A8. I believe that animals have rights like people ($\bar{X}$=4.52)" and "A3. I believe that animals have well-being" ($\bar{X}$=4.50). The mean value was dramatically lower than the other items for "A2.Animals have been created for human use"($\bar{X}$=2.87).

Table 1. The sociodemographic characteristics of the participants
Tablo 1. Katılımcıların sosyodemografik özellikleri

Variable	Groups	Numbers (f)	Percantage (%)
Gender	Female	580	63.31
	Male	336	36.69
Age	18 and younger	48	5.24
	19-25	468	51.09
	26-32	205	22.38
	33 –39	88	9.61
	40-50	67	7.31
	51 and older	40	4.37
Education level	Primary school	31	3.38
	Secondary school	67	7.32
	High-school	180	19.65
	University	638	69.65

Table 2: Descriptive statistics regarding the cognitive dimension of animal welfare attitude scale
Tablo 2: Hayvan refahı tutum ölçeğinin bilişsel boyutuna ilişkin betimsel istatistikler

	Items	Aggrement Level						
		1	2	3	4	5	$\bar{X}$	SD
C1	Conditions of shelter affect animal welfare	0.0	0.8	10.2	25.5	63.5	4.52	0.71
C2	Animal feeding requirements affect animal welfare	0.2	1.0	5.6	27.4	65.8	4.58	0.66
C3	Animal health conditions affect animal welfare	0.1	0.4	6.2	26.6	66.7	4.59	0.63
C4	Staff responsible for the care of animals has an impact on animal welfare	0.3	2.3	8.6	30.9	57.9	4.44	0.77
C5	Conditions of transporting animals affect animal welfare	1.5	3.8	17.7	31.1	45.9	4.16	0.95
C6	Conditions that may lead to nervosity affect animal welfare	1.3	0.9	10.4	28.6	58.8	4.43	0.81
C7	The conditions of reproduction of animals affect animal welfare.	0.7	2.6	13.2	34.6	48.9	4.28	0.84
C8	The conditions of reproduction of animals affect animal welfare.	0.2	3.3	15.0	30.2	51.3	4.29	0.85
C9	Equipment and technology used in animal production affect animal welfare	0.9	2.4	14.3	29.1	53.3	4.32	0.87
C10	The feeling of self-confidence affect animal welfare	0.1	2.0	8.8	29.1	60.0	4.47	0.75
C11	The recognition of the animals as an individual affect animal welfare	2.4	3.1	15.3	28.6	50.6	4.22	0.98
C12	Slaughtering of livestock affects animal welfare.	10.4	9.5	21.3	25.8	33.0	3.62	1.31
C13	Naming animals affect animal welfare	11.1	8.1	23.5	21.9	35.4	3.62	1.33
C14	The conditions during transport affect animal welfare.	1.5	4.3	12.5	29.5	52.2	4.27	0.94
C15	Religious sacrificing of the animals affect animal welfare	18.0	9.3	19.3	22.4	31.0	3.39	1.46
C16	Leave the animals in streets (like cats, dogs) affect animal welfare	4.6	5.1	14.5	26.7	49.1	4.10	1.12
C17	The activities of non-governmental organizations supporting animal protection affet animal welfare	2.9	3.2	13.2	31.0	49.7	4.21	0.99
C18	Legislation regarding animal protection affects animal welfare.	1.1	3.8	11.0	30.2	53.9	4.32	0.89
C19	Purchase of food products have been produced in animal friendly production system (milk, egg, meat etc.)affect animal welfare	1.1	3.1	9.4	29.5	56.9	4.38	0.86
C20	Human-animal interaction affect animal welfare.	4.3	5.4	16.8	29.0	44.5	4.04	1.10

Table 3: Descriptive statistics regarding the behavioural dimension of animal welfare attitude scale
Tablo 3: Hayvan refahı tutum ölçeğinin davranışsal boyutuna ilişkin betimsel istatistikler

	Items	Agreement level (%)						
		1	2	3	4	5	$\bar{X}$	SD
B1	I am interested in animal welfare	2.1	4.9	16.4	26.7	49.9	4.17	1.01
B2	Animal welfare issues affect my choices when purchasing animal food products.	3.5	6.6	21.9	26.1	41.9	3.96	1.10
B3	I tell people around me about animal welfare	3.0	6.6	17.2	27.0	46.2	4.07	1.08
B4	I encourage people to treat animals well.	0.8	2.7	10.2	30.3	56.0	4.38	0.84
B5	I treat to street animals with compassion	1.1	1.6	12.0	27.3	58.0	4.39	0.84
B6	I support the civil society organizations involved in animal protection	6.0	8.7	18.6	25.2	41.5	3.87	1.21
B7	I comply with legislation regarding animal welfare	1.1	2.3	11.5	31.0	54.1	4.35	0.85
B8	I always behave animals well	0.0	2.0	7.7	26.9	63.4	4.52	0.72
B9	I make required attempts against animal violence	1.5	2.4	14.0	28.7	53.4	4.30	0.90
B10	I buy food products that have been produced in compliance with high animal welfare standards.	4.5	5.5	20.5	29.0	40.5	3.96	1.11
B11	I buy food products produced under the animal welfare standards even if they are expensive	6.7	6.8	22.5	27.6	36.4	3.80	1.19

Table 4. Descriptive statistics regarding the affective dimension of animal welfare attitude scale
Tablo 4. Hayvan refahı tutum ölçeğinin duyuşsal boyutuna ilişkin betimsel istatistikler

	Items	Agreement level (%)						
		1	2	3	4	5	$\overline{X}$	SD
A1	I think that animal as an individual	2.4	3.2	15.4	27.9	51.1	4.22	0.98
A2	Animals have been created for human use	32.8	11.6	14.5	18.0	23.1	2.87	1.59
A3	I believe that animals have well-being.	0.7	3.0	7.7	23.5	65.1	4.50	0.81
A4	I belive animals are sentient beings	0.9	2.1	6.8	24.0	66.2	4.53	0.79
A5	I can understand an animal of experience pain and suffering.	1.1	3.1	12.1	31.0	52.7	4.31	0.88
A6	Behave cruel to animals is atrocious	1.1	1.4	4.9	16.4	76.2	4.65	0.74
A7	I believe that there is a relationship between domestic violence and intentional harm against animals	1.6	4.6	11.2	23.1	59.5	4.34	0.96
A8	I believe that animals have rights like people	0.5	2.2	8.3	23.0	66.0	4.52	0.78
A9	I believe that attitudes of people regarding animals affect other peoples' perception of them	0.7	2.9	9.8	25.4	61.2	4.44	0.84
A10	I believe that happy animals will produce higher quality products such as meat, milk, eggs, etc.	1.1	2.5	13.4	27.8	55.2	4.34	0.88

DISCUSSION

Two-thirds of the pet owners participating in this study were women and Lue et al. (2008) reported similar rates of female participants in a study they conducted with US pet owners. This result was interpreted as women adopting more pets as well as being more involved in animal welfare initiatives and research. The results regarding the affective dimension of famale's animal welfare attitude is more dominant can be supported by this argument (Selby et al. 1981). In parallel with the notifications of Prato-Previde et al. (2006) it was thought that female's have more tendency to interact (such as talking, playing, friendship) with pet animals than males.

Participants were mostly young (78.71% younger than 32 years old) and educated (89.30% graduated from high school and university). It was thought that socio-economic and geographical conditions may have lead to this result. It is likely that educated and young participants living in big cities were more motivated to adopt a pet animal to cope with the stress and loneliness caused by living alone and heavy workload (Roudebush et al. 2008, Johnson 2009, Chou 2016). In fact, Erten et al. (2019) reported similar reports on the sociodemographic structure of pet owners living in the Southeastern Anatolia and the Mediterranean regions.

The results regarding the cognitive dimension of the animal welfare attitude scale showed that pet owners believe that the housing, feeding and health conditions of pet animals significantly affect animal welfare. These high animal welfare attitude scores regarding animal health and care are involve favorable opportunities for enhancing pet welfare. Because most of the participants were living in big cities such as Ankara and Izmir and, probably their pets could have been home alone all day and have been significantly restrained. This positive attitudes of pet owners can ensure potential opportunities for cooperation with pet owners to fight againts welfare problems caused by limitation of natural behaviors, insufficient exercise (abnormal behaviors, bone health problems, obesity, etc.) and poor feeding (Kienzle et al. 1998, Rohlf et al. 2010). Also, higher education level of pet owners can provide an important advantage in strengthening responsible ownership. Roudebush et al. (2008) also reported that the education level and motivation of the animal owners are very important to increasing animal welfare.

The results on the cognitive dimension of pet owners suggested, pet owners believe that sacrificing or slaughtering animals and naming animals does not affect animal welfare considerably. This result was found surprising at first glance because there was parallelism in the responses for these items of pet owners with Turkish farmers and farm animal keepers (Kılıç and Bozkurt 2013, Çelik and Bozkurt 2016, Bozkurt et al. 2017). It has been thought that, the participants may have a low level of knowledge and awareness of animal welfare or they may have a perception that naming pet animals are not correlated with animal welfare (Kılıç and Bozkurt 2020). Since, many studies show that people create a strong emotional bond with their pet animals and they behave as if they are family members or their child, and named them usually as if they were individuals (Franklin 1999, Prato-Previde et al. 2006, Johnson 2009). Also, according to the results in the cognitive and behavioral dimensions of the animal welfare attitudes of the participants, it was evaluated that the opinions and behaviors of the participants contained some differences for pet animals and farm animals. Pet owners considered that slaughtering or sacrificing

livestock has less impact on animal welfare. This approach has been evaluated to be anthropocentric and it may be related to their consumer perception and attitudes. Participants may have not seen cats or dogs as food like farm animals (Wrye 2009). Already, Morris et al. (2012) reported that it is common in humans that cats or dogs have more cognitive abilities and therefore are superior to other animals.

The results on the behavioral dimension of the animal welfare attitude scale demonstrated that the participants always behave well with pet animals and stray animals and they encourage other people to do that. This findings highlights that Turkish pet owners can show strong empathy for stray animals. Moreover, Dodd et al. (2019) and Erten et al. (2019) reported that pet owners have a high tendency to adopt animals from free sources such as animals that have been abandoned or have a high potential for abandonment. These results are very positive because it is understood that pet owners can support strategies and projects to be developed in terms of increasing the welfare of owned or unowned pet animals in the future.

Pet owners have been found to have a low attitude towards purchasing foods produced in animal friendly production systems. This result was similar to attitudes of owners regarding the slaughter of livestock. The reasons such as food purchasing preferences (vegetarianism, veganism, etc.) (Preylo and Arikawa 2008, Foer 2010, Dodd et al. 2019), animal welfare knowledge or socio-economic conditions (Franklin 1999, Jacobson and Chang 2018) may also have been effective on pet owner's attitude. Dodd et al. (2019) reported a higher rate of vegetarians or vegans among pet owners compared to the general population structure.

It has been observed that pet owners are compassionate towards stray animals but their attitudes toward supporting NGOs working in animal protection (especially for protection of stray animals) are negative. In general, our results show that Turkish pet owners have an important sensitivity to protection of the stray animal population. In Turkey since 2005, the main EU legislation has been transposed into national legislation, collaboration has increased between stakeholders and many NGOs have been established in animal protection. However, it is understood that the effects of adapted EU acquis are yet low, activities of NGOs do not reach sufficiently to society for reasons such as communicative, financial and organizational problems and the animal activists' interactions with society via media is also weak (Talas 2011, Aksulu 2013, Tekvar 2017, Aşar 2018).

In affective dimension of animal welfare attitude scale, pet owners showed the highest participation for the arguments like animals are sentient creatures, animals have rights like humans and it is brutal to mistreat animals. So, pet owners had a dramatically less contributed to the item that animals were created for human use. According to these results, the pet owners admitted the human-animal relationship far from the anthropocentric view and consider animals valuable with a " be sensitivity" approach (Rohlf et al. 2010, Morris et al. 2012, Aşar 2018).

CONCLUSIONS

The results showed that the affective dimension of the animal welfare attitudes of Turkish pet owners in Central and Western of Turkey is quite strong. However, it seems that the cognitive and behavioral dimensions need to be supported to strengthen the animal welfare attitudes of pet owners. It was concluded that pet owners can support strategies to increase animal welfare and, increasing pet owners' knowledge and awareness relationships between animals and humans, values and norms as well as NGOs can provide valuable opportunities for increasing animal welfare in Turkey.

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REFERENCES

Aksulu M. Yeni toplumsal hareketler: Türkiye'de hayvan hakları savunuculuğu ve sosyal medya. Maltepe Üniversitesi Sosyal Bilimler Enstitüsü Radyo Sinema Televizyon Anabilim Dalı. Yüksek Lisans Tezi. 2013; İstanbul.

American Pet Products Association (APPA). National pet owners survey, 2017-2018. American Pet Products Association, Inc. Greenwich, CT. 2018;(http://americanpetproducts.org/Uploads/MemServices/GPE2017_NPOS_Seminar.pdf. Erişim:16.08.2019).

Anonymous. Pet hayvanı sektörü, 2019(https://www.dunya.com/sirketler/2-milyar-dolarlik-pazarda-hektas-hamlesi-haberi-413047, Erişim:21.03.2019).

Aşar H. Hayvan haklarına yönelik temel görüşler ve yanılgıları. Kaygı, Uludağ Üniversitesi Fen-Edebiyat Fakültesi Felsefe Dergisi. 2018; 30:239-251.

Boissy A, Manteuffel G, Jensen MB, Moe RO, Spruijt B, Keeling LJ, Winckler C. Assessment of positive emotions in animals to improve their welfare. Physiology and Behavior. 2007; 92:375–397.

Bozkurt Z, Koçak S, Kılıç İ, Çelikeloğlu K, Hacan Ö, Lenger ÖF, Tekerli M. Attitudes of staff regarding animal welfare: A

description on poultry farms in Afyonkarahisar. Kocatepe Veteriner Dergisi, 2017; 10(4): 308-316.

Bozkurt Z, Sarıal Kubilay GS. Tek sağlık-Tek refah:Başlıca paydaş olarak pet hayvan sahiplerinin rolü.4th International Anatolian Agriculture, Food, Environment and Biology Congress, 20-22 April 2019, Afyonkarahisar. Congress Proceedings Book, pages;339-342.

Broom DM. Animal welfare in the European Union. Brussels: European Parliament Policy Department, Citizen's Rights and Constitutional Affairs, Study for the PETI Committee. 2017; pp. 2427.

Chou Y. The Changing of Social Meanings of Pets and Their Alternative Futures. (Master's Thesis). Tamkang University Graduate Institute of Futures Studies. 2016. (http://www.metafuture.org/pdf/chouthesis.pdf. Erişim:11.05.2019).

Çelik B, Bozkurt Z. The attitudes and perceptions towards animal welfare of staff employed in the care and handling of animals during animal transport in muş province. Kocatepe Vet J 2016; 9(4): 294-303.

De la Fuente MF, Souto A, Caselli C, Schiel N. People's perception on animal welfare: why does it matter?. Ethnobiology and conservation. 2017;6.

Dodd SA, Cave NJ, Adolphe JL, Shoveller AK, Verbrugghe A. Plant-based (vegan) diets for pets: A survey of pet owner attitudes and feeding practices. PloS one. 2019; 14(1):e0210806.

Erten Ö, Öztürk Y, Yılmaz, O. Türkiye'de pet hayvan sahiplerinin sosyo-demografik yapıları ve pet hayvancılığına bakışları; Alanya-Mardin örneği. Mehmet Akif Ersoy Üniversitesi Veteriner Fakültesi Dergisi. 2019; 4(2), 76-83.

European Dog&Cat Alliance. The welfare of dogs and cats involved in commercial practices:a review of the legislation across EU countries; 2019. (https://www.ispca.ie/uploads/Welfare-EU-dogs-cats.pdf, Erişim: 02.01.2019).

Foer JS. Eating animals. 2010; Penguin UK.

Franklin A. Animals and Modern Cultures: A Sociology of Human-Animal Relations in Modernity.1999; London, UK: Sage Publications.

Glanville CR, Hemsworth PH, Coleman GJ. Conceptualising dog owner motivations: The pet care competency model and role of'duty of care'.Animal Welfare.2020; 29(3), 271-284.

Guzman Z. Owning a pet can cost you $42,000, or 7 times as much as you expect. 2017; (https://www.cnbc.com/2017/04/27/how-much-does-it-cost-to-own-a-dog-7-times-more-than-you-expect.html.).

Haldeman P. The secret price of pets. The New York Times. 2018;(https://www.nytimes.com/2018/07/04/style/how-to-pamper-your-pet.html).

Holbrook MB, Woodside AG. Animal companions, consumption experiences, and the marketing of pets: Transcending boundaries in the animal–human distinction. 2008.

Jacobson KC, Chang L. Associations between pet ownership and attitudes toward pets with youth socioemotional outcomes. Frontiers in Psychology. 2018; 9, 2304.

Johnson J. Dogs, Cats, and Their People: The Place of the Family Pet and Attitudes about Pet Keeping. (A thesis presented to the University of Waterloo in fulfilment of the thesis requirement for the degree of Master of Arts in Public Issues Anthropology. 2009; Waterloo, Ontario, Canada. (https://uwspace.uwaterloo.ca/bitstream/handle/10012/4379/Johnson_Jill.pdf;sequence=1).

Kılıç İ, Bozkurt Z. Assessment of Turkish consumer attitudes using an Animal Welfare Attitude Scale (AWAS). Veterinaria México OA. 2020; 7(1).

Kılıç İ, Bozkurt Z. The relationship between farmers' perceptions and animal welfare standards in sheep farms. Asian Australas. J. Anim. Sci. 2013; 26 (9) : 1329-1338.

Kienzle E, Bergler R, Mandernach AA. comparison of the feeding behavior and the human-animal relationship in owners of normal and obese dogs. The Journal of Nutrition. 1998; 128:2779S–2782S.

Kirk CP. Dogs have masters, cats have staff: Consumers' psychological ownership and their economic valuation of pets. Journal of Business Research. 2019;99:306-318.

Lue TW, Pantenburg DP, Crawford PM. Impact of the owner-pet and client-veterinarian bond on the care that pets receive. Journal of the American Veterinary Medical Association. 2008; 232(4): 531-540.

Mariti C, Gazzano A, Moore JL, Baragli P, Chelli L, Sighieri C. Perception of dogs' stress by their owners. Journal of Veterinary Behavior: Clinical Applications and Research. 2012; 7(4): 213-219.

McMichael AJ. Globalization, climate change, and human health. New England Journal of Medicine. 2013; 368(14):1335-1343.

Michel KE, Willoughby KN, Abood SK, Fascetti AJ, Fleeman LM, Freeman LM, Laflamme DP, Bauer C, Kemp BLE, Van Doren JR. Attitudes of pet owners toward pet foods and feeding management of cats and dogs. Journal of the American Veterinary Medical Association. 2008; 233(11):1699-1703.

Morris P, Knight S, Lesley S. Belief in animal mind: does familiarity with animals influence beliefs about animal emotions?. Society & Animals.2012; 20(3):211-224.

Odendaal JSJ. Die veearts en diereregte (the veterinarian and animal rights). J. S. Afr. vet. Assoc.1988; 59 (2):87-97.

Odendaal JSJ. Demographics of companion animals in South Africa. J. S. Afr. vet. Assoc.1994; 65(2): 67-72.

Paul ES, Serpell JA. Childhood pet keeping and humane attitudes in young adulthood. Animal Welfare. 1993; 2(4): 321–337.

Prato-Previde E, Fallani G, Valsecchi P. Gender differences in owners interacting with pet dogs: an observational study. Ethology. 2006; 112(1):64-73.

Preylo BD, Arikawa H. Comparison of vegetarians and non-vegetarians on pet attitude and empathy. Anthrozoös. 2008; 21(4): 387-395.

Reevy GM, Delgado MM. Are emotionally attached companion animal caregivers conscientious and neurotic? Factors that affect the human–companion animal relationship. Journal of Applied Animal Welfare Science. 2015;18(3):239-258.

Rohlf VI, Bennett PC, Toukhsati S, Coleman G. Why do even committed dog owners fail to comply with some responsible ownership practices?. Anthrozoös. 2010; 23(2):143-155.

Roudebush P, Schoenherr WD, Delaney SJ. An evidence-based review of the use of therapeutic foods, owner education, exercise, and drugs for the management of obese and overweight pets. J. Am. Vet. Med. Assoc. 2008; 233:717–725.

Sanderson S, Finco D, Pogrelis A, Stacy L, Unger C. Owner impressions of three premium diets fed to healthy adult dogs. J Am Vet Med Assoc. 2005;227:1931–6.

Scott P. What are companion animals? The Companion Animal Welfare Council. 2004;(www.awselva.co.uk/cawc.pdf. Erişim: 19.07.2019).

Selby L, Rhoades A, John D. Attitudes of the public towards dogs and cats as companion animals. Journal of Small Animal Practice.1981; 22.3: 129-137.

Slater MR, Weiss E, Lord LK. Current use of and attitudes towards identification in cats and dogs in veterinary clinics in Oklahoma City, USA. Animal Welfare-The UFAW Journal. 2012; 21(1): 51.

Stamps J, Groothuis TGG. The development of animal personality: Relevance, concepts and perspectives. Biol. Rev. 2010; 85:301–325.

Talas, M. Sivil toplum kuruluşları ve Türkiye perspektifi. Türklük Bilimi Araştırmaları, 2011;29: 387-401.

Taylor N, Signal TD. Empathy and attitudes to animals. Anthrozoös. 2005;18(1):18-27.

Tekvar SO. HAYTAP'ın hayvan hakları mücadelesinde çatışmayönetimi: Hayvan hakları savunucularının kendi aralarında uzlaşması mümkün mü?. Uşak Üniversitesi Sosyal Bilimler Dergisi. 2017; 10(2):181-204.

Wrye J. Beyond pets: Exploring relational perspectives of petness. The Canadian Journal of Sociology / Cahiers canadiens de sociologie.2009; 34:1033-1063.

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RESEARCH ARTICLE

Sprayed Intraperitoneal and Incisional Lidocaine Reduces Early Postoperative Pain After Ovariohysterectomy in Dogs

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ABSTRACT

In the present study, it was aimed to investigate the effect of intraperitoneal and incisional sprayed lidocaine on postoperative stress, oxidative stress and pain in dogs undergoing ovariohysterectomy. The control group (n=12) received intraperitoneal and incisional sprayed 0.9 % NaCl, whereas the preparation of lidocaine (8.8 mg/kg) with the equal volume of 0.9 % NaCl following calculation of the individual doses was sprayed in the experiment group (n=12). Modified Melbourne pain assessment was performed before sedation (T), at the end of surgery (T0), 30 minutes after surgery (T½) and at 1 (T1), 2 (T2), 4 (T4) and 6 (T6) hours after the end of surgery. Venous blood samples were collected to measure the concentrations of serum cortisol, total oxidant status (TOS) and total antioxidant status (TAS) at related times. Oxidative stress index (OSI) was calculated by the determination of the rate of TOS/TAS. The concentrations of cortisol, TAS and OSI did not show any significant difference between groups ($p > 0.05$). The concentration of TOS was the highest at T1 in the experiment group ($p<0.05$). The pain scores in the experiment group were lower ($p<0.05$) than those detected at T2 ($p<0.05$), T4 ($p<0.01$) and T6 ($p<0.01$) in the control group. In conclusion, it was stated that the treatment of intraperitoneal and incisional sprayed lidocaine is effective on postoperative pain management in dogs undergoing ovariohysterectomy.

Keywords: Lidocaine, TOS, TAS, OSI, Pain

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İntraperitoneal ve İnsizyonel Sprey Lidokain Uygulaması Köpeklerde Ovaryohisterektomi Sonrası Erken Postoperatif Ağrıyı Azaltır

ÖZ

Sunulan çalışmada, ovariohisterektomi yapılan köpeklerde intraperitoneal ve insizyonel sprey lidokain uygulamasının operasyon sonrası stres, oksidatif stres ve ağrı üzerine etkilerinin araştırılması amaçlandı. Kontrol grubuna (n=12) sprey şeklinde intraperitoneal ve insizyonel % 0.9 NaCl uygulanırken, deneme grubu (n=12) bireysel doz hesaplamasını takiben eşit dozda % 0.9 NaCl ile hazırlanmış lidokain (8,8 mg/kg) aldı. Modifiye Melbourne ağrı skorlaması sedasyon öncesi (T), operasyon bitimi (T0), operasyondan 30 dakika (T½), 1 (T1), 2 (T2), 4 (T4) ve 6 (T6) saat sonra gerçekleştirildi. İlgili zamanlarda venöz kan örnekleri alınarak, serum kortizol, total oksidan durum (TOD) ve total antioksidan durum (TAD) ölçümleri yapıldı. Oksidatif stres indeksi (OSI) TOD/TAD oranına göre belirlendi. Kortizol, TAD ve OSI değerlerinin gruplar arasında istatistiksel olarak fark oluşturmadığı belirlendi ($p > 0.05$). Deneme grubunda TOD düzeyinin T1 zamanında en yüksek seviyede olduğu gözlendi ($p<0.05$). Deneme grubundaki ağrı skorlarının kontrol grubundaki T2 ($p<0.05$), T4 ($p<0.01$) ve T6 ($p<0.01$) zamanlarına göre daha düşük olduğu tespit edildi. Sonuç olarak, ovariohisterektomi yapılan köpeklerde intraperitoneal ve insizyonel sprey lidokain uygulamasının postoperatif ağrı yönetiminde etkili olduğu ifade edilmektedir.

Anahtar Kelimeler: Lidokain, TOD, TAD, OSI, Ağrı

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INTRODUCTION

Ovariohysterectomy is a common surgical procedure in female dogs (Gunay et al. 2011, Yilmaz et al. 2014, Kibar et al. 2019, Korkmaz et al. 2019) due to its potential benefits, including the prevention of unwanted pregnancies, mammary tumour formation, pyometra and the presence of vaginal discharge due to pyometra (Davidson et al. 2004). Internationally, veterinary canine ovariohysterectomies are performed under routine surgical protocols and in controlled environments that produce relatively comparable amounts of stress and tissue injury. Apart from the use of analgesics, medical intervention is generally limited (Gautier et al. 2019). The wide spectrum of unfavorable alterations in normal body homeostasis following surgery is collectively referred to as surgical stress (Anup et al. 1999, 2000). The major source of stress for the animal is not only the surgery procedure itself, but also surgery-associated parameters such as human handling, anesthesia-induced dysphoria, pain, analgesia and mid or long term care in a hospitalization unit (Nenadovic et al. 2017). Moreover, it has been demonstrated that oxidative stress is another unexpected postoperative condition of ovariohysterectomy (Lee and Kim 2014). Cumulatively, tissue damage, oxidative stress and pain during and after surgery may lead to poor postoperative outcomes (Sies 1997).

It has been reported that ovariohysterectomies on dogs are generally performed without specific treatment for pain by practitioners (Carpenter et al. 2004). The reasons for this approach include the expense of analgesics, the data recording requirements of those analgesic drugs, concerns that the recovery process might be impaired (Lamont et al. 2000, Carpenter et al. 2004) and the difficulties in pain recognization (Carpenter et al. 2004). Local anesthetics have been widely used in the veterinary field due to its analgesia potency by regional blockade, easy accessible property and comparatively inexpensive price. Lidocaine is considered a good option as a local analgesic due to its extended time of activity (Wilson et al. 2004). The anesthetic and analgesic effects and postoperative pain relief of various treatment methods of lidocaine have previously been reported, including line block (McKune et al. 2014), local infiltration to mesovarium (Bubalo et al. 2008), intravenous (Tsai et al. 2013, Lu et al. 2016), intraperitoneal (Kibar et al. 2019, Carpenter 2004) and incisional (Carpenter 2004). However, the data of sprayed intraperitoneal and incisional lidocaine treatment under the combination of xylazine and ketamine anesthesia are limited. Therefore, the present study was aimed to demonstrate the effect of sprayed intraperitoneal and incisional lidocaine treatment on the management of postoperative pain and oxidative stress in dogs undergoing ovariohysterectomy.

MATERIALS and METHODS

A total of 24 bitches of various breeds referred to the university animal hospital for elective ovariohysterectomy were used in the study. Animals weighing 26±1.2 kg were randomly separated in two groups. All procedures were approved by the Local Ethic Committee of Afyon Kocatepe University (AKUHADYEK-159-17). All animals were kept at the hospitalization unit of the animal hospital and food or water consumption was not allowed for the eight hours immediately before ovariohysterectomy.

Anesthesia and Surgery Protocol

Sedation was performed by 0.045 mg/kg subcutaneous (s.c.) atropine (Atropin, Vetaş, Turkey) 30 minutes (min) prior to the injection of xylazine HCl (2-3 mg/kg intramuscular (i.m.); Alfazyne 2%, Egevet, Turkey). Meloxicam (0.2 mg/kg, s.c., Maxicam, Sanovel, Turkey) was injected following the sedation. Induction of anesthesia was continued by 10 mg/kg i.m. ketamine HCl (Alfamyne 2%, Egevet, Turkey). An intravenous (IV) catheter was introduced into the cephalic vein for further blood sampling and fluid therapy. Intravenous lactated Ringer's solution (10 mL/kg/h) was provided throughout the procedure. All surgeries were performed by the same surgeon from median line in a routine manner to avoid bias between the groups as previously described elsewhere (Korkmaz et al. 2019). Briefly, the ventral abdomen was prepared aseptically for ovariohysterectomy and a midline incision (1.5 - 2.5 cm) was performed. The uterine ligament was held by a uterine hook following the incision of line alba to reach the abdominal cavity. The cranial part of both ovaries and cervix uteri were ligatured. In the experiment group (n=12), lidocaine (8.8 mg/kg, L-anestin %2, Alke, Turkey) was prepared using the equal volume of saline following calculation of the individual doses. During each step of removing the ovaries and the entire uterus, lidocaine was sprayed to the related parts as well as the left and right or cranial and caudal parts of the abdominal cavity and finally, to the incision line just before closing the skin. The control group received only sprayed saline. The durations of anaesthesia and surgery were recorded. The animals were observed for signs of lidocaine toxicity during the postoperative process. Postoperative care was maintained by daily injections of penicillin + streptomycin (20 mg/kg, i.m. Penoksal, Vilsan, Turkey) for five consecutive days. Sutures were removed ten days after surgery.

Assessment of concentrations of blood cortisol, total oxidant status, total antioxidant status and oxidative stress index

Blood samples were collected following the each pain assessment process. Blood samples were immediately centrifuged at 5000 rpm for 10 minutes and then sera were stored at -20°C until further analysis of the

concentrations of cortisol, total oxidant status (TOS) and total antioxidant status (TAS). The analysis of TOS and TAS (Erel 2004, Erel 2005) before sedation (T), at the end of surgery (T0), 30 minutes after surgery (T½) and at 1 (T1), 2 (T2) and 4 (T4) hours after the end of surgery and as well as the cortisol at T, T0, T½, T1, T2, T4 and T6 was performed by commercial kits using ELISA method (Table 1). Oxidative stress index (OSI) was determined by division of the values of TOS (μmol H_2O_2 Eq/L) to TAS (mmol Trolox Eq/L) (Baysal et al. 2012).

Assessment of pain scoring

Modified Melbourne Pain Scale (MMPS) was used for the evaluation of pain by the same person, who did not know the groups in the study (Table 2). This blind assessment was performed at T, T0, T½, T1, T2, T4 and T6. Butorphanol (0.2 mg/kg, i.v., Butomidor, Richter Pharma A.G. Wels, Austria) was prepared as a rescue analgesic at any time, when the MMPS was scored higher than 9 points.

Statistics

The distribution of normality of data was analysed by Shapiro-Wilk normality test. It was found that all data had the normal distribution. Therefore, differences in duration of surgery, pain scores and the concentrations of cortisol, TOS and TAS as well as OSI rates detected during measurement times between the groups were compared by using t test. A repeated measures two way ANOVA test was used to compare differences within the groups (SPSS 16.0). Values were described by mean ± Standard Error Mean (SEM). The data were considered to be significantly different at $p < 0.05$.

Table 1: The information of sensitivity, coefficient of variations and provider of commercial test kits for the measurement of cortisol, total oxidant status (TOS) and total antioxidant status (TAS).

Test	Sensitivity	Coefficient of variations		Provider
		Intraassay	Interassay	
Cortisol	2.5 ng/ml	8.1 %	6.6 %	EIA-1887, DRG, USA
TAS	4μmol/L	3.3 %	2.8 %	Mega Tıp, Gaziantep, Turkey
TOS	1.20 mmol/L	3.9 %	3.2 %	Mega Tıp, Gaziantep, Turkey

Table 2: Modified Melbourne Pain Scoring Scale

Dog Name/ID: _____ Date: _____ Time Point: _____ Breed: _____	
Total UMPS Score: _____	
Category and descriptor Score _____	
From outside the cage	
Vocalization (choose only one)*	
Not vocalizing	0
Slight vocalization but dysphoric	1
Intermittent vocalization	2
Continuous vocalization	3
Posture	
a) Guarding or protecting affected area	2
b) Position (choose only one)	
Lateral recumbency	0
Sternal recumbency	1
Sitting, standing, or comfortable	1
Standing with head hanging	2
Moving	1
Abnormal posture and/or uncomfortable, continuous position change	2
Activity (choose one)	
At rest	
Sleeping	0
Semi-conscious	0
Awake	1

Eating	0
Restless (pacing continuously; getting up and down)	2
Rolling and thrashing	3
From inside the cage	
Mental status (choose only one)** Baseline minus current score = overall score	
Too sedate to evaluate or dysphoric	0
Submissive	1
Uninterested in people (unusual for this dog)	2
Overtly friendly	3
Wary or Aggressive	4
*Response to palpation (choose only one)****	
Normal, allows palpation of surgical site	0
Allows but then moves away, tenses or looks when surgical area touched	1
Increased whining or painful expression when surgical area touched	2
Will not allow general surgical area to be touched	3
*Vocalization (choose only one)**	
Not vocalizing	0
Vocalizing but responds to quiet voice and/or stroking	1
Vocalizing when touched	2
Intermittent vocalization	2
Continuous vocalization	3

The minimum possible score is 0; the maximum possible score is 20.*Does not include alert barking. **For this category, score recorded is the score obtained after surgery minus the score obtained before surgery. ***Palpate around the general surgical area starting at the dorsal end and working toward incision site

RESULTS

Duration of ovariohysterectomy in the control group was 25.50±3.50 minutes, whereas it was 26.30±3.35 minutes in the experiment group. It was found that the duration of surgery did not differ significantly between groups. It was observed that the concentrations of cortisol in the control group slightly increased until T2 and decreased after T4. However, these changes were not significant statistically ($p>0.05$). A similar pattern was also observed in the experiment group but only the concentrations detected at T1 and T2 were higher ($p<0.001$) than those obtained at T (Table 3). Although lower concentrations of cortisol were detected at T4 and T6 in the experiment group as compared to the control group, the differences at other measurement times between the control and the experiment groups were not significant statistically ($p>0.05$) (Figure 1).

It was seen that the concentration of TOS detected at T slightly increased at subsequent measurement times in the control group but all those changes were not significant statistically ($p>0.05$) (Table 4). The concentrations of TOS detected in the experiment group showed that the highest concentration of TOS was at T1 ($p<0.05$) and this was statistically similar to the other measurement times, except T. In addition, the TOS value at T1 in the experiment group was higher ($p<0.05$) than those detected in the control group. On the other hand, the concentrations of TAS did not show any significant difference within or between the groups ($p>0.05$) (Table 5).

The OSI value in the control group increased at the end of surgery and was higher at other measurement times than those detected at the sedation time, but those changes were not statistically significant ($p>0.05$). Similarly, non-significant higher values at T1 and T4 in the experiment group were observed. Moreover, there was no significant difference between groups ($p>0.05$) (Table 6).

The scores of pain assessment initiated at the end of surgery in the control and the experiment groups are shown in Table 7. Accordingly, it was observed that the pain scores gradually increased until T4 ($p<0.001$) and remained high in the control group, whereas the pain scores in the experiment group increased ($p<0.001$) until T1 and remained high (Table 7). However, the comparison of pain scores between control and experiment groups revealed that pain scores obtained at T2 ($p<0.05$), T4 ($p<0.01$) and T6 ($p<0.01$) in the control group were higher than those detected in the experiment group (Figure 2).

Table 3: The concentrations of cortisol (ng/dL) detected before sedation (T), at the end of surgery (T0), 30 minutes after surgery (T ½) and at 1 (T1), 2 (T2), 4 (T4) and 6 (T6) hours after the end of surgery following the pain assessment process (Mean±SEM).

Blood Sampling Time (hour)	Control (n=12)	Experiment (n=12)
T	136.45 ± 21.19	100.34 ± 12.36[b]
T 0	184.96 ± 24.28	187.44 ± 26.73[ab]
T ½	196.69 ± 29.62	206.98 ± 38.77[ab]
T 1	212.36 ± 31.23	223.80 ± 24.98[a]
T 2	241.45 ± 33.48	266.77 ± 27.18[a]
T 4	239.64 ± 24.74	200.32 ± 22.36[ab]
T 6	211.12 ± 28.41	158.62 ± 20.71[ab]

Small ([ab]) letters in superscript indicate significant differences ($p<0.001$) within the experiment group.

Table 4: The concentrations of total oxidant status (TOS) (µmol/L) detected before sedation (T), at the end of surgery (T0), 30 minutes after surgery (T ½) and at 1 (T1), 2 (T2) and 4 (T4) hours after the end of surgery following the pain assessment process (Mean±SEM).

Blood Sampling Time (hour)	Control (n=12)	Experiment (n=12)
T	2.65 ± 0.21	2.72 ± 0.37[b]
T 0	3.77 ± 0.51	4.47 ± 0.72[ab]
T ½	3.63 ± 0.68	3.54 ± 0.16[ab]
T 1 *	3.13 ± 0.41	4.93 ± 0.61[a]
T 2	3.44 ± 0.43	3.42 ± 0.34[ab]
T 4	3.82 ± 0.69	4.73 ± 0.58[ab]

Small ([ab]) letters in superscript indicate significant differences ($p<0.05$) within the experiment group. * indicates significant difference ($p<0.05$) between control and experiment groups.

Table 5: The concentrations of total antioxidant status (TAS) (mmol/L) detected before sedation (T), at the end of surgery (T0), 30 minutes after surgery (T ½) and at 1 (T1), 2 (T2), and 4 (T4) hours after the end of surgery following the pain assessment process (Mean±SEM).

Blood Sampling Time (hour)	Control (n=12)	Experiment (n=12)
T	1.84 ± 0.06	1.83 ± 0.15
T 0	1.80 ± 0.09	1.85 ± 0.01
T ½	1.85 ± 0.09	1.86 ± 0.02
T 1	1.96 ± 0.23	1.78 ± 0.07
T 2	1.84 ± 0.01	1.86 ± 0.01
T 4	1.72 ± 0.10	1.80 ± 0.10

Table 6: The oxidative stress index (OSI) [TOS (µmol H_2O_2 Eq/L)/TAS (mmol Trolox Eq/L)] detected before sedation (T), at the end of surgery (T0), 30 minutes after surgery (T ½) and at 1 (T1), 2 (T2), and 4 (T4) hours after the end of surgery following the pain assessment process (Mean±SEM).

Blood Sampling Time (hour)	Control (n=12)	Experiment (n=12)
T	1.74 ± 0.13	1.84 ± 0.44
T 0	2.09 ± 0.24	2.41 ± 0.39
T ½	1.88 ± 0.36	1.89 ± 0.09
T 1	1.76 ± 0.38	2.80 ± 0.36
T 2	1.87 ± 0.24	1.83 ± 0.18
T 4	2.27 ± 0.42	2.65 ± 0.28

Table 7: Distribution of pain scores (Mean±SEM) detected before sedation (T), at the end of surgery (T0), 30 minutes after surgery (T ½) and at 1 (T1), 2 (T2), 4 (T4) and 6 (T6) hours after the end of surgery in the control and experiment groups.

Pain Scoring Time (hour)	Control (n=12)	Experiment (n=12)
T 0	0.00 ± 0.00^{e}	0.08 ± 0.08^{c}
T ½	1.41 ± 0.28^{d}	1.75 ± 0.30^{b}
T 1	2.75 ± 0.39^{c}	3.00 ± 0.17^{a}
T 2	4.08 ± 0.33^{b}	3.25 ± 0.18^{a}
T 4	5.08 ± 0.31^{ab}	3.83 ± 0.32^{a}
T 6	5.66 ± 0.25^{a}	3.91 ± 0.39^{a}

Small (^{abc}) letters in superscript indicate significant differences ($p<0.001$).

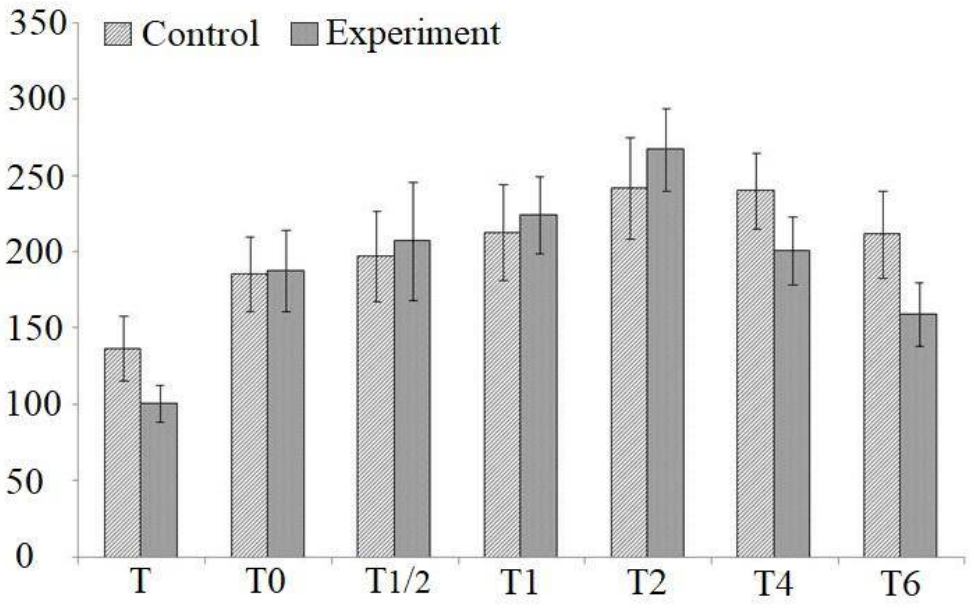

Figure 1. Representative changes of concentrations of cortisol (ng/dL) before sedation (T), at the end of surgery (T0), 30 minutes after surgery (T ½) and at 1 (T1), 2 (T2), 4 (T4) and 6 (T6) hours after the end of surgery between the control and experiment groups.

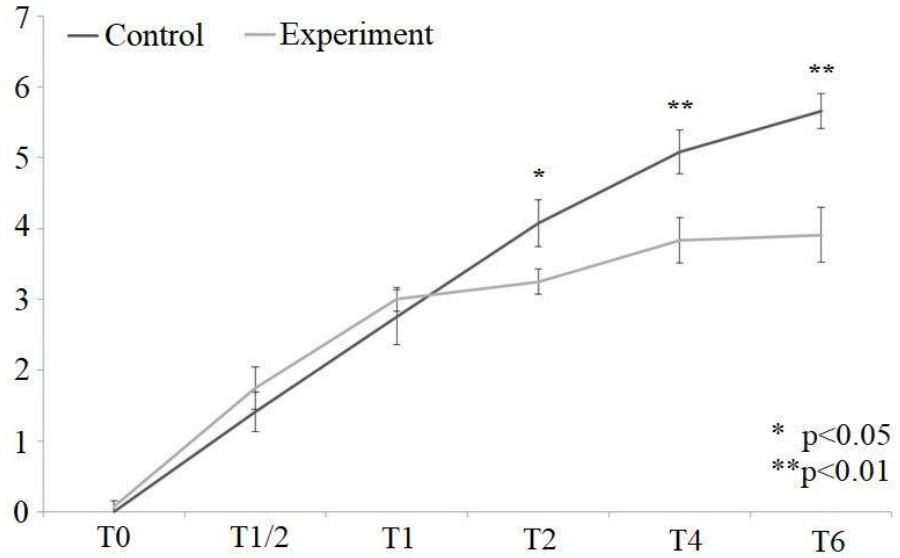

Figure 2. The comparison of pain scores detected before sedation (T), at the end of surgery (T0), 30 minutes after surgery (T ½) and at 1 (T1), 2 (T2), 4 (T4) and 6 (T6) hours after the end of surgery between control and experiment groups.

DISCUSSION

Ovariohysterectomy is a common elective surgery in which tissue trauma and inflammation due to surgical manipulations cause pain in dogs (Lemke et al. 2002). It has been reported that the postoperative pain following 24 hours changes animal behaviours (Lemke et al. 2002; Tsai et al. 2013). It has been postulated that ovariohysterectomy is also a common model for the evaluation of postoperative pain and efficacy of analgesic drugs, since the surgery is performed in healthy dogs without the evidence of pain (Devitt et al. 2005, Michelsen et al. 2012, Tsai et al. 2013, Yilmaz et al. 2014, Korkmaz et al. 2019). Therefore, the effect of intraperitoneal and incisional administration of lidocaine on postoperative pain was evaluated in dogs undergoing ovariohysterectomy in this study.

It has been reported that standard ovariohysterectomy takes 18.3±3.9 minutes, while 20.8±4 minutes is needed for laparoscopic ovariohysterectomy (Devitt et al. 2005). In another report, it has been stated that ovariohysterectomies are performed by experienced veterinary surgeons in 13 to 28 minutes (Michelsen et al. 2012). In the present study, ovariohysterectomies were performed in 25.50±3.50 minutes and 26.30±3.35 minutes in the control and the experiment groups, respectively. The duration of surgery between groups did not show any significant difference and these durations in both groups were consistent with above-mentioned reports (Devitt et al. 2005, Michelsen et al. 2012).

Local anesthetics such as lidocaine have been widely used in the veterinary field however, lidocaine disparately from other analgesic drugs, completely blocks the sensory nerve fibres and inhibits the development of pain by preventing central sensitization (Lemke and Dawson 2000). The pharmacokinetics of lidocaine 2 % with the combination of epinephrine (1:200.000-1:400.000) have been showed that plasma concentrations rapidly decrease with no toxic concentrations (Wilson et al. 2004). The high volume of local anaesthetics needed for intraperitoneal anesthesia has been observed to cause side effects such as sedation, vomiting, tremors and seizures in a dose dependent manner (Carpenter et al. 2004, Kim et al. 2012). In the present study, it was observed that lidocaine did not cause any of the above-mentioned side effects.

In dogs, major abdominal surgeries such as ovariohysterectomy cause significant hormonal changes in response to surgical manipulation and these changes reach their peak near the end of the surgery or shortly after the recovery from anesthesia. The short-lived stress response to ovariohysterectomy returns to its preoperative values by 5 hours after surgery (Benson et al. 2000) and the concentrations of cortisol returns the baseline value by 12 (Yilmaz et al. 2014) or 24 hours postsurgery (Church et al. 1994, Fox et al. 1994, Benson et al. 2000). Since the hypothalamus-epiphysis-adrenal axis causes a response during environmental changes, anesthesia and surgery (Church et al. 1994), the serum cortisol concentrations seem to be an important stress marker of this response in bitches. Therefore, serum cortisol concentrations were measured to evaluate the postoperative stress response to surgery in the present study. Furthermore, prostaglandins that are produced under the circumstances of stress (Bugajski et al. 2004, Rettori et al. 2009), stimulate the secretion of corticotrophin releasing hormone (CRH), vasopressin and adrenocorticotrophin hormone (ACTH) (Gadek-Michalska et al. 2005) and the release of corticosterone, by acting directly in the adrenal gland (Wang et al. 2000, Mohn et al. 2005). Therefore, the concentrations of cortisol are indirectly decreased by the inhibition of prostaglandin synthesis via cyclooxygenase (COX) enzyme inhibition (Yilmaz et al. 2014). It is well known that meloxicam has been used in dogs for medium to long term treatment of pain and inflammation and has selectivity against COX2 versus COX1 (Distel et al. 1996, Yilmaz et al. 2014). On the other hand, it has been reported that the concentrations of cortisol rise for 2.5 hours following ovariohysterectomy in meloxicam-injected dogs (Yilmaz et al. 2014). In the present study, it is thought that the acute inhibition of prostaglandin in the control and experiment groups could not be achieved because of the injection of meloxicam during premedication. It has been indicated that the concentrations of cortisol increase at one and two hours after standard or laparoscopic ovariohysterectomy as compared to the basal values (Devitt et al. 2005). Moreover, a rapid decrease in the concentrations of cortisol has been reported following the administration of intraperitoneal local anesthetics during laparoscopic ovariohysterectomy in dogs (Kim et al. 2012). In the present study, similar to previous reports (Devitt et al. 2005, Kim et al. 2012, Yilmaz et al. 2014), increasing postoperative concentrations of cortisol in the control and experiment groups was observed. Although the concentrations of the cortisol measured at T4 and T6 in the experiment group were lower than those detected in the control group, the differences were not statistically significant. It was reported that postoperative concentrations of cortisol increased in ovariohysterectomies performed by unexperienced surgeons (Michelsen et al. 2012). Therefore, it is suggested that the administration of lidocaine is not effective to decrease the postoperative concentrations of cortisol under less traumatic surgery circumstances. Additionally, it is postulated that less traumatic manipulations and more reasonable durations of surgery might be enough to control the concentrations of cortisol.

The trauma caused by the surgical procedure is known to support an oxidative process due to ischaemia or reperfusion (Halliwell 1994). Oxidative stress is a condition which is caused by cellular

destruction following any surgical approach (Sies 1997) and negatively affects the postoperative process (Lee and Kim, 2014). The organism produces antioxidants as a response against oxidative stress to protect the organs (Erel 2004). Since an oxidant-antioxidant balance is involved in the body, lower TOS values would be expected after lidocaine treatment in this study. Nevertheless, in the present study, lidocaine did not decrease the TOS values which were an unexpected result. It has been reported that lidocaine releases the endogenous opioids by inhibition of production of thromboxane A2 via neurokines (Lauretti 2008) and shows a fast and moderate analgesic effect as compared to other local anesthetics (Hellyer et al. 2007). Furthermore, it has been stated that the infiltration of lidocaine to the mesovarium before ovariohysterectomy may cause hematomas (Bubalo et al. 2008). Additionally, it has been indicated that intramuscular or subcutaneous administration of local anesthetics might cause complications such as dense inflammation, spleen laceration and hernia (Fitzpatrick et al. 2010). Therefore, the cause of higher TOS values in the experiment group may possibly be the inflammation process due to the lidocaine treatment.

In the present study, the concentrations of TAS and OSI values in the control and experiment groups did not show any significant differences. It has been reported that surgery performed in abdominal organs leads to the oxidative stress, especially in erythrocytes for 24 hours (Anup et al. 1999). However, total antioxidant values do not depend on whether the surgery is performed or not (Szymczyk et al. 2003). Although significant difference was observed in TOS values, it is suggested that the treatment of lidocaine is not effective for the management of oxidative stress due to the unchanged TAS and OSI values.

It was observed that the pain scores in the experiment group were lower at T2, T4 and T6, as compared to the control group. This finding was consistent with the previous data (Carpenter et al. 2004, Campagnol et al. 2012), that the lower postoperative pain scores were evident by the administration of intraperitoneal and incisional local anesthetics. Moreover, pain scoring systems are mainly based on the behavioral changes including palpation (Odete and Lesley 2013, Mathews et al. 2014, Epstein et al. 2015). Therefore, it is suggested that local anesthetics may be used for the management of pain level, since the anesthetic agent causes the loss of regional sensation.

It is postulated that surgeries performed at optimal time and less manipulation may be of importance to manage the post-operative stress. Furthermore, it is concluded that the treatment of intraperitoneal and incisional lidocaine is effective on postoperative pain management in dogs undergoing ovariohysterectomy.

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Conflict of Interest: The authors declare that they have no conflict of interest.

REFERENCES

Anup R, Aparna V, Pulimood A, Balasubramanian KA. Surgical stress and the small intestine: role of oxygen free radicals. Surgery. 1999;125:560-569.

Anup R, Balasubramanian KA. Surgical stress and the gastrointestinal tract. J Surg Res. 2000; 92:291-300.

Baysal E, Taysi S, Aksoy N, Uyar M, Celenk F, Karatas ZA, Tarakcioglu M, Bilinç H, Mumbuc S, Kanlikama M. Serum paraoxonase, arylesterase activity and oxidative status in patients with obstructive sleep apnea syndrome (OSAS). Europ Rev Med Pharmacol Sci. 2012; 16:770-774.

Bubalo V, Moens YP, Holzmann A, Coppens P. Anaesthetic sparing effect of local anaesthesia of the ovarian pedicle during ovariohysterectomy in dogs. Vet Anaesth Analg. 2008; 35:537-542.

Bugajski J, Gadek-Michalska A, Bugajski AJ. Nitric oxide and prostaglandin systems in the stimulation of hypothalamic-pituitary-adrenal axis by neurotransmitters and neurohormones. J Physiol Pharmacol. 2004;55:679-703.

Campagnol D, Teixeira-Neto FJ, Monteiro ER, Restitutti F, Minto BW. Effect of intraperitoneal or incisional bupivacaine on pain and the analgesic requirement after ovariohysterectomy in dogs. Vet Anaesth Analg. 2012;39(4):426-430.

Carpenter RE, Wilson DV, Evans AT. Evaluation of intraperitoneal and incisional lidocaine or bupivacaine for analgesia following ovariohysterectomy in the dog. Vet. Anaesthesia and Analg. 2004;31(1):46-52.

Church DB, Nicholson AI, Ilkiw JE, Emslie DR. Effect of non-adrenal illness, anaesthesia and surgery on plasma cortisol concentrations in dogs. Res Vet Sci. 1994;56:129-131.

Davidson EB, Moll HD, Mark EP. Comparison of laparoscopic ovariohysterectomy and ovariohysterectomy in dogs. Vet Res. 2004;33:62-69.

Devitt CM, Cox RE, Hailey JJ. Duration, complications, stress, and pain of open ovariohysterectomy versus a simple method of laparoscopic-assisted ovariohysterectomy in dogs. J Am Vet Med Assoc. 2005;227(6):921-927.

Distel M, Mueller C, Bluhmki E, Fries J. Safety of meloxicam: a global analysis of clinical trials. Br J Rheumatol. 1996;35(Suppl 1):68-77.

Epstein ME, Rodan I, Griffenhagen G, Kadrlik J, Petty M, Robertson SA, Simpson W. 2015 AAHA/AAFP pain management guidelines for dogs and cats. J Am Anim Hospital Assoc. 2015;51(2):67-84.

Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem. 2004;37:277-285.

Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem. 2005;38:1103-1111.

Fitzpatrick CL, Weir HL, Monnet E. Effects of infiltration of the incision site with bupivacaine on postoperative pain and incisional healing in dogs undergoing ovariohysterectomy. J Am Vet Med Assoc. 2010;237:395-401.

Fox SM, Mellor DJ, Firth EC, Hodge H, Lawoko CRO. Changes in plasma cortisol concentrations before, during and after analgesia, anaesthesia and anaesthesia plus ovariohysterectomy in bitches. Res Vet Sci. 1994;57(1):110-118.

Gadek-Michalska A, Spyrka J, Bugajski J. Psychosocial stress affects the involvement of prostaglandins and nitric oxide in the lipopolysaccharide-induced hypothalamic-pituitary-adrenal response. J Physiol Pharmacol. 2005;56:287-298.

Gautier A, Graff EC, Bacek L, Fish EJ, White A, Palmer L, Kuo1 K. Effects of ovariohysterectomy and hyperbaric oxygen therapy on systemic inflammation and oxidation in dogs. Front Vet Sci. 2019;6:506.

Gunay A, Gunes N, Gunay U. Effect of ovariohysterectomy on lipid peroxidation and levels of some antioxidants and biochemical parameters in bitches. Bull Vet Inst Pulawy. 2011;55:695-698.

Halliwell B. Free radicals and antioxidants: a personal view. Nutr Rev. 1994;70:257-265.

Hellyer PW, Robertson SA, Fails AD. Pain and Its Management, In: Lumb & Jones' Veterinary Anesthesia and Analgesia, Ed; Tranquilli WJ, Thurmon JC, Grimm KA, 4th Ed., Blackwell Publishing, UK. 2007; pp. 31-60.

Kibar M, Tuna B, Kısadere İ, Güzelbekteş H. Comparison of instilled lidocaine and procaine effects on pain relief in dogs undergoing elective ovariohysterectomy. Israel J Vet Med. 2019;74(3):148-154.

Kim YK, Lee SS, Suh EH, Lee L, Lee HC, Lee HJ, Yeon SC. Sprayed intraperitoneal bupivacaine reduces early postoperative pain behavior and biochemical stress response after laparoscopic ovariohysterectomy in dogs. Vet J. 2012;191(2):188-192.

Korkmaz M, Yilmaz O, Saritas AK, Demirkan I, Jaroszewski JJ. Evaluation of intraperitoneal and incisional bupivacaine or levobupivacaine for postoperative analgesia in ovariohysterectomized dogs. Acta Sci Vet. 2019;47:1666.

Lamont LA, Tranquilly WJ, Grimm KA. Physiology of pain. Vet Clin North Am Small Anim Pract. 2000;30:703-728.

Lauretti GR. Mechanisms of analgesia of intravenous lidocaine. Rev Bras Anestesiol. 2008;58(3):280-286.

Lee JY, Kim MC. Comparison of oxidative stress status in dogs undergoing laparoscopic and open ovariectomy. J Vet Med Sci. 2014;76(2):273-276.

Lemke KA, Dawson SD. Local and regional anesthesia. Vet Clin North Am Small Anim Pract. 2000;30:839-857.

Lemke KA, Runyon CL, Horney BS. Effects of preoperative administration of ketoprofen on anesthetic requirements and signs of postoperative pain in dogs undergoing elective ovariohysterectomy. J Am Vet Med Assoc. 2002;221(9): 1268-1275.

Lu D, Wu C, Yin Y, Ma X. Analgesic Effects of Lidocaine and Fentanyl Alone or in Combination Undergoing Ovariohysterectomy in Female Dogs. Pakistan Vet J. 2016;36(4):435-439.

Mathews KA, Paley DM, Foster RA, Valliant AE, Young SS. A comparison of Ketorolac with Flunixin, Butorphanol, and Oxymorphone in controlling postoperative pain in dogs, Can. Vet J. 1996;37:557-567.

McKune CM, Pascoe PJ, Lascelles BDX, Kass PH. The challenge of evaluating pain and a pre-incisional local anesthetic block. Peer J. 2014;2:e341.

Michelsen J, Heller J, Wills F, Noble G. Effect of surgeon experience on postoperative plasma cortisol and C-reactive protein concentrations after ovariohysterectomy in the dog: A randomised trial. Australian Vet J. 2012;90(12):474-478.

Mohn CE, Fernandez-Solari J, De Laurentiis A, Prestifilippo JP, de la Cal C, Funk R, Bornstein SR, McCann SM, Rettori V. The rapid release of corticosterone from the adrenal induced by ACTH is mediated by nitric oxide acting by prostaglandin E2. Proc Natl Acad Sci USA. 2005;102:6213-6218.

Nenadović K, Vučinić M, Radenković-Damnjanović B, Janković L, Teodorović R, Voslarova E, Becskei Z. Cortisol concentration, pain and sedation scale in free roaming dogs treated with carprofen after ovariohysterectomy. Vet World. 2017;10(8):888-894.

Rettori V, Fernandez-Solari J, Mohn C, Zorrilla Zubilete MA, de la Cal C, Prestifilippo JP, De Laurentiis A. Nitric oxide at the crossroad of immunoneuroendocrine interactions. Ann NY Acad Sci. 2009;1153:35-47.

Sies H. Oxidative stress: oxidants and sntioxidants. Exp Physiol. 1997;82:291-295.

Szymczyk G, Beltowski J, Marciniak A, Kotarski J. Assessment of serum lipid peroxide levels and antioxidant status in females who had undergone total abdominal hysterectomy without closing of the peritoneum. Ginekol Pol, 2003; 74:1397-1403.

Tsai TY, Chang SK, Chou PY, Yeh LS. Comparison of postoperative effects between lidocaine infusion, meloxicam, and their combination in dogs undergoing

ovariohysterectomy. Vet Anaesth Analg. 2013;40:615-622.

Wang H, Walker SW, Mason JI, Morley SD, Williams BC. Role of arachidonic acid metabolism in ACTH-stimulated cortisol secretion by bovine adrenocortical cells. Endocr Res. 2000;26:705-709.

Wilson DV, Barnes KS, Hauptman JG. Pharmacokinetics of combined intraperitoneal and incisional lidocaine in the dog following ovariohysterectomy. J Vet Pharmacol Ther. 2004;27(2):105-109.

Yilmaz O, Korkmaz M, Jaroszewski JJ, Yazici E, Ulutas E, Saritas ZK. Comparison of flunixin meglumine and meloxicam influence on postoperative and oxidative stress in ovariohysterectomized bitches. Polish J Vet Sci. 2014;17(3):493-499.

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RESEARCH ARTICLE

Investigation of the Prevalence of Ketosis in Cows in Ardahan Region

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ABSTRACT

In the postpartum period, the energy requirement increases in high yielding dairy cows. According to the negative energy balance (NED) degree, clinical or subclinical ketosis may occur during this period. In this study; The aim was to investigate the prevalence of ketosis in dairy cows in Ardahan Region. The animal material to be used in the study was determined as 200 as a result of statistical analyzes of TUIK data. Animal material was selected from Ardahan city center, Göle, Çıldır, Hanak and Damal districts. The enterprises where, study is carried out are similar in terms of milk yield, management, maintenance and nutrition factors. Blood samples were collected on days 7 and 14 postpartum to determine the prevalence of ketosis. Beta hydroxybutyrate (BHB), non-esterified fatty acid (NEFA) and glucose levels were determined from the obtained samples. Patients with BHB concentration with 1.0<1.4 mmol / L were considered to have subclinical ketosis. Patients with BHB concentration with ≥1.4 mmol / L were considered to have clinical ketosis. The prevalence of clinical ketosis in the postpartum period in Ardahan was 1% (2/200) and the prevalence of subclinical ketosis in Ardahan was 10% (20/200). Blood glucose levels of animals with ketosis were significantly lower than the healthy animals.

Keywords: β-hydroxybutyrate, clinical ketosis, subclinical ketosis, non-esterified fatty acid, negative energy balance

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Ardahan Yöresindeki İneklerde Ketozis Yaygınlığının Araştırılması

ÖZ

Postpartum dönemde, yüksek verimli süt ineklerinde enerji gereksinimi artmaktadır. Bu dönemde oluşabilen negatif enerji dengesinin (NED) derecesine göre, klinik veya subklinik ketozis meydana gelebilmektedir. Bu çalışmada; Ardahan yöresinde süt ineklerindeki ketozis yaygınlığının araştırılması amaçlanmıştır. Çalışmada kullanılacak hayvan materyali sayısı TÜİK verilerine göre yapılan istatiksel analizler sonucunda 200 olarak belirlendi. Hayvan materyali, Ardahan Merkez, Göle, Çıldır, Hanak ve Damal ilçelerinden seçildi. Çalışma yapılan işletmeler; süt verimi, yönetim, bakım ve beslenme faktörleri açısından birbirine benzer olarak seçildi. Ketozisin prevelansını belirlemek amacıyla kan numuneleri doğumdan sonra 7 ve 14. günlerde toplandı ve elde edilen örneklerden Beta Hidroksibütirat (BHB), NEFA ve glukoz seviyeleri belirlendi. BHB konsantrasyonu, 1.0-1.4 mmol/L arasında olanlar subklinik ketozisli olarak kabul edildi. BHB konsantrasyonu ≥1.4 mmol/L olanlar ise klinik ketozisli olarak kabul edildi. Çalışma sonucunda Ardahan yöresinde postpartum dönemde klinik ketozis yaygınlığı %1 (2/200) ve subklinik ketozis yaygınlığı ise %10 (20/200) olarak tespit edildi. Ketozisli hayvanların kan glukoz seviyesi sağlıklı hayvanlara göre önemli derecede düşük tespit edildi.

Anahtar Kelimeler: β-hydroxybutyrate, klinik ketozis, subklinik ketozis, esterleşmemiş yağ asidi, negatif enerji dengesi

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INTRODUCTION

In dairy cows, the period including three weeks before and after birth is called the transition period. Metabolic changes occurring in this period are observed to be higher than those occurring during pregnancy and lactation (Grummer 1995). In particular, problems originating from metabolism lead to a significant yield decrease, and to reproductive losses (Drackley 1999, İssi et al. 2016). Any health problem that occurs in the cows in the transition period decreases milk yield by an average of 7.2 L daily during the first 20 days of lactation (Vernon 2005).

Infertility and metabolic diseases which are characterized by decreased milk yield and by yield losses, are the most important problems of the transition period (Issi et al. 2016). The decrease in dry matter intake (DMI) is the most important risk factor in the development of these diseases. Metabolic diseases are observed in the first weeks of lactation, where milk synthesis increases rapidly (Şahal et al. 2011, Yıldız et al. 2019).

Ketosis develops in cows with high milk yield as a result of the disruption of carbohydrate and volatile fatty acid metabolism in the two-month postpartum period, especially in the 2nd to 4th weeks. The disease is characterized by decreased blood glucose level, depletion of liver glycogen and glucose reserves, decreased gluconeogenic activity, fatty degeneration in the liver and increased ketone bodies in the body. Ketosis is a disease of metabolism that has an acute, subacute and chronic course (Blood and Radostits 1989, Drackley et al. 1992, Yuhang et al. 2015, Hossain and Samad 2019).

Ketone bodies (BHB, acetoacetic acid and acetone) are formed as a result of fatty acid oxidation. Low blood glucose level during ketosis triggers the mobilization of fat reserves in the body and thus the level of NEFA increases (Ospina et al. 2010).

With this study, the prevalence of ketosis in the cows in the Ardahan region and the economic losses of this disease were investigated by examining the levels of BHB, NEFA, Glucose, Triglyceride, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Calcium (Ca) and Phosphorus (P) on the 7th and 14th days after birth.

MATERIALS and METHODS

Animal material

According to the dataof Ardahan Agriculture Provincial Directorate, there are 131,942 dairy cows between the ages of 3-7 in Ardahan region, considering the number of animals from which blood and milk will be taken, it has been calculated that 196 dairy cows will be sufficient at %95 confidence interval. In this study, 200 cows belonging to a total of 97 breeders in the age range of 3-7 years were used.

Ethical Approval

This research has been approved by Kafkas University Animal Experiments Local Ethics Committee (KAU-HADYEK/2018-092).

Collection of blood samples

Cows were sampled on postpartum days 7 and 14. For examination, 5 mL of blood was taken from V. jugular for his sampling. Blood samples were taken 4-5 hours after feeding (Duffield, 2000; Öğün, 2008). Blood samples were taken into an antiquagulant-free (BD Vacutainer, UK) and Sodium fluoride (NaF) tubes, and incubated at room temperature for 20 minutes and then centrifuged at 3000 rpm for 15 minutes (Custer et al., 1983). After serum samples were taken, glucose and BHB were determined. The sera were then stored at -20 ° C until further analysis.

BHB, NEFA, Glukoz, Trigliserit, AST, ALT, Ca and P measurements

Serum BHB (Randox®, United Kingdom), NEFA (Randox®, United Kingdom), Glukoz (DDS®, Turkey), Trigliserit (DDS, Turkey), AST (DDS, Turkey), ALT (DDS, Turkey) Calcium (DDS, Türkiye) and Fosfor (DDS, Türkiye) were measured using commercial ELISA kits.

Statistical analysis

Data was analysed using statistical software package SPSS® (SPSS 22, USA). The comparison of two parameters was made using t test. One-way analysis of variance (ANOVA) was used to determine changes in parameters over time. The results obtained were expressed as mean and standard error (X±SE). Values of $P<0.05$ and below were considered statistically significant.

RESULTS

In the study, as stated in the method, those with BHB levels between 1.0-1.4 mmol/L were considered as subclinical, those with ≥1.4 mmol/L were considered as clinical ketosis and those with <1.0 mmol/L as healthy. According to this procedure, the prevalence of ketosis on the 7th day is presented in Table 1. On the 7th day of postpartum, subclinical ketosis prevalence in Ardahan region was found to be at the rate of 10% (20/200) and clinical ketosis prevalence was found to be at the rate of 1% (2/200) (Table 1).

Table 1: Prevalence of ketosis according to BHB level on postpartum 7th day
Tablo 1: Postpartum 7. gün BHB seviyesine göre ketozis prevalansı

	N	Rate (%)	Min	Max.	Mean	SE
Healthy	178	89	0.62	0.84	0.74	0.06
Subclinical ketosis	20	10	1.05	1.39	1.23	0.10
Clinical ketosis	2	1	1.41	1.91	1.66	0.35

The prevalence of ketosis on postpartum 14th day is presented in Table 2. In this period, subclinical ketosis prevalence in Ardahan region was found to be at the rate of 8.5% (17/200) and clinical ketosis prevalence was found to be at the rate of 0.5% (1/200) (Table 2).

Table 2: Prevalence of ketosis according to BHB level on postpartum 14th day
Tablo 2: Postpartum 14. gün BHB seviyesine göre ketozis prevalansı

	N	Rate (%)	Min	Max	Mean	SE
Healthy	182	91	0.56	0.96	0.65	0.06
Subclinical ketosis	17	8.5	1.02	1.36	1.13	0.10
Clinical ketosis	1	0.5	1.78	1.78	1.78	0.00

In the study, comparison of some biochemical parameters in the postpartum 7th and 14th days in cows with and without ketosis (healthy) is shown as Mean ± SE in Table 3. On the postpartum 7th and 14th days, it was determined that NEFA, triglyceride, AST and ALT levels increased significantly (P <0.001), while glucose level decreased (P <0.001) compared to healthy cows. However, at that sampling time, there was no significant change encountered in Ca and P levels (Table 3).

Table 3: Comparison of some biochemical parameters in the postpartum 7th and 14th days in cows with and without ketosis
Tablo 3: Sağlıklı ve ketozisli ineklerin postpartum 7 ve 14. günlerde bazı biyokimyasal parametrelerinin karşılaştırılması

Parameters	Healthy	Ketosis (Subclinical and Clinical)	P
Postpartum 7th day			
NEFA (mmol/L)	0.42±0.01	0.81±0.01	P<0.001
Glukoz (mg/dL)	50.69±0.13	45.45±0.4	P<0.001
Trigliserid (mg/dL)	22.69±0.10	31.86±0.58	P<0.001
AST (U/L)	81.19±0.42	116.68±2.11	P<0.001
ALT (U/L)	26.03±0.22	36.68±1.05	P<0.001
P (mmol/L)	2.50±0.01	2.49±0.02	P>0.05
Ca (mmol/L)	1.47±0.01	1.47±0.02	P>0.05
Postpartum 14th day			
NEFA (mmol/L)	0.45±0.00	0.77±0.01	P<0.001
Glukoz (mg/dL)	55.33±0.11	46.05±0.33	P<0.001
Trigliserid (mg/dL)	19.85±0.11	29.95±0.50	P<0.001
AST (U/L)	78.33±0.35	109.37±2.27	P<0.001
ALT (U/L)	24.40±0.19	34.64±1.00	P<0.001
P (mmol/L)	2.50±0.01	2.50±0.19	P>0.05
Ca (mmol/L)	1.48±0.01	1.49±0.02	P>0.05

According to the survey information obtained from the producers, daily milk yields in the cows with ketosis (subclinical and clinical) and in healthy cows in winter (from 1 November to 31 March) and pasture periods (from 1 April to 30 October) are presented in Table 4. In the performed analyses, it was determined that the milk yield decreased significantly (P <0.01) in animals with ketosis (Table 4).

Table 4. Periodical daily milk yield in ketosis and healthy cows (L/day)
Tablo 4. Ketozisli ve sağlıklı ineklerde dönemsel günlük süt verimleri (L/gün)

Period	Availability	N	Mean	SE	P
Pasture Period	Healthy	75	11.14	0.24	P<0.01
	Ketozis	22	9.45	0.48	
Winter Period	Healthy	75	5.81	0.20	P<0.01
	Ketozis	**22**	**4.40**	**0.32**	

DISCUSSION

Ketosis is a metabolism disease which is acute, subacute and has chronic course and characterized by disruption of carbohydrate and volatile fatty acid metabolism, depletion of glycogen and glucose reserves in the liver, fat degeneration and decreased glucose level due to these disorders, and increased ketone bodies (Blood and Radostits 1989).

Symptoms of clinical ketosis are clinical symptoms and ketonuria in urine and milk. However, subclinical ketosis, which leads to secondary diseases (Mastitis, Metritis etc.) and progresses latently without showing clinical information, causes serious economic losses (Öğün 2008). This is usually because subclinical ketosis is not diagnosed and is overlooked.

It has been reported that almost half of the high-milk-producing cows, in particular, carry a risk of subclinical ketosis during the early lactation period (Emery et al. 1968, Öğün 2008). In a study conducted in the neighbouring city of Kars, where BHB was used as a criterion, the prevalence of subclinical ketosis was determined as 12.02% on the postpartum 7th day and 10.3% on the 14th day (Öğün 2008). In a study conducted in 12 countries in North America and Western Europe between 2011 and 2013, the prevalence of subclinical ketosis in the holstein breed has been determined as 24.1% (Brunner et al. 2019). In a study conducted in the Mediterranean, Aegean and Marmara regions in the postpartum period, the clinical ketosis prevalence has been found as 3.8%, 7.3% and 9.7%, respectively. In the same study, the prevalence of subclinical ketosis has been found to be 14.8%, 16.6% and 22.3%, respectively (Şentürk et al. 2016). In our study, the clinical ketosis rate was determined as 1% and 0.5%, and the rate of subclinical ketosis as 10% and 8.5%, respectively, in postpartum 7th and 14th days. The determined rates, along with being close to those of Turkey, are seen to be lower than of those in Europe. This situation is thought to be closely related to nutritional programs and milk yield. It has been reported that the incidence of ketosis can be determined at the highest level in the first week of postpartum (Emery et al. 1968, Öğün 2008).

It was reported that the level of BHB decreased significantly in the postpartum 14th day compared to the 7th day (Cavestany et al. 2005, Öğün 2008). Also in this study, it was found that the level of BHB decreased similarly in different breeds and in total.

It has been reported that the level of NEFA and then BHB increase in ketosis (Veenhuisen et al. 1991). Increased levels of BHB may be accompanied by increased NEFA and decreased glucose (Aeberhard et al. 2001, Busato 2002, Öğün 2008, Akgül 2014). In this presented study, it was determined that there was a decrease glucose level and an increase in NEFA and BHB in cows with ketosis (subclinical and clinical). Similar findings were obtained in ketosis (clinical and subclinical ketosis) studies conducted in Bursa in 2014 and in Saudi Arabia in 2017 (Akgül 2014, El-deep and El-bahr 2017).

It was determined that the level of NEFA increased at the postpartum 14th day compared to the 7th day. However, in another study, it has been reported that NEFA level increased on the postpartum 1st day and decreased gradually in the next 3 weeks (Vaquez-anon at al. 1994).

In the transition period, especially during the lactation phase, when the energy used in the body tissues and milk production cannot be met with ration, the energy deficit is met by the mobilization of fats (Bertics et al. 1992, Öğün 2008). However, the amount of fatty acid that can enter the TCA cycle is limited. When this limit is exceeded, the level of NEFA increases (Goff and Horst 1997). In the presented study, it was found that the level of NEFA in cows with ketosis increased compared to the healthy ones. It has been reported that the NEFA level had increased in cows with NEB and this was associated with increased fat mobilization (Aeberhard et al. 2001).

It has been determined that the glucose level was the lowest at the postpartum 8th day and increased until the 21st day (Seifi et al. 2007). In addition, it has been reported that glucose level decreased by 25% in the first week of lactation compared to the prenatal period and increased in the second week of lactation (Vaquez-anon et al. 1994). The level of glucose and ketone bodies may provide information about the amount of energy required in animals (Hertd et al. 1981). Low glucose and high BHB levels also indicate that energy is not taken enough (Whitaker et al. 1983). It has been stated that glucose level is a good indicator in determining the severity of the disease in clinical ketosis (Kelly 1977). Decrease in glucose value has been determined to be parallel with the increase in the level of BHB (Andre et al. 1987). It has been reported that glucose level decreased dramatically in cows with ketosis in the postpartum period (Akgül 2014). In another study conducted in the same period, it has been determined that glucose level decreased significantly in cows with ketosis (El-deep and El-bahr 2017). In this presented study, it was determined that the cows with ketosis had a low glucose level in the postpartum 7th and 14th days compared to healthy ones. In addition, glucose level was determined to be increased significantly on the 14th day compared to the 7th day. This indicates that the glucose level decreases in the postpartum process and increases gradually after the first week. It has been reported that decreased glucose is associated with insufficient liver function, low energy in feed and increased glucose requirement (Aslan and Nizamlıoğlu 1985, Duffield 2000, Veenhuisen et al. 1991, Öğün 2008). During the transition phase of dairy cows, the need for glucose increases for lactose production (Busato et al. 2002). This leads to the development of NEB and a decrease in glucose as a result of insufficient gluconeogenesis (Andersson and Emanuelson 1985, Brumby et al. 1975, Drackley 1999).

In this study, it was determined that the level of triglyceride increased on the postpartum 7th day and decreased on the 14th day. In the postpartum first two weeks in the cows with ketosis, triglyceride levels were found higher than healthy ones. It has been reported that circulating triglycerides are taken by the mammary glands in high-milk-yield cows in lactation period and used in milk fat synthesis (Grummer 1993). NEFA is the main component for triglyceride (Akgül 2014). Goff and Horst (1997) have reported that triglyceride level varies depending on nutrition.

It has been determined that in the postpartum first two weeks in cows with ketosis (subclinical and clinical), AST and ALT activities are considerably high compared to healthy ones. In addition, it has been determined that the level of AST and ALT was high on the postpartum 7th day and then decreased on the 14th day. AST activity has been reported to be increased on the postpartum 7th day and then decreased gradually (Seifi et al. 2007). In subclinical ketosis, AST and ALT activities have been found to be increased (Öğün 2008). Similarly, it has been reported that AST and ALT activity was increased in cows with subclinical ketosis, however, this increase was lower in ALT (Kennerman 1999). Studies and our study show that the increase in AST and ALT activity is associated with liver fattening and ketosis (Kauppinen 1984, Steen et al. 1997, El-deep and El-bahr 2017).

It has been reported that the level of Ca in cows varies between 2-3 mmol/L and the P level between 1.16-2.32 mmol/L (Barton et al. 1981, Can et al. 1987, Öğün 2008). The level of Ca and P has been reported to be decreased on the postpartum 8th day, then increased gradually, and this was due to the use of molecules in milk synthesis (Seifi et al. 2007). In this presented study, no changes in Ca and P values were detected in the postpartum 7th and 14th days in cows with ketosis and in healthy animals and in between these days. In a similar study, it has been reported that Ca level was lower in cows with ketosis than healthy ones, however, the difference was not significant (Akgül 2014).

CONCLUSION

As a result, it was determined that the prevalence of ketosis can be determined by the level of BHB. In the cows in Ardahan region, the clinical ketosis prevalence was determined on the postpartum 7th day as 1%, subclinical ketosis prevalence was determined as 10%, while the clinical ketosis prevalence was determined on the 14th day as 0.5% and subclinical ketosis prevalence was determined as 8.5%. According to the measurements performed on the postpartum 7th and 14th days with the BHB test, the most sensitive breed to clinical ketosis risk was detected to be Brown Swiss hybrid and the most sensitive breed to the risk of subclinical ketosis was detected to be Brown Swiss. In the cows with ketosis, BHB level decreases on the postpartum 7th day compared to 14th day. In our study, it was determined that milk yield decreased by 25% in ketosis disease. When this result was adapted to TSI data, it was found that a daily financial loss of 76,193.25 TL occurred in Ardahan and its vicinage.

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REFERENCES

Aeberhard K, Bruckmaier R.M, Blum J.W. Enzymatic and endocrine status in high-yielding dairy cows-part 2. J. Vet. Med. A, 2001; 48(2):111-127.

Akgül G. Subklinik ve klinik ketozisli ineklerde adiponektin düzeyinin ölçülmesi, nefa, bhba ve adiponektin düzeyleri aralarındaki ilişkilerin belirlenmesi. PhD thesis, Uludağ University Health Science Institute, Bursa, 2014.

Andersson L, Emanuelson U. An epidemiological study of hyperketonaemia in Swedish dairy cows; determinants and the relation to fertility. Prev. Vet. Med, 1985; 3(5):449–462.

Andre E, Bazin S, Siliart B. Interest and limits of blood chemistry in high producing cows. Israel Journal of Veterinary Medicine, 1987; 43:110-116.

Aslan V, Nizamlioglu M. İneklerde gebelik ve laktasyon dönemlerinde kan glukoz degerleri ve subklinik ketozisin teshisi üzerinde arastırmalar, Selçuk Üniv. Vet. Fak. Derg, 1985; 1(1):57-64.

Barton B.A, Horst R.L, Jorgensen N.A, Deluca H.F. Concentration of calcium, phosphorus and 1,25-Dihydroxy vitamin D in plasma of dairy cows during the lactation cycle. J Dairy Sci, 1981; 64(5):850-852.

Bertics S.J, Grummer R.R, Valino C.C, Stoddard E.E. Effect of prepartum dry matter intake on liver triglyceride concentration and early lactation. Journal of Dairy Science, 1992; 75(7):1914-1922.

Blood D.C, Radostits O.M. Veterinary Medicine, 7th Ed, Bailliere Tindall, Philadelphia, 1989; 1128-1138.

Brumby P.E, Anderson M, Tucklet B, Storry J.E, Hibbitt K. Lipid metabolism in the cow during starvation-induced ketosis. Biochemic Journal, 1975; 146(3):609-615.

Brunner N, Groeger S, Raposo J.C, Bruckmaier R.M, Gross J.J. Prevalence of subclinical ketosis and production diseases in dairy cows in Central and South America, Africa, Asia, Australia and New Zealand, and Eastern Europe. Transl. Anim. Sci, 2019; 3(1):84–92.

Busato A, Faissler D, Kupfer U, Blum J.W. Body conditions scores in dairy cows: associations with metabolic and endocrine changes in healthy dairy cows. J. Vet. Med. A., 2002; 49(9):455-460.

Can R, Yılmaz K, Erkal N. Primer ketozisli süt ineklerinin bazı kan özellikleri ve sağaltımı üzerinde klinik araştırmalar, Ankara Üniv Vet Fak Derg, 1987; 34(3):433-448.

Cavestany D, Blanch J.E, Kulcsar M, Uriarte G, Chilibroste P, Meikle A, Febel H, Ferraris A, Kral E. Studies of the transition cow under a pasture-based milk production system: metabolic profiles. J. Vet. Med. A, 2005; 52(1):1–7.

Drackley J.K, Richard M.J, Ber D.C, Young J.W. Metabolic Changes in Dairy Cows with Ketonemia in Response to Feed Restriction and Dietary 1,3 Butanediol. Journal of Dairy Science, 1992; 75(6):1622-1634.

Drackley J.K. Biology of dairy cows during transition period: the final frontier?, Journal of Dairy Science, 1999; 82(11):2259-2273.

Duffield T.F. Subclinical ketosis in lactating dairy cattle. Veterinary Clinics North America Food Animal Practice, 2000; 16(2):231-253.

El-Deeb W.M, El-Bahr S.M. Biochemical markers of ketosis in dairy cows at post paturient period: oxidative stress biomarkers and lipid profile. Vet Arhiv, 2017; 87(4):431-440.

Emery R.S, Bell J.W, Thomas J.W. Benefits derived from routine testing form ilk ketones, J. Dairy Res., 1968; 51(8):1308-1309.

Goff J.P, Horst R.L. Physiological changes at parturition and their relationship to metabolic disorders, Journal of Dairy Sci., 1997; 80(7):1260-1268.

Grummer R.R. Etiology of lipid-related metabolic disorders in periparturient dairy cows. *Journal of dairy science*, 1993; 76(12):3882-3896.

Grummer R.R. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. Journal of Animal Science, 1995; 73(9):2820-2833.

Herdt T.H, Steven J.B, Olson W.G, Larson V. Blood concentration of β hydroxybutyrate in clinical normal holstein freisian herds and in those with a high prevalance of clinical ketosis. American Journal of Veterinary Research, 1981; 42(3):503-506.

Hossain S.M.S, Samad M.A. Prevalence of sub-clincial ketozis and its associated cow level risk factors in lactatıng dairy cross-bred cows ın bangladesh. J. Vet. Med. OH Res, 2019; 1(1):29-38.

İssi M, Gül Y, Başbuğ O. Evaluation of renal and hepatic functions in cattle with subclinical and clinical ketozis. Turk J Vet Anim Sci, 2016; 40(1):47-52.

Kauppinen K. ALAT, AP, ASAT, GGT, OCT activities and urea and total bilirubin concentration in plasma of normal ana ketotic dairy cows. Zentralblatt Fur Veterinarmedizin Reihe A, 1984; 31(1-10):567-576.

Kelly J.M. Changes in serum β hydroxybutyrate concentration in dairy cows kept under commercial farm conditions. Veterinary Record, 1977; 101(25):409- 502.

Kennerman E. Incidence, early diagnosis of subclinical ketosis and determinations of liver dysfunctions in Bursa region. *UÜ Vet Fak Derg*, 1999; 18:97-107.

Ospina P.A, Nydam D.V, Stokol T, Overton T.R. Evaluation of nonesterified fatty acids and β-hydroxybutyrate in transition dairy cattle in the northeastern United States: Critical thresholds for prediction of clinical diseases. Journal of Dairy Science, 2010; 93(2):546-554.

Öğün, M. Kars yöresindeki ineklerde subklinik ketozis prevalansının biyokimyasal yöntemlerle araştırılması. PhD thesis, Kafkas University Health Science Institute, Kars, 2008.

Seifi H.A, Gorji-Dooz M, Mohri M, Dalir-Naghadeh B, Farzanch N. Variations of energy-related biochemical metabolites during transition period in dairy cows, Comp. Clin. Pathol., 2007; 16(4):253-258.

Steen A, Gronstol H, Torjesen P.A. Glucose and insulin responses to glucagons injection in dairy cows with ketosis and fatty liver, Zentralbl Vet Med, 1997; 44(1-10):521–530.

Şahal M, Çolakoğlu C.E, Alihosseini H. Ketozis ve yağlı karaciğer sendromunun tedavisinde güncel yaklaşımlar ve tedavideki başarısızlığın nedenleri, Turkiye Klinikleri J Vet Sci., 2011; 2(2):140-150.

Şentürk S, Cihan H, Mecitoğlu Z, Çatık, Akgül G, Kasap S, Topal O. Prevalence of ketosis in dairy herds in Marmara, Aegean and Mediterranean regions of Turkey. Ankara Üniv Vet Fak Derg, 2016; 63(1):283-288.

Vazquez-anon M, Bertics S, Luck M, Geummwe R.R, Pinheiro J. Peripartum liver triglyceride and plasma metabolites in dairy cows. Journal of Dairy Science, 1994; 77(6):1521-1528.

Veenhuisen J.J, Drackley J.K, Richard M, Sanderson T.P, Miler L.D, Young J.W. Metabolic changes in blood and liver during devolopment and early treatment of fatty liver and ketosis in cows. J. Dairy Sci., 1991; 74(12):4238-4253.

Vernon R.G. Lipid metabolism during lactation: A review of adipose tissue-liver interactions and the development of fatty liver. Journal of Dairy Research, 2005; 72(4):460-469.

Whitaker D.A, Kelly J.M, Smith E.J. Subclinical ketosis and serum beta-hydroxybutyrate levels in dairy cattle. British Vet. J., 1983; 139(5):462–463.

Yıldız R, Ider M, OK M. Beta hidroksi bütirik asit düzeyinin diğer metabolik test parametreleri üzerine etkisi. Vet Hekim Der Derg, 2019; 90 (1):15-21.

Yuhang S, Bo W, Shi S, Hongyou Z, Chuang X, Ling W, Cheng X. Critical thresholds of liver function parameters for ketozis prediction in dairy cows using receiver operating characteristic (ROC) analysis. Veterinary Quarterly, 2015; 35(3):159-164.

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RESEARCH ARTICLE

Application of Electron Beam Irradiation Technique for Shelf-Life Extension of Animal Food Products

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ABSTRACT

For food processing gamma rays, electron beam and X-rays are used for disinfection of microorganisms and for extension of the shelf-life of the food. Electron beam irradiation process and its facilities are discussed widely for animal food products, nowadays. Although there are many advantages of this technique, high doses may be needed to achieve desired purposes. The irradiation conditions such as irradiation energy, dose and speed, penetration depth, processing speed should be optimized for such an irradiation apparatus design. The type and the size of the food; if it is fresh, frozen or in packets; transition process to atmospheric medium and irradiation parameters are the issues that need to be adjusted carefully according to aims. In this work, design parameters of an electron beam irradiation system are described and simulation of a low energy electron curtain accelerator is introduced. This study was carried out as the first step with the construction idea of a system that is planned to be made most appropriately in the current laboratory conditions. Also is to demonstrate the operability of such a system at low energies for sterilization on the surface of the target material.

Key words: Electron beam, electron accelerator, electron beam irradiation of food, food irradiation, animal products.

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Elektron Demeti ile Işınlama Tekniğinin Hayvansal Ürünlerin Raf Ömrünün Uzatılmasında Kullanımı

ÖZ

Gıda ışınlama sürecinde mikroorganizmaların dezenfeksiyonu ve gıdanın raf ömrünün uzatılması için gama ışınları, elektron ışını ve X ışınları kullanılır. Günümüzde, hayvansal gıda ürünleri için elektron demeti ile ışınlama işlemi ve tesisleri yaygın olarak tartışılmaktadır. Bu tekniğin birçok avantajı olmasına rağmen, hedefe ulaşmak için yüksek dozlara ihtiyaç duyulabilmektedir. Işınlama enerjisi, dozu ve hızı, penetrasyon derinliği, işlem hızı gibi ışınlama koşulları, bu tip bir ışınlama sistemi tasarımı için optimize edilmelidir. Gıdaların taze, dondurulmuş veya paketler halinde olması, tipi ve büyüklüğü, atmosfer ortamına geçiş süreci ve ışınlama parametreleri hedefe göre dikkatlice ayarlanması gereken parametrelerdir. Bu çalışmada, elektron demeti ile ışınlama sisteminin tasarım parametreleri açıklanmış ve düşük enerjili bir elektron hızlandırıcısının simülasyonu tanıtılmıştır. Bu çalışma, mevcut laboratuvar koşullarında yapılması planlanan bir sistemin tasarımı fikrinin ilk adımı olarak gerçekleştirilmiştir. Ayrıca böyle bir sistemin hedef malzemenin yüzeyi üzerine uygulanması ile sterilizasyon gibi uygulamalar için düşük enerjilerde çalışabileceği gösterilmiştir.

Anahtar Kelimeler: Elektron demeti, elektron hızlandırıcısı, gıdaların elektron demeti ile ışınlaması, gıda ışınlaması, hayvansal ürün.

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INTRODUCTION

Food irradiation is one of the foreseen methods that can control the growth of microorganisms, parasites, insects and some losses that may occur during slaughtering, processing and transporting. By food irradiation, prevention of foodborne illness, and food hygiene security are aimed. Irradiation applications are also used to extend the shelf life of food products. Irradiation has about 100 years of history and is specified for safe food processes (Molins et al. 2001, Farkas and Farkas2011). It is confirmed by organizations of the World Health Organization (WHO), the Food and Agriculture Organization (FAO), and the International Atomic Energy Agency (IAEA) as being effective in achieving the aforementioned purpose and a safe, nutritional suitable method since 1980s.

Irradiation does not cause radioactivity in foods, actually it is an energy input on them. The amount of this energy is irradiation absorption defined as dose in the unit of gray (Gy), (Ferreira et al. 2017). The superiority of the strong ability of irradiation to penetrate in materials breaks both structural, metabolic functions and lead to fragmentation of DNA. Hence, results as the eventual death of microbial cells. Intensive and much-localized energy, namely uniform energy is imparted to the material and in this way; a sufficient level of sterilization impact can be obtained with a little increase in the whole temperature of that material. Herewith, the irradiation process is very convenient for fresh fruits and vegetables, meat, and other animal products, and frozen foods.

Irradiation process helps to reduce the microorganism activities, to prevent parasite contamination sources and prevent diseases, to remove pests and sterile products, to eliminate some of the chemicals used for foods, and also to reduce fungicide residue problem with food (Lagunas-Solar 1995, Olson 1998, Korel and Orman 2005, Goresline2018). The US Department of Agriculture's (USDA's) Food Safety and Inspection Service announced a voluntarily recall of approximately 46000 lbs of ground beef for possible *Escherichia coli* O157:H7 contamination. The detection of such pathogenic bacteria in ground beef caused a reduction in beef consumption and huge economic losses.

In some studies, it has been seen that irradiation significantly reduces foodborne pathogen concentrations (Kwon et al. 2008, Arthur 2005). In recent years, irradiation has been approved for the processing of chilled or frozen uncooked meat and meat by-products (U.S. Department of Agriculture, Food Safety and Inspection Service. 1999). To process these products, high penetration, and high energy radiation might be needed to provide the entire meat product and both exposed surface and internal regions are irradiated. The increasing number of foodborne bacteria as *Escherichia coli* O157:H7, *Pseudomona*s spp., Listeria *monocytogenes*, *Staphylococcus aureus*, and *Bacillus cereus* (Taha 1999, Woodburn and Raob 1997, Park et al. 2010) particularly *Salmonella* has increased interest in food irradiation as an effective technique for the disposal (Min et al. 2005). For eliminating pathogens in heat-sensitive products like eggs, the irradiation process can be more attractive (Radomyski et al. 1994, Arvanitoyannis 2010). However, the effects of irradiation on the physicochemical and functional properties of shell eggs and liquid egg yolk and white are still argumentative.

Gamma, X-rays, and accelerated electron beams are used mostly in the industry for various applications as well as food irradiation (Ozer 2017). Gamma rays from Co-60 or Cs-137 (below 5 MeV energy level) are commonly used since they have high penetrating power, low dose rate, and foods can be irradiated with their thick coverage packages. On the other hand, electron beams are generated (below 10 MeV energy level) with low penetrating power, and higher dose rate. Electron beams can be easily started and stopped by turning on and off the power supply thus represents a safer irradiation process. Besides, low penetrating power can be used as selective irradiation of surfaces. X-rays provide high penetrating power as the Gamma rays and it can be also stopped as electron beams (Sommers and Fan 2008). However, their energy conversion efficiency is low and the cost is much for nowadays technological conditions.

The electron beam irradiation (EBI) of foods has been shown an alternative and convenient method to reduce microorganism load without affecting the flavor of the foods. It is a fast and effective method for sterilization, of a variety of many foods. This method seems more reliable and safe to the consumers, because no radioactive source is needed and also it is more preferable for temperature-sensitive products (Silindir and Özer 2009). Some of the scientific committees have done some studies about EBI and have concluded that no problems in the irradiated foods with 10 MeV electron beam (high energy level) at 30 kGy in terms of nutritional changes, mutagenicity, microbiological safety and radioactivity (Kobayashi 2018).

Some studies have presented the efficacy of EBI for reducing pathogenic microorganisms in animal products. Wong and Kitts 2003; Kim et al. 2010 stated in their recent studies that EBI proved to be an effective method for controlling microbial growth in shell-eggs without adversely affecting physicochemical and functional properties and also for ground beef of low-fat content. In the studies,

3 kGy absorbed dose for irradiation of the fresh-shell-eggs resulted in minor changes of proteins, lipids, or carbohydrates.

Although there is wide use of pathogen interventions as antimicrobial solutions for beef meat, outbreaks establish a connection with them exposed to such pathogen interventions continue. EBI is proposed to be the method of resolving these restrictions. Especially, EBI is proven by many studies as reducing the pathogens in meat and poultry products without damaging their quality. (Lewis 2002, Arthur et al. 2005, Duong et al. 2008, Kundu and Holley 2013, Maxim et al. 2014).

MATERIALS and METHODS

For industrial and medical applications electron beam accelerators process 0.15 to 10 MeV electron beam energies. Energy and current are the important primary characteristics for these type of electron accelerators.

Electron beam applications of the food irradiation process can be classified as low energy surface applications and high energy irradiation. For surface treatment up to 400 keV energetic electron beam energies are known to be sufficient (Blackburn 2017, Miller 2017). By application of low energetic electron beam to the surface of the food material, biological contaminants are removed efficiently with a little penetration depending on the electron beam energy. Approximately 15 mm penetration on the surface of the carcass sides is obtained including considerable radiation dose with this process. The pathogen contamination of carcass is a surface phenomenon. Thus, lower pathogen load without adverse affection of product organoleptic quality is expected by this application technique (Arthur et al. 2005).

The technical criteria for food irradiation are the irradiation dose and speed, penetration depth, processing speed. The average dose is the amount of energy absorbed by the material is divided by its mass, in the units of Gy as previously stated. High energy electrons immediately interact with the irradiated matter. Therefore, the absorbed dose is highest on the surface of the material and falls vertically towards the material depth. The input dose in the irradiated material is directly proportional to the electron beam current. Dose speed starts 10^3 Gy/s for electrostatic accelerators where it is 10 Gy/s for radioisotope gamma sources. This is one of the advantages of electron accelerators in applications.

The penetration depth of the electrons in the material is directly proportional to the electron energy and inversely proportional to the density of the material to be irradiated. The penetration depth for given electron energy is given in terms of the weight of the material to be irradiated per unit area (gr/cm^2). The biggest disadvantage of electron accelerators compared to gamma sources is that the material thickness to be irradiated is limited. Electron accelerators are more suitable for irradiation of grain, spices, small-sized fruits and vegetables, meat, and seafood packaged for daily consumption (Salimov et al. 2000). On the other hand, the material can be irradiated by scanning in two dimensions (with the inclusion of magnetic field) with accelerated electron beams. It causes the efficiency of the accelerators is to be larger compared to gamma sources. For example, by 50 kW electron beam, irradiation efficiency is 0,35 and irradiation dose is 2 kGy; around 30 tons of fresh white-poultry meat and red meat can be irradiated per hour for disinfection (Turhan et al.2020).

Nowadays, low dose, low penetration EBI technology has evolved to the point where large nonuniform surface areas can be effectively treated and an entire carcass side. A study indicated that low dose electron beam irradiation (lower than 2kGy) could reduce some pathogens especially *E.coli* and *S.typhimurium* on inoculated shell eggs (Kim et al. 2010, 2016).

Irradiation of fish and meat need medium doses up to 10 kGy to control pathogenic microorganisms in fresh or frozen meat. As referenced in Ferraira et al. 2018 for fish lower doses than 7kGy and lower than 3 kGy for eggs are needed. However, the permitted levels of irradiation are not sufficient to control the pathogenic viruses. Studies on EBI of beef surfaces presented that by this technique carcasses can be only irradiated on the surface and for lower depths (Arthur et al. 2005). By electron beams, surface structures such as fat and other external tissues are removed. For deep penetration, higher energetic electron beams are needed. To determine this, one may need to look at the curves for the distribution of absorbed dose along the beam direction (Ferraira et al. 2018).

For EBI process, as a source of radiation electron beams are needed to be generated by an electron beam linear accelerator or a Van de Graff generator. The electrons are accelerated and can reach to energies up to 10 MeV. The choice of a type of accelerator for a particular application is usually based on the application considerations (Cleland and Parks 2003, Cleland et al. 2003). There is a big interest in computer modeling for designing the irradiation apparatus and its operating environment. By modeling, the performance of an irradiator can be predicted and the optimization of the process can be done.

A schematic view of an accelerator-based system consists of an electron accelerator, a scanning system and a material handling system that the beam can move through the material. The electron accelerator

is usually located inside the high voltage vacuum. In the accelerator tube generated electrons are accelerated and gain energy. Then these accelerated electrons escape through a thin window, usually titanium is preferred, and move through the target material.
To model an electron beam irradiation apparatus, important variables are electron beam energy, beam current and geometry, speed of the process, exit window foil thickness, the distance between exit window and the target material.

RESULTS and DISCUSSION

In this study, design and simulation of a low-energy electron-curtain-accelerator is presented. Design facilities are discussed in details, in conjunction with SIMION 3D electron trajectory simulation software. By SIMION 3D, calculation of charged particle trajectories in electric fields with a given configuration of electrodes, particle initial conditions, including optional magnetic field can be done (Scientific Instrument Services Inc.).

The majority of industrial installations in the low energy range base on the linear cathode concept. In this way, electrons distribute over a wide web of material that moves through the beam. A free electron is emitted by thermionic emissions by heating tungsten filament on the negative cathode side. The free electron is accelerated in the electric field and gain energy in electron volt (eV). The production and acceleration of electrons are done inside a long evacuated vacuum tube (Ozer et al. 2017). The electrons emitted from the thermionic cathode are accelerated over a single stage to pass through the anode (a thin metallic window) producing a continuous stream of electrons to irradiate materials or products in atmospheric conditions (Auditore et al. 2013).

In Fig. 1 the Computer-Aided Design (CAD) model of the system (Dassault Systems Corp.).The designed electron accelerator consists of a 0.25 mm diameter W wire wrapped to provide a spring-shaped filament 150 mm long with a 1 mm diameter, emitting electrons by the thermionic effect. To convey the electrons emitted from the filament as a focal (parallel) beam on the anode a focusing electrode is used. The shape of this electrode is optimized for the desired beam shape. Also, a cylindrical high-voltage terminal that assists in focusing and reducing the potential gradient concerning the grounded shell is used.

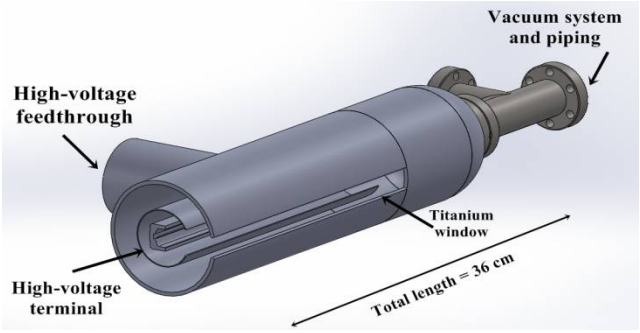

Figure 1. CAD model of the designed accelerator system.

For high voltage isolation, an electrically grounded cylindrical concentric shell to vacuum enclosure and minimizing voltage gradient is employed. To extract the accelerated electrons to the air a thin anode that forms a window as 150mm x 10mm is created. A titanium foil with 20μmthicknesses is preferred. The system is expected to work in pulse mode so a pulsed power supply system is expected to be used during the real construction. The electron beam is supposed to be uniformly distributed on the target material.

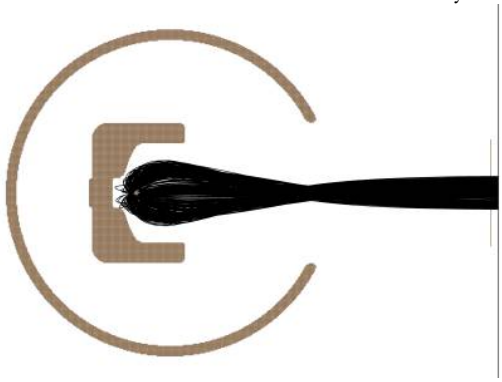

Figure 2. View of generated electron beam in SIMION and beam profile through the system.

The focusing electrode and high voltage terminal design, and corresponding electron beam profile, as simulated with SIMION software, are shown in Fig. 2 and Fig. 3. About 90% of accelerated electrons from filament housing could successfully penetrate the titanium foil when the filament structure is -200 kV (or may arrange up to -500 kV) relative to earth. Then the maximum electric field in vacuum is about 6.5 MV/m near the round corner of high-voltage terminal, which is less than the critical electrical field (20 MV/m) in a high vacuum.

Dose distribution is one of the most important parameters of the irradiation process as mentioned previously, hence for reproducible process and heat diffusion on the exit window. In Fig. 3, beam distribution has shown at the exit foil of the window (red dots). Designed low energy electron accelerator has a compact design and expected as reliable in terms of beam uniformity, robustness, and high voltage safety.

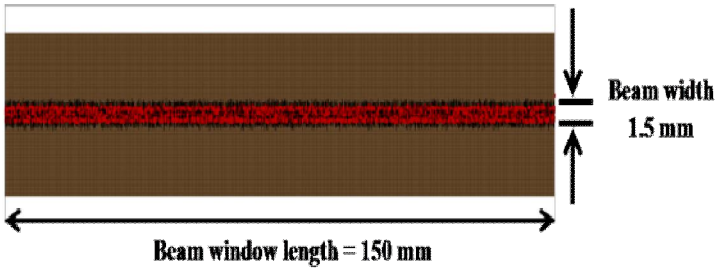

Figure 3. Beam distribution has shown at the exit foil of the window (red dots).

The exit window includes an exit window foil and a support grid contacting and supporting the exit window foil. The support grid has first and second grids, each having respective first and second grid portions that are positioned in an alignment and thermally isolated from each other. The first and second grid portions each have a series of apertures that are aligned for allowing the passage of a beam therethrough to reach and pass through the exit window foil. The second grid portion contacts the exit window foil. The first grid portion can mask the second grid portion and the exit window foil from heat caused by the beam striking the first grid portion. To prevent undue energy loss, the foil is preferably made as thin as possible while at the same time providing sufficient mechanical strength to withstand the pressure differential between the vacuum enclosure and irradiation atmosphere. As illustrated in the Fig.4, the heat problem can be solved by utilizing a titanium foil that has a thickness of 17 micrometers or less.

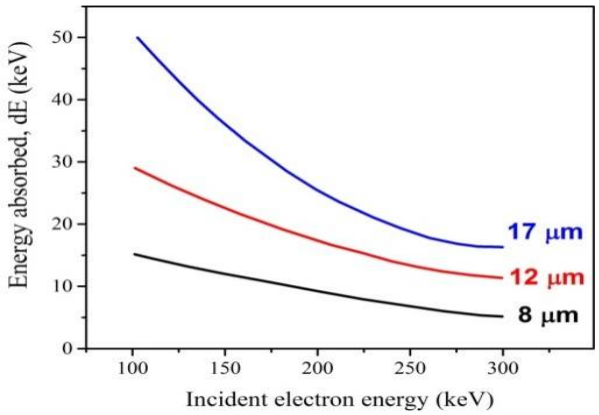

Figure 4. Exit window assembly.

In accordance with the principles of this study, the particle beam generating device can be made smaller in size and operate at a higher efficiency level when the thin foil of the exit window assembly is made of titanium or alloys thereof and having a thickness of 17 μm or less, preferably in a range of 3–10 micrometers. Alternatively, the foil may also be constructed of aluminum or alloys thereof having a thickness of 25 μm or less.

Here, the design of a low electrostatic accelerator modeled in SIMION 3D program is described and some efforts to modulate exit window assembly have been done to optimize the heat dissipation on the exit foil. Electrons from a modified electron gun are injected into the tube and accelerated through the tube. Targets can be irradiated using an electron beam which energy can range below 500 keV safely. Future studies are needed to be done for describing the dependence different beam current, dose and the

adaption of exit window is In this process, thermal analysis of heat dissipation on foil and therewith active/passive cooling system for foil window is an important issue to be considered.

This study was carried out as the first step with the construction idea of a system that is planned to be made most appropriately, in the current laboratory conditions not thinking of commercial sterilization. The primary purpose of this study is to demonstrate the operability of such a system at low energies for applications such as sterilization on the surface of the target material. EBI facilities that serve in sector for commercial use are available in different purposes depending on the needs in the world.

This study is expected to be assisting further work for planned further work for the irradiation process of sterilization on the surface. It is possible to specify design of electron beam systems that have higher energies and serves medium doses in this field of the food industry for animal products. In this scale, for example, they may be used for surface sterilization of marketable eggs (only the shell and the outer thin white). This can be done with a proper selection of the energy and dose of electrons obviating considerable irradiation of the main egg contents.

REFERENCES

Anup R, Aparna V, Pulimood A, Balasubramanian KA. Surgical stress and the small intestine: role of oxygen free radicals. Surgery. 1999;125:560-569.

Arthur TM, WheelerT, Shackelford S D, Bosilevac J M, Nou X, Koohmaraie M. Effects of low-dose, low-penetration electron beam irradiation of chilled beef carcass surface cuts on Escherichia coli O157: H7 and meat quality. Journal of food protection. 2005; 68(4): 666-672.

Arvanitoyannis IS. Irradiation of food commodities: techniques, applications, detection, legislation, safety and consumer opinion. Academic Press, 2010.

Auditore L, Barnà RC, De Pasquale D, Interdonato S, Italiano A, Trifiró A,Trimarchi M. Compact 300 keV electron gun for radiation processing. Review of scientific instruments. 2005; 76(12): 123301.

Blackburn C. Food Irradiation Technologies: Concepts, Applications and Outcomes. Royal Society of Chemistry. 2017.

Caja MM, Del Castıllo MR, Blanch GP. Solid-phase microextraction as a methodology in the detection of irradiation markers in ground beef. Food chemistry. 2008; 110(2): 531-537.

Cleland MR, Parks LA. Medium and high-energy electron beam radiation processing equipment for commercial applications. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions With Materials And Atoms. 2003; 208: 74-89.

Cleland MR,Parks LA,Cheng S. Applications for radiation processing of materials. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms. 2003; 208:66-73.

Dassault Systèmes Corp., USA. SolidWorks, www.solidworks.com Accessien date: 15.01.2020.

Duong DQ, Crandall PG, Pohlman FW, O'Bryan CA, Balentine CW, Castillo A. Improving ground beef safety and stabilizing color during irradiation using antioxidants, reductants or TSP. Meat science. 2008; 78(4): 359-368.

Farkas J and Farkas CM. History and future of food irradiaiton, Trends in Food Science and Techonology 2011; 22: 121-126.

Farkas J. Charged particle and photon interactions with matter. Food irradiation. In A. Mozumder, Y. Hatano (Eds.); Basel: Marcel Dekker, Inc. New York, 2004;pp. 785-812.

Farkas J. Irradiation of poultry meat. In G. C. Mead (Ed.), Food safety control in the poultry Boca Raton/Cambridge: CRC Press/Woodhead Publ. Ltd., 2005; pp. 433-453.

Ferraira ICFR, Antonio AL, Verde SC. Food Irradiation Technologies Concepts, Applications and Outcomes, The Royal Society of Chemistry. 2018.

Goresline, HE. Historical aspects of the radiation preservation of food. In: preservation of food by ionizing radiation. CRC Press. 2018; p. 1-46.

Kim HJ, Yong HI, Jayasena DD, Lee HJ, Lee H, Jo C. Microbial safety and physicochemical characteristics of electron beam irradiated whole egg powder. Food science and biotechnology. 2016; 25(29: 637-642.

Kim HJ, Yun HJ, Jung S, Jung YK, Kim KH, Lee J W, JCU. Effects of electron beam irradiation on pathogen inactivation, quality, and functional properties of shell egg during ambient storage. Food Science of Animal Resources, 2010; 30(4): 603-608.

Kim HJ, Yun HJ, Jung S, Jung YK, Kim KH, Lee J W, Jo CU. Effects of electron beam irradiation on pathogen inactivation, quality, and functional properties of shell egg during ambient storage. Food Science of Animal Resources. 2010; 30(4): 603-608.

Kobayashi K, Yasuda H. Formation of a superstructure in 1T-TiSe2 induced at room temperature by electron beam irradiation. Materials Research Express. 2018; 5(8): 085006.

Komolprasert V, Morehouse KM, Morehouse KMatthew. Irradiation of Food And Packaging. DC: American Chemical Society, Washington. 2004.

Korel F, Orman S. Gıda Işınlaması, Uygulamaları ve Tüketicinin Işınlanmış Gıdaya Bakış Açısı Harran Üniversitesi Ziraat Fakültesi Dergisi. 2005; 9(2): 19-27.

Kundu D, Holley R. Effect of low-dose electron beam irradiation on quality of ground beef patties and raw, intact carcass muscle pieces. Journal of food science. 2013; 78(6): S920-S925.

Kwon JH, Kwon Y, Nam KC, Lee EJ, Ahn DU. Effect of electron-beam irradiation before and after cooking on the chemical properties of beef, pork, and chicken. Meat science. 2008; 80(3): 903-909.

Lagunas-Solar MC. Radiation processing of foods. An overview of scientific principles and current status. Journal of Food Protection. 1995; 58:186–192.

Lewis SJ, Velasquez A, Cuppett SL. Effect of electron beam irradiation on poultry meat safety and quality. Poultry science. 2002; 81(6): 896-903.

Maxim JE, Neal JA, Castillo A. Development of a novel device for applying uniform doses of electron beam irradiation on carcasses. Meat science. 2014; 96(1): 373-378.

Miller RB, Antonio AL, Carreño I, Craven E, Strasser A, Kim J, Gryczka U. Food Irradiation Technologies: Concepts, Applications and Outcomes. Royal Society of Chemistry. 2017.

Min BR, Nam KC, Lee EJ, Ko GY, TrampelDW, Ahn DU. Effect of irradiating shell eggs on quality attributes and functional properties of yolk and white. Poultry science. 2005; 84(11): 1791-1796.

Molins R, Motarjemi Y, Käferstein F. Irradiation: a critical control point in ensuring the microbiological safety of raw foods. Food Control 2001; 12:347–356.

Olson D. Food irradiation future still bright. Food Technology. 2004;58(7): 112.

Olson DG. Irradiation of food. Food Technology. 1998;52: 56–62.

Ozer ZN, Yavuz M, Ozkan M, Yalım HA. Design and simulation of low-energy electron accelerator for industrial applications, 3rd International Conference on Theoretical and Experimental Studies in Nuclear Applications and Technology, 2017; 130.

Ozer ZN. Electron beam irradiation processing for industrial and medical applications. In: EPJ Web of Conferences. EDP Sciences. 2017; p. 01019.

Park JG, YoonY, Park JN, Han IJ, Song BS, Kim J H, Lee JW. Effects of gamma irradiation and electron beam irradiation on quality, sensory, and bacterial populations in beef sausage patties. Meat science. 2010; 85(2): 368-372.

Radomyski T, Murano EA, Olson DG, Murano PS. Elimination of pathogens of significance in food by low-dose irradiation: a review. Journal of food protection. 1994; 57(1):73-86.

Salimov RA, Cherepkov VG, Kuksanov NK, Kuznetzov SA. The use of electron accelerators for radiation disinfestation of grain. Radiation Physics and Chemistry. 2000, 57(3-6): 625-627.

Scientific Instrument Services Inc., USA. Simion, www.simion.com. Accession date: 02.03.2020.

Silindir M, Özer AY. Sterilization methods and the comparison of e-beam sterilization with gamma radiation sterilization. Fabad Journal of Pharmaceutical Sciences 2009; 34(1): 43.

Sommers CH, Fan X. Food irradiation research and technology. John Wiley & Sons ed., 2008.

Taha SM. Incidence, toxigenicity and control of certain pathogenic bacteria in different environmental sources. Ph.D. Thesis, Faculty of Science, Ain Shams University, Cairo, Egypt, 1999.

Turhan Ş, Karabacak H, Erel Y, Ocak ., Ünal S, Zengin T. Elektron hızlandırıcılarının gıda ışınlanması için değerlendirilmesi. 2002.

U.S. Department of Agriculture, Food Safety and Inspection Service. Food irradiation of meat food products, final rule. Fed. Regist.1999;64:72149–72166.

Wong PYY, Kitts DD. Physicochemical and functional properties of shell eggs following electron beam irradiation. Journal of the Science of Food and Agriculture. 2003; 83(1): 44-52.

Woodburn MJ, Raob CA. Household food following widely publicized outbreaks of foodborne illness. Journal of Food Protection, 1997; 60: 1105–1109.

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RESEARCH ARTICLE

Phytotherapy with *O. sanctum* and *O.onites* in Cows with Subclinical Mastitis

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ABSTRACT

Mastitis is a disease defined as inflamation of the mammary gland, which is common in dairy farms and negatively affects yield and quality. Herbal medicines have gained importance in our country due to lower toxicity, less side effects and absence of residue in milk.

This study was conducted on the essential oils obtained by extraction of *Ocimum sanctum* and *Origanum onites*, which are medicinal plants against mastitis pathogens. The aim of the study was to investigate the effect of *O. sanctum* and *O. onites* on the number of somatic cells, which is an indicator of subclinical mastitis. In the study, 18 dairy cattle and 6 control cows were selected by CMT (California mastitis test) in 80 dairy cows farm. The solution prepared from essential oils obtained from *O. sanctum* and *O.onites* plants was applied to the sick udder of 18 cows with subclinical mastitis, 12 ml / day x 3 days, 1st, 5th, 9th, 26th days. SHS was determined by taking the sample. SHS was measured as 2185.78 cells / ml before solution application and 809.72 cells / ml after solution applications. In conclusion, intramamarian administration of *O. sanctum* and *O.onites* in the treatment of subclinical mastitis can be considered as an alternative or an addition to antibiotics.

Key words: *Ocimum sanctum*, *Origanum onites*, subclinical mastitis

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Subklinik Mastitisli İneklerde *O. sanctum* ve *O. onites* ile Fitoterapi

ÖZ

Süt işletmelerinde yaygın olarak görülen, verim ve kaliteyi olumsuz etkileyen faktörlerden mastitis, meme bezinin yangısı olarak tanımlanan hastalıktır. Ülkemizde bitkisel ilaçlar, daha düşük toksisite, daha az yan etki ve sütte kalıntı olmaması nedeniyle önem kazanmıştır.

Bu çalışma, mastitis patojenlerine karşı tıbbi bitkilerden olan *Ocimum sanctum* ve *Origanum onites*' in ekstraksiyonu ile elde edilen uçucu yağları üzerine yürütülmüştür. Çalışmanın amacı, subklinik mastisin göstergesi olan somatik hücre sayısı üzerine *O. sanctum* ve *O. onites* in etkisi araştırılmıştır. Çalışmada, 80 başlık süt ineği çiftliğinde kuru döneme çıkacak 18 hasta süt ineği ve 6 kontrol grubu inek CMT (California Mastitis Test) ile seçilerek çalışmalar yürütülmüştür. *O. sanctum* ve *O. onites* bitkilerinden elde edilen uçucu yağlardan hazırlanan solüsyon, önceden tespiti yapılan 18 subklinik mastitli ineğin hasta olan memesine 12 ml/gün x 3 gün olacak şekilde meme içine uygulanmıştır 1., 5., 9., 26. günlerde süt numunesi alınarak SHS tayini yapılmıştır. SHS, solusyon uygulamadan önce 2185.78 hücre/ml, solusyon uygulamalarından sonra 809.72 hücre/ml olarak ölçülmüştür. Sonuç olarak, *O. sanctum* ve *O. onites* in subklinik mastit tedavisinde intramamarian uygulanması antibiyotiklere alternatif veya ek olarak değerlendirilebilir**.**

Anahtar Kelimeler: *Ocimum sanctum, Origanum onites,* subklinik mastitis

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GİRİŞ

Meme bezinin yangısı olarak da tanımlanan mastitis süt verimini, bileşimini etkileyen, hayvanların sürü dışı bırakılması ile tedavi masraflarından dolayı ciddi ekonomik kayıplara sebep olan ve süt sığırı işletmelerinde sık görülen bir hastalıktır (Gürbulak vd. 2009; Fratini vd, 2014; Akdağ vd. 2016; Younus vd. 2018; Borne vd. 2019). Mastitis, etkenlerin bulaşma şekilleri, korunma önlemleri ve tedavisi bakımından oldukça karmaşık bir yapı göstermektedir. Sığır mastitinin etiyolojisinde farklı mikroorganizmalar bulunmaktadır, *Stafilococcus aureus, Stafilococcus agalactiae, Streptococcus disgalactiae*, *Streptococcus uberis* ve *Escherichia coli* vakaların yaklaşık% 80'ine neden olur (Ranjan, vd. 2006). Subklinik mastitislere bağlı olarak sütte şekillenen değişimler, memeye plazma proteinlerinin geçmesi, iyon konsantrasyonundaki farklılaşma, lokal hücrelerin yıkımı sebebiyle intrasellüler bileşiklerin süte geçmesi, meme epitelinin sentez kapasitesinin azalması en önemlisi ise sütte somatik hücrelerin artması şeklinde görülmektedir (Gürbulak vd. 2009; Peker vd. 2016).

Mastitisli sütlerde, polimorf nükleer çekirdekli lökosit (PMN) sayısının artışını tespit eden testlerden California Mastitis Testi (CMT); 1957 yılında anormal sütlerin belirlenmesi amacıyla geliştirilmiştir. CMT, basit ve kolayca uygulanabilen, hızlı ve oldukça ucuz bir testtir ve subklinik mastitislerin belirlenmesinde SHS (Somatik Hücre Sayısı) hakkında kabaca bilgi vermektedir. CMT, tam olarak SHS hakkında kesin bir bilgi vermemekle birlikte sadece değerin düşük ya da yüksek olduğunu göstermektedir (Nizamlıoğlu vd.1992, Alkan vd., 2014). Somatik hücre sayısı, 1 ml süt içerisindeki sayılan hücre olarak ifade edilmektedir. Süt endüstrisi gelişmiş ülkelerde; sürü yönetimi, meme sağlığı ve süt kalitesinin değerlendirilmesinde somatik hücre sayımı en önemli kriterlerin başında gelmektedir (Alkan vd., 2014).

Bitki ve bitki ürünlerini kullanmayı içeren eski bir tedavi yöntemi olan fitoterapi, insanlığın varoluşundan bu yana hem insan hem de veteriner hekimliği alanında kullanılmaktadır (Kuru ve Oral 2013, Taçbaş ve Baydan 2018). Tıbbi bitkiler, alternatif tıbbın ana kaynağını oluşturmakta ve eski zamanlardan beri insan ve hayvan hastalıklarını tedavi etmek için kullanılmaktadır. Bitkisel ilaçlar, daha düşük toksisite, daha az yan etki ve sütte kalıntı bırakmaması nedeniyle önem kazanmıştır. Benzer nedenlerden dolayı, Dünya Sağlık Örgütü şifalı bitkilerin kullanımını vurgulamıştır. Mushtaq ve ark. (2018), mastitis patojenlerine karşı *Origanum vulgare, Lippia graveolens, Thymus vulgaris* tıbbi bitkilerin uçucu yağlarındaki karvakrol, timol ve sinnamaldehit gibi bileşenlerinin antimikrobiyal aktivitelerinin olduğunu belirtmişlerdir.

Ocimum sanctum (Hint Fesleğeni), çok çeşitli hayvan hastalıklarında kullanılan değerli bir bitkisel ilaçtır (Shafi vd. 2016). *O. sanctum'* un ana bileşenleri uçucu yağlar (Eugenol% 80), flavonoidler ve triterpen' dir (Ursolik asit). *O. sanctum'* un bitkisel özü immünomodülatör özelliklere, antimikrobiyal özellik ve antiinflamatuar özelliklere sahiptir (Aprajita vd.2017).

Origanum onites (Türk kekiği), uçucu yağ içeriği ve bileşimi en önemli kalite kriterlerinden biridir. Kuru kekik yaprakları % 2.0 - 4.5 uçucu yağ oranına sahip olmalıdır. Güçlü antimikrobiyal özellikleri nedeniyle, ana bileşen olan karvakrol, uçucu yağın içeriği bir kalite kriteri olarak kabul edilmektedir (Kaçar vd., 2006).

Uçucu yağlar, çeşitli ekstraksiyon teknikleri kullanılarak aromatik bitkilerden izole edilmektedir. Damıtma, bir karışımın bir şişede ısıtılması yoluyla buharlaştırmayı veya bunun ardından da buharın yoğunlaştırılmasını içermektedir. Uçucu yağların çıkarılması için standart damıtma sistemi olan hidrodistilasyon, genellikle Clevenger tipi cihaz ile gerçekleştirilmektedir (Clevenger, 1928; Gavahian, vd. 2015). Ekstraksiyon teknikleri arasında, hidrodistilasyon (HD), uçucu yağları şifalı bitkilerden ayırmak için kullanılan en yaygın yöntemdir.

Çalışmada, güçlü antibakteriyel etkinliği ile bilinen *O. sanctum* ve *O. onites* bitki ekstraktlarının antimikrobiyal etkinliğinin kuru dönemdeki ineklerde subklinik mastitis üzerine etkisi araştırılmıştır.

MATERYAL VE YÖNTEMLER

Bitki Materyallerinin Toplanması

Balıkesir Büyükşehir Belediyesi Kırsal Hizmetler Daire Başkanlığı Çiftçi Eğitim ve Üretim Merkezi (BAÇEM) Tıbbi Aromatik Bitki yetiştirilen parsellerden *O. sanctum* ve *O. onites* tarihlerinde hasat edilmiştir. BAÇEM Tıbbi Aromatik Bitkiler Ar-Ge laboratuvarında ekstraksiyon için gönderilmiştir.

O. sanctum ve *O. onites* Bitkilerinin Ekstraksiyonu ve Solüsyonun Hazırlanması

O. sanctum ve *O. onites* bitkilerin, uçucu yağın en fazla olduğu bölge olan herba kısımları kullanılmıştır. *O. sanctum* bitkisi ve *O. onites* bitkisi Clevenger düzeneğinde 100 ^{o}C sıcaklıkta 4 saat süre sonunda uçucu yağ elde edilmiştir (Clevenger, 1928). Abboud ve ark. (2015), solüsyon %20 dilüasyon oranında dilüe edilmiştir. Çalışma sonucu solüsyon etkinliğini gösteren inhibisyon zon çapının yüksek değerde olduğu belirlenmiştir (Abboud vd., 2015).

Gupta ve ark. (2020) göre, *O. onites* bitkisindeki etken maddelerden olan karvakrol, timol ve limonen; *O. sanctum* bitkisindeki etken maddelerden olan öjenol ve limonen mastitis hastalığının etkenlerinden olan *Escherichia coli* bakterisine karşı baskılayıcı konsantrasyonu 0.00063-0.01 ml arasında belirlenmiştir. Aynı zamanda *O. onites*' in etken maddesi olan karvakrol ve timolün *Streptecoccus* spp.

karşı baskılayıcı konsantrasyonu sırasıyla 0.00016ml, 0.00031ml olarak belirlenmiştir. Bu verilerden yola çıkılarak çalışmada kullanılması planlanan bitkilerdeki etken madde içerikleri Bezmialem Üniversitesi Fitoterapi Eğitim Uygulama ve Araştırma Merkezine gönderilerek uçucu yağ analizleri yapılmıştır. Analiz sonucuna göre *O. onites'* te limonen %0.812, timol %11.408, karvakrol %21.838; *O. sanctum* da limonen %0.36, öjenol %20.691 olarak tespit edilmiştir. Çalışmada kullanılması planlanan bitki uçucu yağlarının dozajını belirlemek ve çalışılacak bitki uçucu yağlarındaki önemli etken maddeler arasındaki konsantrasyonu en düşük olan limonen etken maddesinin baskılayıcı konsantrasyonuna ulaşabilmek için uçucu yağ dozu bitki başına 1' er ml olarak belirlenmiştir. Bu çalışmadan yola çıkılarak çalışmada kullanılacak dozaj her 1cc uçucu yağına 5 ml saf su olacak şekilde dilüe edilerek %20 dilüasyon oranı sağlanmıştır. Günlük her bir uygulama için 1 cc kekik uçucu yağı ve 1cc hint fesleğeni uçucu yağı 10 ml saf suya karıştırılarak solüsyon hazırlanmıştır (Gupta vd., 2020).

Subklinik Mastitisli İneklerin Seçimi

Balıkesir Edremit, Çıkrıkçı Köyü 120 başlık Burak Bey Çiftliğindeki Holstein ırkı, besleme sistemleri TMR (Total Mixed-Ration) esaslı olan, 3. ve 4. laktasyondaki, kuru ot, konsantre yem ve silaj içeren rasyonla beslenen, besi randımanı 2 olan, ortalama süt verimi 18-22 kg/gün olan 80 inek üzerinde ilk olarak süt numuneleri CMT yapılarak değerlendirilmiştir Çalışmada, CMT +1 altı kontrol grubu, +:1, ++:2, +++:3 olarak değerlendirilenler deney grubu olarak ele alınmıştır (Alkan vd,2014).

CMT sonuçlarına göre küpe numaraları belli olan ineklerden sağım sonrası alınan 50 şer ml süt numuneleri somatik hücre tayini için Balıkesir Damızlık Sığır Yetiştiricileri Birliği' ne gönderilmiş ve subklinik mastitis olan ineklerin pozitifliği tespit edilerek 18 subklinik mastitisli inek ve 6 kontrol grubu inek ile çalışma yürütülmüştür.

Solüsyonun Intramamarian Uygulaması

Subklinik mastitis olan ineklerin memeleri sağım öncesi teat dipping uygulanmıştır. Sağım yapıldıktan sonra Danışman Veteriner Hekim eşliğinde 20 ml' lik enjektörlere hazırlanmış olan solüsyon steril bir şekilde 12 ml/gün x 3 gün olacak şekilde meme başından meme içine uygulanmıştır (20). Yapılan araştırmalar doğrultusunda yapılacak çalışmanın uygulamanın 3. gününde yangısal reaksiyona bağlı somatik hücre sayısının artacağı muhtemeldir (Mukherjee ve Ram 2009; Perini vd., 2014; Abboud vd., 2015; Akdağ vd., 2016; Shafi vd 2016; Mukesh vd.2018; Gupta vd 2020).

Süt Numunelerinin Somatik Hücre Tayini

İlk gün sağım yapılmadan ve solusyonlar meme içine uygulanmadan önce 1. numune alınmıştır. Daha sonra bunu takip eden 5-9-26. günlerde diğer numuneler alınıp somatik hücre tayini için Balıkesir Damızlık Sığır Yetiştiricileri Birliğine gönderilmiştir. Analiz Bentley 400 somatik hücre tayin cihazıyla tayin edilip numuneler değerlendirilmiştir.

İstatistik Analiz Yöntemi

Verilerin istatistiksel analizi IBM SPSS Statistics 24 paket programı aracılığıyla gerçekleştirilmiştir. Verilerin analizinde tanımlayıcı istatistikler (Ortalama ve Standart Sapma) hesaplanmıştır. Deney ve kontrol gruplarında tekrarlı ölçülen somatik hücre tayinlerinin analizinde tekrarlı ölçümlerde iki yönlü varyans analizi (Two-Way Repeated Measures ANOVA) kullanılmıştır. Anlamlılık düzeyi $p<0,05$ ve $p<0,01$ olarak belirlenmiştir.

BULGULAR ve TARTIŞMA

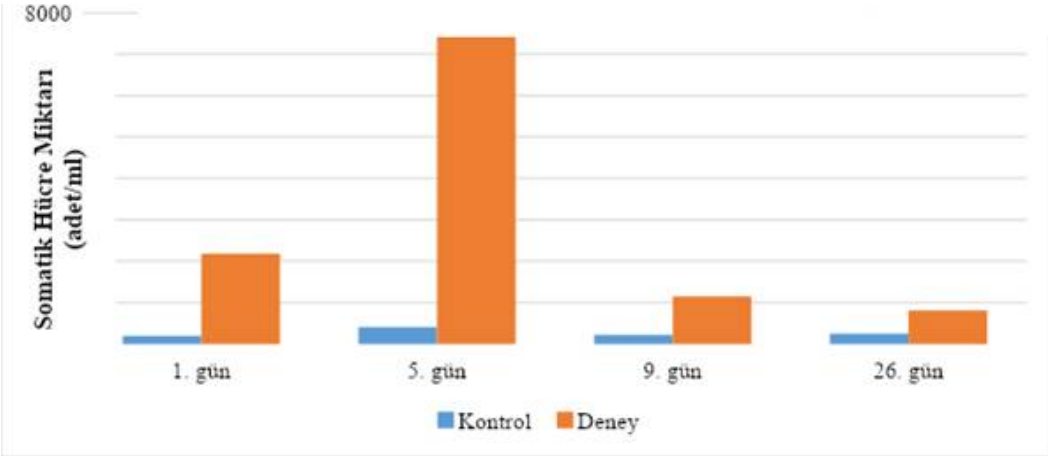
O. sanctum ve *O. onites* bitkilerin, uçucu yağın en fazla olduğu bölge olan herba kısımlarından Clevenger düzeneği ile ekstraksiyon sayesinde uçucu yağları ve kantitatif miktar tayinleri başarılı bir şekilde gerçekleştirilmiştir.

Tablo 1. Büyükbaş hayvan gruplarının ve ölçüm zamanlarına göre somatik hücre tayinlerinin karşılaştırılması.

Gruplar / Zamanlar	Deney(18 adet inek)	Kontrol(6 adet inek)	Toplam(24 adet inek)	F	p
	$\overline{X}$ ± S.S.	$\overline{X}$ ± S.S.	$\overline{X}$ ± S.S.		
1. gün	2185.78±2252.10	192.50±127.31	1687.46±2128.32[b]		
5. gün	7715.67±2877.07	403.67±304.99	5887.67±4074.19[a]	67.644	.001**
9. gün	1152.33±971.64	216.67±179.04	918.42±935.98[b]		
26. gün	809.72±1144.58	248.00±158.79	669.29±1017.61[b]		
Toplam				Etkileşim	
	F=11.563; p< .001**			F=10.941; p< .001**	

**p< .01; $\bar{x}$: Ortalama; S.S.: Standart Sapma; ab: Farklı harfleri içeren ortalamalar arasında fark vardır.

Tablo 1 incelendiğinde, Hayvan gruplarına göre Somatik Hücre Sayısı ortalamalarının istatistiksel olarak fark gösterdiği tespit edilmiştir (F=11.563; p< .001). Bu sonuç deney grubunda yer alan 18 büyükbaş hayvanın daha yüksek somatik hücre ortalamasına sahip olduğunu göstermektedir. Dahası, somatik hücre ortalamalarının ölçüm zamanlarına göre de istatistiksel farklılık gösterdiği tespit edilmiştir (F=67.644; p< .001). Buna sonuca göre, en yüksek Somatik Hücre ortalamalarının 5. ölçüm gününde olduğu ve diğer ölçüm günleri arasında fark olmadığı tespit edilmiştir (p< .05). Ek olarak, büyükbaş hayvan grupları(18 adet deney grubu, 6 adet kontrol grubu inek) ile somatik hücre tayin günleri arasındaki etkileşim istatistiksel olarak anlamlı bulunmuştur (F=10.941; p< .001).



Bu veriler laktasyonun son evresindeki süt ineklerinin subklinik mastitise karşı bitkisel tedavinin faydalı etkilerini incelediğimiz bu önemli çalışmadan elde edilmiştir. Organik üretim yapan ve sütte kalıntı problemi yaşayan büyük işletmelerde *O. sanctum* ve *O. onites* bitkilerinden hazırladığımız solüsyonun pozitif etkisi meme içi enfeksiyonların eliminasyonu ile doğrulanarak potansiyel immünomodülasyon ve antienflamatuar, antibakteriyel etkiye sahip bitkilerde lipofilik bileşenlerin varlığı ile ilişkilendirilebilir.

Mukherjee ve ekibi (2010)'ın yaptığı çalışmada, Sığır subklinik mastitinde *Tinospora cordifolia*'nın (*T. cordifolia*; kök) hidrometanolik ekstraktının hastalıklı meme bezi bağışıklığının arttırılması ve terapötik potansiyeli araştırılmıştır. *T. cordifolia* tedavisinin hidrometanolik özütünün intramammarian infüzyonu başlangıçta SCC'yi artırmıştır; daha sonra, tedavi periyodunun 15. gününde hücre sayısında önemli bir azalma (P <0.05) gözlenmiştir.

Perini ve ekibi (2014), uçucu yağlar antibiyotiklere alternatif olarak, Gram + ve Gram - patojenlerine karşı antibakteriyel etkiye sahip oldukları için mastitis tedavisinde kullanılmıştır. Bu çalışmada, *Staphylococcus aureus* ve *Streptococcus agalactiae* nın sebep olduğu klinik ve subklinik mastitiste *Salvia sclarea*, *Eugenia caryophyllata*, *Thymus vulgaris, Cymbopogon winterianus, Elettaria cardamomum, Cymbopogon flexuosus, Rosmarinus officinalis* ve *Cinnamomum cassia* esansiyel yağları her iki patojene karşı yüksek antibakteriyel etki göstermiştir. *S. sclarea* ve *R. officinalis* yağları, her iki mikroorganizmaya karşı önemli bir antibakteriyel aktivite göstermemiştir. *C. cassia, C. flexuosus* ve *E. caryophyllata* esansiyel yağlarının kombinasyonunda *S. aureus* ve *S. agalactiae*'ye karşı antimikrobiyal etkide yüksek sinerjizm gözlenmiştir. *C. cassia* esansiyel yağının antimikrobiyal etkide orta düzeyde sinerjizm gözlenmiştir. Sonuç olarak, uçucu yağların kullanımı gram-pozitif ve gram-negatif bakterilere karşı antibakteriyel etkiye sahiptirler ve insan sağlığı üzerinde zararlı etkileri bulunmamaktadır. Sonuçlar, *S. aureus* ve *S. agalactiae*' nin neden olduğu sığır mastitisini kontrol etmek için uçucu yağların kullanılabileceğini göstermiştir.

Abboud ve ekibinin çalışmasında (2015), Sığır Staphylococcus ve Streptococcus mastitis patojenlerine karşı antibiyotiklere alternatif olarak *Thymus vulgaris* ve *Lavandula angustifolia* uçucu yağlarının antimikrobiyal aktivitelerini araştırmak için yürütülmüştür. CMT kullanılarak klinik mastitis tespitinde 5 ay süreyle haftada 1 çalışılmıştır. *T. vulgaris* ve *L. angustifolia* buhar ekstraksiyonu yapılan uçucu yağlar aynı zamanda tek başına veya 3 farklı %10, 20, 30 konsantrasyondaki kombinasyonları uygulanmıştır. Timus ve lavanta uçucu yağının %10'u timus ve lavanta karışımının intramamarian uygulaması, birbirini takip eden 4 tedaviden sonra farklı süt numunelerinin bakteriyel koloni sayısında önemli bir azalmaya neden olmuştur. Vazelinli lavanta masaj uygulaması, timus solüsyonunun daldırma yada masaj uygulaması; Staphylococcus ve Streptococcus 2 bakteriyel patojenine karşı güçlü bir antibakteriyel aktivite göstermiştir. Daldırma solüsyonu ve masaj uygulaması, intramamarian uygulamasına göre tedavi edici özelliği daha fazla olduğu belirlenmiştir.

Shafi ve ekibi (2016), antibiyotik tedavisinin ilaca dirençli mikropların gelişmesine neden olabileceği ve polimorf hücrelerin aktivitesini azaltacağını açıklamıştır. Yaptıkları çalışmada, süt ineklerinde mastitis de *O. sanctum* immünoterapötik etkisini potansiyelini araştırmışlardır. İneklerin yarısı kontrol ve diğer yarısı deney grubu olarak ayrılmıştır. Deney grubuna *O. sanctum* yaprak tozu, günlük 2 doza bölündü ve 7 gün boyunca ağızdan 600 mg/kg olarak rasyona katılmıştır. Uygulama sonucunda tedavi mastitis($\chi 2 = 5.07$; $P \leq 0.5$) %69,23 uzaklaştırmış, SCC değeri ve seroplazmin konsantrasyonu önemli ölçüde düşürdüğü tespit edilmiştir. Böylece meme yangısını düşürmüş ve süt kalitesini iyileştirmiştir.

Akdağ ve ekibini yaptığı çalışmada (2017), CMT skorlarının süt laktoz, yağ ve donma noktası ile ilişkili olduğu, laktoz oranı ve donma noktası değerlerindeki düşüş ile birlikte yağ oranındaki artışın mastitis göstergesi olarak kabul edilebileceği belirtilmiştir. Bu çalışmada subklinik mastitisin teşhisi ve tedavisinde özellikle sütte antibiyotik kalıntısı bırakmayan fitoterapik çalışmaların insan sağlığı açısından ortaya çıkabilecek riskleri engelleyebilmek ve süt ve süt ürünlerinin kalitesinin hatalı olarak değerlendirilmesinin önüne geçebilmek için önem kazanmıştır.

Mukesh ve ekibi (2018), 3 Hindistan ırkı olan 20 inekte *Terminalia chebula* (Combretaceae) bitkisinin subklinik mastitis üzerindeki etkisi araştırılmıştır. Araştırmada bitkinin etil asetat ekstresinin *in vitro* antibakteriyel aktivitesi disk difüzyon yöntemi ile incelenmiştir. Toplamda 3 gün süren uygulamanın 3. gününde somatik hücre sayısının genel olarak aşırı artışı görülmüştür. Üç farklı konsantrasyon arasında 500 µg / mL konsantrasyon ekstresi standart amoksisilin kadar etkili olduğu tespit edilmiştir. Sonuçlar, sığır subklinik mastitine karşı antibiyotik tedavisine alternatif olarak *T. chebula*'dan bitki ekstraktının kullanımını desteklemektedir.

SONUÇ

Araştırma sonuçlarının hem bilimsel birikime, hem de ekonomik alana katkı potansiyeli bulunmaktadır. Mastitisin olumsuz etkilerinin önlenmesinde hastalığın subklinik teşhisinin önemi kabul edilen bir gerçektir. Bu nedenle sütçü işletmeler düşük maliyetli ve saha şartlarında kolay uygulanan bir yöntemle hayvanlarının mastitis olup olmadıklarını mümkün olan en erken dönemde belirlenmelidir (Gürbulak vd. 2009; Fratini vd, 2014; Akdağ vd. 2016; Younus vd. 2018; Borne vd. 2019).

Klinik vakalarda ve kuru dönemdeki ineklerde sistemik antibiyotik veya intramammarian infüzyonları sığır mastitinin kontrolü için yaygın olarak kullanılan alternatif tedavilerdir (Perini vd., 2014). Gebe süt ineklerinde ardışık laktasyonlar arasında ve sonraki emzirme döneminde süt verimini en üst düzeye çıkarmak ve meme bezi epitelyumunun laktasyona uygun şekilde girmesine izin vermek için kuru dönem önerilmektedir. Kuru dönem ayrıca, mevcut meme enfeksiyonlarının yaygınlığını azaltmak ve yeni enfeksiyon insidansını azaltmak için, süt kalıntıları riski olmadan uzun dönem intramammarian antibiyotik tedavisine izin vermektedir (Pinedo, vd. 2011). Meme enfeksiyonlarında antibiyotik tedavisinin orta derecede etkisi ve uzun süreli antibiyotik kalıntısı olduğu için sütün değerlendirilememesi söz konusu olmaktadır. Sığır mastitis tedavisindeki antibiyotik kullanımı, hayvanın doğal savunma mekanizmasına olan olumsuz etkilerini azaltmak ayrıca endüstri ve toplum sağlığında büyük endişe yaratan sütte dirençli bakteri ve kalıntıların olması nedeniyle mastitisin kontrolüne yönelik alternatif antibiyotik strateji kavramları dikkat çekmektedir (Perini vd. 2014; Shafi vd. 2016). Hem klinik hem de subklinik mastitisi tedavi etmek için kullanılan antibiyotiklerin daha az etkin olması nedeniyle bitkisel esansiyel yağlar gibi verimli alternatifler bulma konusunda çok sayıda araştırma yapılmıştır (Giupana vd.2018).

Sonuç olarak, *O. sanctum* ve *O. onites* bitkilerinden elde ettiğimiz bu solüsyon subklinik mastitis tedavisinde alternatif tedavi yöntemi olarak kullanılabileceği sonucuna varılmıştır. Tedavi etkinliğinin daha iyi bir şekilde değerlendirilebilmesi için daha geniş ve kapsamlı çalışmalara ihtiyaç duyulmaktadir (Mukherjee vd. 2005; Oral vd.2014).

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KAYNAKLAR

Abboud, M., El Rammouz, R., Jammal, B., &Sleiman, M. *In vitro* and *In vivo* Antimicrobial Activity of two Essential Oils *Thymus vulgaris* and *Lavandula angustifolia* against Bovine Staphylococcus and Streptococcus Mastitis Pathogen. Middle East.2015; J, 4(4): 975-983.

Akdağ, F., Gürler, H., Bülent, T.E.K E., Uğurlu, M., & Koçak, Ö. Jersey Irkı İneklerde CMT Skorlarının ve Skorların Değerlendirilmesindeki Farklılığın Süt Verimi, Süt Bileşimi ve SubklinikMastitis Tanısına Etkisi. İstanbul Üniversitesi Veteriner Fakültesi Dergisi, 2017; 43(1): 44-51.

Alkan, H., Baştan, A., Salar, S., Özdal, M., & Kaymaz, M. Kuru Döneme Çıkarken Enfekte ve Sağlıklı Meme Loblarında California Mastitis Test ve Somatik Hücre Sayısı ile Bakteriyolojik Muayene Sonuçlarının Karşılaştırılması. Ankara Üniversitesi Veteriner Fakültesi Dergisi, 2014; 61(3): 179-183.

Aprajita, J., Singh, A. P., Gaur, A., Kachhawa, J. P., Ankita, S., Sharma, P., &Joshi, R. K.. Study of Thepotential of *Ocimum sanctum* in Subclinical mastitis in Rathi cattle. Veterinary Practitioner, 2017; 18(1): 15-17.

Borne, B. H.,Schaik, G., Lam, T. J., Nielen, M., &Frankena, K.. Intramammary Antimicrobial Treatment of Subclinical Mastitis and Cow Performance Later in Lactation. Journal of dairy science, 2019; 102(5): 4441-4451.

Clevenger, J. F. Apparatus For The Determination of Volatile Oil. Journal of Pharmaceutical Sciences, 1928; 17(4): 345-349.

Fratini, F.,Casella, S., Leonardi, M., Pisseri, F., Ebani, V. V., Pistelli, L., &Pistelli, L. Antibacterial Activity of Essential Oils, Their Blends and Mixtures of Their Main constituents Against Some Strains Supporting Livestock Mastitis. Fitoterapia, 2014; 96: 1-7.

Gavahian, M., Farahnaky, A., Farhoosh, R., Javidnia, K., & Shahidi, F.. Extraction of Essential Oils From *Mentha Piperita* Using Advanced Techniques: Microwave Versus Ohmic Assisted Hydrodistillation. Food and Bioproducts Processing 2015; 94: 50–58.

Giupana, R.,Mihaela, N., Emoke, P., Negrutiu, V., Popescu, S., Vasiu, A., Raluca, P., Carmen, D.S., & Marina, S. Extract Nature İnfluence The Effects of *Melissa Officinalis* L. on the Milk Microbiomethe Milk Microbiome in Cows with Subclinical Mastitis. Annals of Phytomedicine-An International Journal,2018; 7(2): 124-128.

Gupta R., Kumar S. ve Khurana. Essential Oil and Mastitis In Dairy Animals: A Review. *Haryana Vet.*2020; 59(SI): 1-9.

Gürbulak, K.,Canoğlu, E., Abay, M., Atabay, Ö., Bekyürek, T.. İneklerde Subklinik Mastitisin Farklı Yöntemlerle Saptanması. Kafkas Univ Vet Fak Derg, 2009; 15(5): 765-770.

Kaçar, O., Göksu, E., Azkan, N.. İzmir Kekiği (*Origanum onites* L.) Farklı Sıklıkların Bazı Agronomik ve Kalite Özellikleri Üzerine Etkisinin Belirlenmesi. U.Ü. Ziraat Fakültesi Dergisi, 2006; 2 (21): 51-60.

Kuru, M. ve Oral H.. Mastitis Tedavisinde Fitoterapi ve Homeopatinin Kullanımı. Harran Üniversitesi Veteriner Fakültesi Dergisi,2013; *2*(2): 112-116.

Mukesh KN.,Sheth, N. R., N Bhatt, V. D. *In vitro* Antibacterial Evaluation of *Terminalia Chebula* as an Alternative of Antibiotics Against Bovine Subclinical Mastitis. *Animal Biotechnology*, 2019; *30*(2): 151-158.

Mukherjee, R., De, U. K., & Ram, G. C. Evaluation of Mammary Gland Immunity and Therapeutic Potential of *Tinospora cordifolia* against bovine subclinical mastitis. *Tropical animal health and production*, 2010; *42*(4); 645-651.

Mukherjee, R.,Dash, P. K., & Ram, G. C. Immunotherapeutic Potential of *Ocimum sanctum* (L) in Bovine Subclinical Mastitis. *Research in veterinary science*, 2005; *79*(1): 37-43.

Mushtaq, S.,Shah, A. M., Shah, A., Lone, S. A., Hussain, A., Hassan, Q. P., & Ali, M. N.. Bovine Mastitis: An Appraisal of Its Alternative Herbal Cure. Microbial Pathogenesis, 2018; 114: 357-361.

Nizamlıoğlu, M., Kalaycıoğlu, L., Dinç, D. A., Erganiş, O., & Özeren, F. İneklerde Subklinik Mastitisin Erken Teşhisi Amacıyla Sütte N-Asetil B-D Glukozaminidaz (NAG ase) Enzim Aktivitesinin Tayini. Selçuk Üniversitesi Fakülte Dergisi 1992; 8(2): 60-63.

Oral, H., , Çolak, A., Polat, B., Cengiz, M., Cengiz, S., Baştan, A., & Kaya, S. Sütçü İneklerde Subklinik Mastitisin Tedavisinde Aloe Vera Kullanımının Etkinliği. Erciyes Üniversitesi Veteriner Fakültesi Dergisi, 2014; 11(3): 157-161.

Peker Akalın, P., Ergün, Y., Başpınar, N., Doğruer, G., Küçükgül, A., Cantekin, Z., & Salar, S.. Subklinik Mastitisli İneklerde Süt ve Süt Hücrelerinde Vitamin C Düzeyleri. Etlik Veteriner Mikrobiyoloji Dergisi, 2016; 27(1): 21-26.

Perini, S.,Piccoli, R. H., Nunes, C. A., Bruhn, F. R. P., Custodio, D. A. C., & Costa, G. M. Antimicrobial Activity of Essential Oils Against Pathogens Isolated From Bovine Mastitis. J Nat Prod Plant Resour, 2014; 4(2): 6-15.

Pinedo, P., C. Risco, and P. Melendez. "A Retrospective Study on the Association Between Different Lengths of the Dry Period and Subclinical Mastitis, Milk Yield, Reproductive Performance, and Culling in Chilean Dairy Cows."Journal of dairy science. 2011; 94(1): 106-115.

Ranjan, R.,Swarup, D., Patra, R. C., &Nandi, D.. Bovine Prototheca Mastitis: a review. Perspectives in Agriculture, VeterinarySciences, Nutrition And Natural Resources, 2006; 1(17): 1-7.

Shafi, T. A.,Bansal, B. K., Gupta, D. K., &Nayyar, S.. Evaluation of İmmunotherapeutic Potential of *Ocimum Sanctum i*n Bovine Subclinical Mastitis. Turkish Journal of Veterinary and AnimalSciences, 2016; 40(3), 352-358.

Taçbaş, E. ve Baydan, E.. Organik Hayvan Yetiştiriciliğinde Hastalıkların Sağaltımında Kullanılabilecek Maddeler. Lalahan Hayvancılık Araştırma Enstitüsü Dergisi, 2018; 58(2): 117-122.

Younus, M.,Ahmad, T., Sharif, A., Bilal, M. Q., Nadeem, M., Ashfaq, K.. Comparative Therapeutic Efficacy of Homeopathic Complex, Herbal Extract And Antibiotic in The Treatment of Subclinical Mastitis in Dairy buffaloes. BuffaloBulletin, 2018; 37(2): 221-234.

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RESEARCH ARTICLE

Effects of Unbalanced and Balanced Applied Loads on Norbergs Angle in Ventrodorsal Hip-Extended Radiographies

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ABSTRACT

Two radiographic distraction techniques (Standard Ventrodorsal Hip-Extended Radiography, SHER, and Balanced Hip-Extended Radiography, BHER) were evaluated for Canine Hip Dysplasia (CHD) diagnosis on 100 hip joints of 50 dogs to determine the most reliable method in the detection of higher hip laxity employing Norbergs-Olsson angle (NoA) evaluation (FCI hip scoring). Anesthesia was standardized due to uniformity in muscle relaxation and applied to extend loads (SHERKg and BHERKg) were measured on hind legs using electronic weight scales (EWS) simultaneously with ventrodorsal (V/D) radiography. Results of NoA scores were evaluated statistically together with weight scale (WS) values. Significant differences were found between groups of methods for both in WS results ($p<.001$) and NoA evaluations ($p<.001$). The difference between right and left sides in WS results was not found significant ($p>.05$). Even if there was no significant difference occurred in this, balancing the loads with the BHER method caused a significant difference in NoA values between tested methods ($p<.001$). Moreover, the linear, positive, and strong correlation between SHER and BHER methods was shown the reliability of the BHER method in NoA evaluations ($rs= .910$, $p<.001$). In conclusion, by having a positive and strong correlation with the standard method, and better outcomes in FCI hip scoring with lower misdiagnose frequency thus affecting the clinical outcome, the BHER method was offered as a reliable method in the diagnosis of CHD.

Keywords: canine hip dysplasia, norberg's angle, balanced hip extended, ventrodorsal hip extended, radiography

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Dengelenmış ve Dengesiz Gerilim Kuvvetlerinin Köpeklerde Ventro-Dorsal Kalça Görüntülenmesinde Norbergs Açısı Üzerine Etkileri

ÖZ

İki radyografik distraksiyon tekniği (Standart, SHER ve Dengelenmiş Radyografi, BHER) köpek kalça displazisinin değerlendirilmesinde 50 köpeğin 100 kalça ekleminde değerlendirildi ve sonuçlar Norberg-Olsson (NoA) açı derecesi temelinde ele alındı (FCI skorlama). Anestezik yöntem kas gevşemesi yönünden standardize uygulandı. Arka ayaklara uygulanan çekme kuvvetleri (SHERKg ve BHERKg) radyografiler sırasında elektronik yük ölçer (WS) alet vasıtası ile ölçüldü. NoA ölçüm sonuçları yük ölçer sonuçları birlikte istatistiksel yönden değerlendirildi. NoA açısı ($p<.001$) ve WS sonuçlarında gruplar arasında istatistiksel yönden anlamlı sonuçlar belirlendi. WS sonuçlarında sağ ve sol ayak tarafları arasında fark belirlenmedi ($p>.05$). BHER metodunda kullanılan dengeleyici çekme kuvveti sonucunda NoA ölçümlerinde anlamlı farklılık oluştuğu görüldü ($p<.001$). SHER ve BHER metodları arasında istatistiksel yönden lineer, pozitif ve güçlü bir korrelasyon bulunması BHER metodunun güvenilirliği yönünde değerlendirildi ($rs= .910$, $p<.001$). Karar aşamasında; uygulanan BHER metodunun FCI skorlaması yönünden daha az yanlış tanı bulgusu göstermesi neticesinde klinik aşamada köpek kalça displazisinin belirlenmesinde güvenilir bir metod olduğu ve meslektaşlarımıza önerilebileceği sonucuna varıldı.

Anahtar kelimeler: köpek kalça displazisi, norbergs açısı, dengelenmiş kalça gerdirme, ventrodorsal kalça gerdirme, radyografi

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INTRODUCTION

Canine hip dysplasia (CHD) is frequently seen as one of the most common developmental hereditary diseases in dogs which can lead to debilitating osteoarthritis in the advanced stage. Early diagnosis is crucial in the evaluation of the CHD (Farese et al. 1998, Smith et al. 1993). Many clinical reports and studies have been published about early diagnosis of Canine Hip Dysplasia in comparison with palpation and radiographic methods (Adams et al. 1998, Corfield et al. 2007, Culp et al. 2006, Meomartino et al. 2002, Ohlerth et al. 2003, Reagan 2017, Thompson et al. 2007, Verhoeven et al. 2007) in the diagnosis of hip laxity, which is one of the predictors of secondary joint disease (Dueland et al. 2001). The degree of hip joint subluxation is a predictive risk factor for CHD expression in dogs as young as 16 weeks of age (Reagan 2017, Risler et al. 2009). Many genetic screening programs and study groups such as Federation Cynologique Internationale (FCI) and Pennsylvania Hip Screening Program (Penn-Hip) have utilized various radiographic methods for the evaluation of joint laxity and defined the criteria for several classifications in the diagnosis of Canine Hip Dysplasia (Adams et al. 1998, Farese et al. 1998, Reagan 2017, Risler et al. 2009, Yaprakci and Tekerli 2015). CHD is frequently evaluated based on SHER (Adams et al. 1998, Genevois et al. 2007, Genevois et al. 2008) and hip laxity is measured employing Norbergs-Olsson Angle (NoA) (Culp et al. 2006, Genevois et al. 2006, Verhoeven et al. 2007). Norberg Angle is found to be not an accurate predictor of canine hip conformation based on distraction index and dorsolateral subluxation score (Gaspar et al. 2016). A basis deficit of Standard V/D Hip-Extended Radiography remains unnoticed till today.

The main aim of this study was to demonstrate the difference of hip scores between two tested radiography methods (SHER and BHER) using NoA measurements according to Federation Cynologique Internationale (FCI) recommendations. The results of applied loads used to extend hind legs were conducted with associated NoA evaluations. Differences and correlations of the groups were evaluated statistically.

MATERIALS and METHODS

This study was carried out by the principles of the Basel Declaration and recommendations of Warsaw University of Life Sciences, Faculty of Veterinary Medicine Animal Hospital Guidelines and the diagnostic protocol was approved by the Head Committee for Clinical Studies in 2008. All subjects' owners gave written informed consent under the Declaration of Helsinki. A comparative study was held on 100 hip joints of 50 dogs from different breed, sex, and age that were brought to clinics for canine hip dysplasia evaluation. The mean age was 20.2 months old and the mean bodyweight of the group was 30.12 Kg (Std. Error=1.77, Std. Deviation=12.54). Cases were anesthetized using a standard combination of dexmedetomidine (Dexdomitor^{tm}, Pfizer, 325 ug/m2), butorphanol (Butomidor 0.2-0.4 mg/Kg) and midazolam (Dormicum^{tm}, Roche 0.1-0.25 mg/Kg) administrated intravenously. Referring to the sedation scale that is used in a related study (Genevois et al. 2006) an excellent muscle relaxation is crucial and was chosen for the convenient radiography procedure for all cases. WS measurements were obtained simultaneously with radiographs. A slight inward rotation to hind legs was supplied to centralize the kneecaps on femoral notches. This was done by hobbling the hind limbs with a string at the level of stifle joints (Figure 1, ty). A cloth strap (cs) was tautened over the stifle joints to stabilize the back part of the animal on the table and to parallel hind legs to the table (Figure 1, cs). Legs were tied with band strings (Figure 1, c) connected over EWS (Figure 1, EWS) to the adjustable poles (p) of the radiography table (Figure 1, p). The WS measurements were registered in kilograms within the accuracy of two decimals.

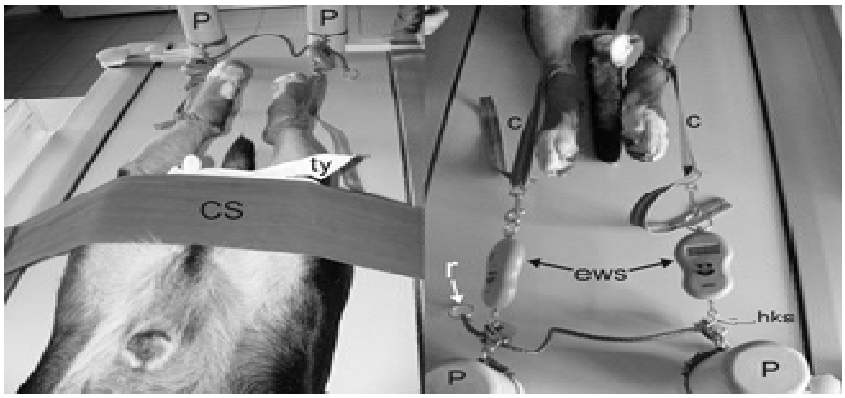

Figure 1. Ventrodorsal hip-extended radiography procedure and weight scale evaluation

Figure 1, Legend: cs: cloth strap, ty: hobbling string, p: table poles, hks: hook, ews: electronic weigth scale (two sides)

Two types of methods (SHER and BHER) were taken consecutively. None of the elements used to stabilize the patient on the table were changed between methods. In BHER, radiographs were taken using the "Balance Distractor" device on hind legs. The Balance Distractor device (Figure 1) was made of two pulleys (Figure 2, pu) and a string (Figure 2, a) passing through these pulleys which were attached to the pole rings (Figure 2, pr). On both ends of the string, there were rings (Figure 1, r) to supply an attachment to the hooks of EWS. In performing the radiography, firstly SHER evaluation was made and the hooks of the WSs (Figure 1, Figure 2, hks) were detached from pole rings (Figure 2, pr) by relaxing the poles and subsequently re-attached to the rings (Figure 1, r) of the string (Figure 2, a) of the Balance Distractor device to perform BHER method. The second measurement of WS was done for the BHER method in the same manner as it was done for the SHER method.

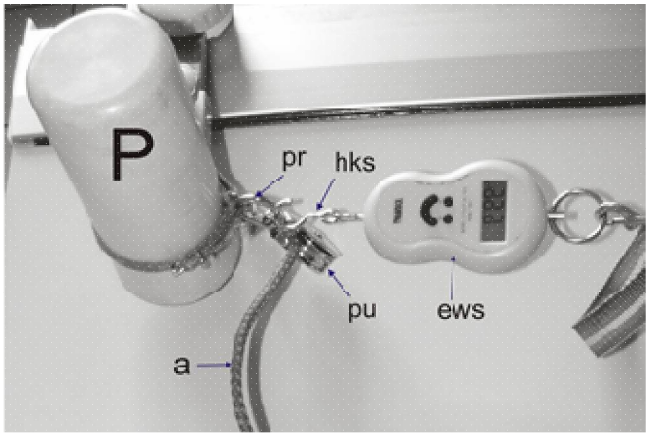

Figure 2. Parts of the Balance Distractor device (single side represented)
Figure 2, Legend: ews: electronic weight scale, pu: pulley, hks: hook, pr: pole ring, a: string of the balance distractor, p: pole

All radiographs were taken by digital radiography device (GE Prestige II, General Electric Company, Easton Turnpike Fairfield, Connecticut, USA) and by radiology technicians that were unaware about the features of the study to avoid flank judgment. NoA measurements were obtained by a radiologist as in guideline described in related studies (Adams et al. 1998, Culp et al. 2006, Henry 1992, Verhoeven et al. 2007) using a standard measuring template and 1 degree of accuracy was chosen for the evaluation. The rotated radiographs were repeated and found concurrent diseases such as pelvic fractures, obvious degenerative joint disease, or coxofemoral luxations were excluded from the study. The normality of the data was tested with the Kolmogorov-Smirnov test. To compare differences between the NoA angles of SHER and BHER methods, Wilcoxon Signed Rank test was used. To compare differences of the WS used with SHER and BHER methods, paired sample test was used. To find out the relationship between NoA angles of SHER and BHER methods, Spearmans rho coefficient (rs) was used. To find out the relationship between the WS values between methods, Pearson correlation coefficient(r) was used. The joints were considered as dysplastic if 105°<NoA value was observed for clinical results evaluation. SPSS 12.0 software was used for statistical evaluations.

RESULTS

The groups' descriptive values were represented in Table 1.

Table 1: Descriptive values for the study groups

Descriptive Statistics				
Study Groups	SHERKG	BHERKG	SHERNoA	BHERNoA
N	100	100	100	100
Mean	4,1730	1,9750	98,0347	95,6582
Std. Error of Mean	,21814	,04722	1,10584	1,04710
Median	3,7500	1,9000	100,5850	97,4750
Std. Deviation	2,18137	,47221	11,05839	10,47095
Minimum	,62	1,00	72,76	71,64
Maximum	9,55	2,99	122,62	116,30

Legend: std.: standard, N: number, SHERKG: Standard Hip Extended Kg, BHERKG: Balanced Hip Extended Kg, SHERNoA: Standard Hip Extended Norbergs Angle, BHERNoA: Balanced Hip Extended Norbergs Angle

Significant difference was found between BHERKg (M=1.97, SE=.47) and SHERKg(M= 4.17, SE=0.22),t(99)= -10,45, p<.001, r = -0.72. However when the differencebetween right and left hip sides taken into account by loads, SHERKgRight (M=3,8404, SE= 0,3271) and SHERKgLeft (M=4,5056, SE=0,337), t(49)= -1.916, p>.05, r=-0.26, and the difference between BHERKgRight (M=1,9690, SE= 0, 065) and BHERKgLeft (M=1,9810, SE=0, 068), t(49)= -0.719, p>.05, r=-0.10, was not found statistically significant (Table 2).

Table 2: Detailed descriptive values for weight scale evaluations

Descriptive Statistics

WS groups	Mean	N	Std. Deviation	Std. Error Mean
SHERKgRight	3,8404	50	1,91989	,27151
SHERKgLeft	4,5056	50	2,38790	,33770
BHERKgRight	1,9690	50	,46355	,06556
BHERKgLeft	1,9810	50	,48535	,06864

Legend: std: standard, N: number, WS: weight scale, SHERKG: Standard Hip Extended Kg, BHERKG: Balanced Hip Extended Kg

The study showed that there was a statistically significant difference between SHERNoA (Median=100.59) and BHERNoA (Median=97.48), z= -5.089, p<.001, r=-0.76. A significant, positive, and strong correlation was found between SHERNoA and BHERNoA, rs= .910, p<.001 (Figure 3).

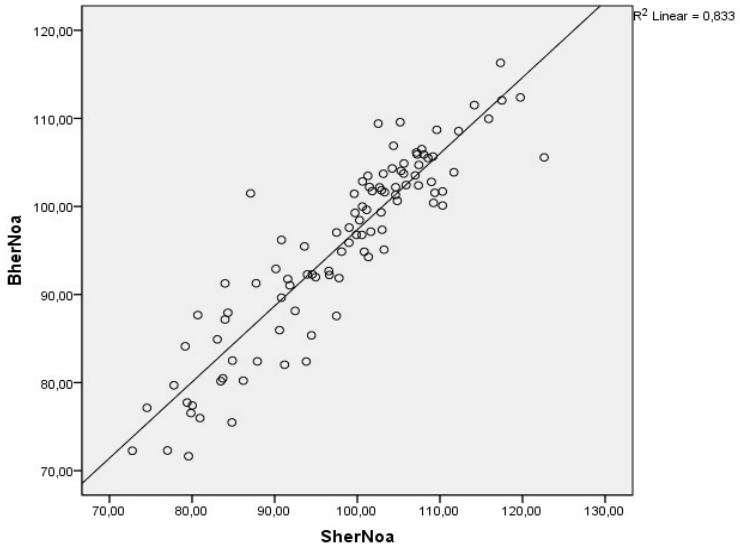

Figure 3. Graph of the correlation between SHERNoA and BHERNoA.
Figure 3, Legend: BHERNoa: Balanced Hip Extended Norbergs Angle Values, SHERNoa: Standard Hip Extended Norbergs Angle Values

In clinical results, according to the SHER method, 72 hip joints (72%) were found diseased and 28 hip joints (%28) were healthy, according to the BHER method; 83 hip joints (83%) were found diseased and 17 (%17) were healthy. The 72 diseased hip joints of the SHER method were also found as diseased by 97.2% (70 joints) but yet 2 joints (2.8%) of this group were healthy according to the BHER method. The 28 healthy hip joints in the SHER method were also found as healthy with the BHER method by 53.6% (15 joints) but 13 joints (46.4%) from this group were found as diseased (see Table 3).

Table 3: Cross table of the ratio of diseased and healthy hip joint statuses according to SHER and BHER methods

Method			BHER		
			Diseased	Healthy	Total
SHER	Diseased	Count	70	2	72
		% within SHERDiseased	97,2%	2,8%	100,0%
		% within BHERDiseased	84,3%	11,8%	72,0%
		% of Total	70,0%	2,0%	72,0%
	Healthy	Count	13	15	28
		% within SHERDiseased	46,4%	53,6%	100,0%
		% within BHERDiseased	15,7%	88,2%	28,0%
		% of Total	13,0%	15,0%	28,0%
Total		Count	83	17	100
		% within SHERDiseased	83,0%	17,0%	100,0%
		% within BHERDiseased	100,0%	100,0%	100,0%
		% of Total	83,0%	17,0%	100,0%

Legend: SHERKG: Standard Hip Extended, BHERKG: Balanced Hip Extended

DISCUSSION

To the author's knowledge, this is the first study in which the measurement of applied extending loads in standard ventro-dorsal hip-extended radiography was utilized and their effects have been evaluated. In this study, a study group of 50 dog's 100 hip joints were evaluated with NoA evaluation for the diagnosis of CHD. All dogs were chosen randomly from big and giant breeds (mean body weight was 31.38 Kg, Labrador retriever (26%), German Shepherd (12%), English Bulldog (10%), Shar Pei (6%), Newfoundland (6%)) when they were brought for CHD control to clinics by their owners. The anesthesia protocol was standardized due to uniformity in muscle relaxation, which is found crucial according to related studies **(Genevois et al. 2006, Todhunter et al. 2003)** when assessing subjective data e.g. when measuring coxometric values such as NoA **(Genevois et al. 2006)** in scoring hip joints. The evaluations were made by radiology and biomechanical analysis was not conducted. The inter-observer bias was not appointed since all radiographs were evaluated by the same scrutinizer using a standard measuring template as shown in related studies **(Verhoeven et al. 2007, Verhoeven et al. 2009)**.

FCI grading system is found to be the most reliable method at the mean age of 244 days, with high negative and positive predictive values **(Ohlerth et al. 2003)**. The PennHip and distraction index method are found very sensitive techniques in predicting true negatives but both techniques have been found to overestimate the fraction of dysplastic dogs at the age of 384 days which would lead to the elimination of a high proportion of working dogs, therefore a combination of both methods is offered to select the dogs whether for breeding or working **(Reagan 2017)**. The use of 105 degrees of NoA threshold value would result in high false-negative diagnoses in German Shepherd dogs and cause to the removal of the dogs from the gene pool that is not CHD susceptible which is found counterproductive **(Culp et al. 2006, Reagan 2017, Yaprakci and Tekerli 2015)**. Our study group means age was 20.4 months (612 days) which is found in term the CHD expression is conclusive **(Manley et al. 2007)**.

Within the results, BHER method showed significantly lower (mean: 1, 97 kg SE: 0,04722) loads compared to SHER method (mean: 4, 17 kg SE: 0,21814) $p<.001$, $r= -0.72$. The difference of loads between hip sides (SHERKg Right and Left & BHERKg Right and Left) were not found significant ($p>.05$) in both techniques but there was significant difference between methods SHERKg and BHERKg where BHERKg mean was significantly lower on both sides comparing to SHERKg. Conversely, our data confirmed that, even if there is no significant difference occurred in extending loads between hip sides, balancing these loads caused a significant difference in NoA values between tested methods (SHERNoA - BHERNoA; $z= -5.089$, $p<.001$, $r=-0.76$).

The linear relationship and strong effect size confirm the corroboration between SHER and BHER techniques in which the 72 diseased hip joints of SHER method was also found 97.2% diseased by BHER method. In the healthy group of SHER method which the 28 healthy hip joints were later found 46% (13 hip joints) diseased by BHER method. The true positives of SHER method were common between methods by 17% but 2.8% of disparity was occurred in BHER method (Table 3).

The hip laxity may change due to patients positioning which leg extension twists the hip joint capsule resulting in forcing the femoral heads into the acetabulum **(Farese et al. 1998, Gaspar et al. 2016)**.

Bilateral symmetry of pelvis is seen altered unacceptably depending on pelvic rotation as shown in a study **(Gaspar et al. 2016)**. In our study the positioning was set the same in both techniques. Advancement of the disease causes the thickening of the joint capsule **(Corfield et al. 2007)** and changes the subluxation degree of femoral heads. We believe that balancing the extending loads on hip sides prevented the shift of excessive load on to opposite side of the hip which was proven to cause a radiographical error in the observation of coxometric values.

CONCLUSION

The results of this study indicate the corroboration of both methods with strong and positive correlation in true diagnoses that was shown the sensitivity of BHER method. The results of NoA evaluation with BHER method showed higher sensitivity compared to SHER method. Even when a lower force was applied, the subluxations were found significantly higher than those in SHER. Higher sensitivity of BHER method may let better diagnosis of hip joint laxity, and the conclusion of this study is BHER method was found to be more reliable method in the diagnosis of CHD.

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Conflict of Interest: Authors declare no conflict of interest.

Author Contributions: M.V.Y.; development of the concept of balancing the applied loads on radiography, designing the experimental setup, performing tests, evaluation of the statistical results, manuscript preparation and submission
M.G.; designing the experimental setup, performing tests, evaluation of the statistical results

REFERENCES

Adams, W. M., R. T. Dueland, J. Meinen, R. T. O'brien, E. Giuliano and E. V. Nordheim (1998). "Early detection of canine hip dysplasia: Comparison of two palpation and five radiographic methods." J Am Anim Hosp Assoc 34(4): 339-347.

Corfield, G. S., R. A. Read, K. A. Eastley, J. L. Richardson, I. D. Robertson and R. Day (2007). "Assessment of the hip reduction angle for predicting osteoarthritis of the hip in the labrador retriever." Aust Vet J 85(6): 212-216.

Culp, W. T., A. S. Kapatkin, T. P. Gregor, M. Y. Powers, P. J. Mckelvie and G. K. Smith (2006). "Evaluation of the norberg angle threshold: A comparison of norberg angle and distraction index as measures of coxofemoral degenerative joint disease susceptibility in seven breeds of dogs." Vet Surg 35(5): 453-459.

Dueland, R. T., W. M. Adams, J. P. Fialkowski, A. J. Patricelli, K. G. Mathews and E. V. Nordheim (2001). "Effects of pubic symphysiodesis in dysplastic puppies." Vet Surg 30(3): 201-217.

Farese, J. P., R. J. Todhunter, G. Lust, A. J. Williams and N. L. Dykes (1998). "Dorsolateral subluxation of hip joints in dogs measured in a weight-bearing position with radiography and computed tomography." Vet Surg 27(5): 393-405.

Gaspar, A. R., G. Hayes, C. Ginja, M. M. Ginja and R. J. Todhunter (2016). "The norberg angle is not an accurate predictor of canine hip conformation based on the distraction index and the dorsolateral subluxation score." Preventive Veterinary Medicine 135: 47-52.

Genevois, J. P., T. Cachon, D. Fau, C. Carozzo, E. Viguier, F. Collard and D. Remy (2007). "Canine hip dysplasia radiographic screening. Prevalence of rotation of the pelvis along its length axis in 7,012 conventional hip extended radiographs." Vet Comp Orthop Traumatol 20(4): 296-298.

Genevois, J. P., G. Chanoit, C. Carozzo, D. Remy, D. Fau and E. Viguier (2006). "Influence of anaesthesia on canine hip dysplasia score." J Vet Med A Physiol Pathol Clin Med 53(8): 415-417.

Genevois, J. P., D. Remy, E. Viguier, C. Carozzo, F. Collard, T. Cachon, P. Maitre and D. Fau (2008). "Prevalence of hip dysplasia according to official radiographic screening, among 31 breeds of dogs in france." Vet Comp Orthop Traumatol 21(1): 21-24.

Henry, G. A. (1992). "Radiographic development of canine hip dysplasia." Vet Clin North Am Small Anim Pract 22(3): 559-578.

Manley, P. A., W. M. Adams, K. C. Danielson, R. T. Dueland and K. A. Linn (2007). "Long-term outcome of juvenile pubic symphysiodesis and triple pelvic osteotomy in dogs with hip dysplasia." J Am Vet Med Assoc 230(2): 206-210.

Meomartino, L., G. Fatone, A. Potena and A. Brunetti (2002). "Morphometric assessment of the canine hip joint using the dorsal acetabular rim view and the centre-edge angle." J Small Anim Pract 43(1): 2-6.

Ohlerth, S., A. Busato, M. Rauch, U. Weber and J. Lang (2003). "Comparison of three distraction methods and conventional radiography for early diagnosis of canine hip dysplasia." J Small Anim Pract 44(12): 524-529.

Reagan, J. K. (2017). "Canine hip dysplasia screening within the united states: Pennsylvania hip improvement program and orthopedic foundation for animals hip/elbow

database." Veterinary Clinics of North America: Small Animal Practice 47(4): 795 - 805.

Risler, A., J. M. Klauer, N. S. Keuler and W. M. Adams (2009). "Puppy line, metaphyseal sclerosis, and caudolateral curvilinear and circumferential femoral head osteophytes in early detection of canine hip dysplasia." Vet Radiol Ultrasound 50(2): 157-166.

Smith, G. K., T. P. Gregor, W. H. Rhodes and D. N. Biery (1993). "Coxofemoral joint laxity from distraction radiography and its contemporaneous and prospective correlation with laxity, subjective score, and evidence of degenerative joint disease from conventional hip-extended radiography in dogs." Am J Vet Res 54(7): 1021-1042.

Thompson, R., S. C. Roe and I. D. Robertson (2007). "Effects of pelvic positioning and simulated dorsal acetabular rim remodeling on the radiographic shape of the dorsal acetabular edge." Vet Radiol Ultrasound 48(1): 8-13.

Todhunter, R. J., J. E. Bertram, S. Smith, J. P. Farese, A. J. Williams, A. Manocchia, H. N. Erb, N. L. Dykes, N. I. Burton-Wurster and G. Lust (2003). "Effect of dorsal hip loading, sedation, and general anesthesia on the dorsolateral subluxation score in dogs." Vet Surg 32(3): 196-205.

Verhoeven, G., F. Coopman, L. Duchateau, J. H. Saunders, B. Van Rijssen and H. Van Bree (2007). "Interobserver agreement in the diagnosis of canine hip dysplasia using the standard ventrodorsal hip-extended radiographic method." J Small Anim Pract 48(7): 387-393.

Verhoeven, G. E., F. Coopman, L. Duchateau, T. Bosmans, B. Van Ryssen and H. Van Bree (2009). "Interobserver agreement on the assessability of standard ventrodorsal hip-extended radiographs and its effect on agreement in the diagnosis of canine hip dysplasia and on routine fci scoring." Vet Radiol Ultrasound 50(3): 259-263.

Yaprakci, M. V. and M. Tekerli (2015). "Köpeklerde kalça displazisine yol açan kalıtsal ve çevresel faktörler üzerine bir derleme." Lalahan Hayvancilik Araştirma Enstitüsü Dergisi 55(1): 37 - 43.

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SHORT COMMUNICATION

Elaphostrongylus cervi (Cameron, 1931) in Red deer (*Cervus elaphus*): First Record in Turkey

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ABSTRACT

This study was carried out to determine the presence of Elaphostrongylus cervi infection found in Red deer at Eskişehir Catacık Red Deer Production Station between June-July 2020. Based on the red deer traces and signs in the study area, 32 fresh faeces of animals were collected from the places where the faeces density was found. Stool samples were examined for cestode rings macroscopically, and microscopically by sedimentation, saturated salt flotation, and Baermann Wetzel methods. Larvae were found in all the collected faeces. The larvae obtained by the Baerman technique were identified at the species level using the relevant literature. E. cervi identified in red deer with this study is the first record in our country. Thus, it will provide valuable data for the red deer in Turkey
Keywords: Elaphostrongylus cervi, Red deer, First record, Eskişehir

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Kızıl geyiklerde (*Cervus elaphus*) *Elaphostrongylus cervi* (Cameron, 1931): Türkiye'de İlk Kayıt

ÖZ

Bu çalışma Haziran-Temmuz 2020 tarihleri arasında Eskişehir Çatacık Kızıl Geyik Üretim İstasyonu'ndaki Kızıl geyiklerde bulunan Elaphostrongylus cervi enfeksiyonunun varlığını belirlemek amacıyla yapılmıştır. Çalışma alanında bulunan Kızıl geyik iz ve belirtilerinden hareket edilerek dışkı yoğunluğunun bulunduğu yerlerden hayvanlara ait 32 taze dışkı toplanmıştır. Dışkı örnekleri cestod halkaları yönünden makroskobik olarak, sedimentasyon, doymuş tuzlu su flotasyon ve Baermann Wetzel yöntemleri ile mikroskobik olarak incelenmiştir. Toplanan dışkıların hepsinde larvalara rastlanmıştır. Baerman tekniği ile elde edilen larvalar ilgili literatürlerden faydalanılarak tür düzeyinde teşhis edilmiştir. Yürütülen bu çalışmayla Kızıl geyiklerde teşhis edilen E. cervi ülkemizde ilk kayıttır. Bu sebeple Türkiye'de Kızıl Geyikler için değerli bir veri oluşturacaktır.
Anahtar Kelimeler: Elaphostrongylus cervi, Kızıl geyik, İlk kayıt, Eskişehir

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INTRODUCTION

Elaphostrongylus cervi which is an important helminth parasite in Red Deer (*Cervus elaphus*) is found in muscles, brain and the epidural space of the spinal cord in many deer species including Caspian red deer *(Cervus elaphus maral)*, Canadian deer *(Cervus elaphus canadensis)*, Japanese deer *(Cervus nippon)* and roe deer *(Capreolus capreolus)*. The intermediate hosts are various land snails (Mason, 1989). Mature parasites are 4-6 cm long, pale, and in thread-shaped structure. Transmission of the parasite is through the infective larvae reaching the lungs through the bloodstream. Symptoms include lung inflammation and nervous disorders in the brain (Boden 2005). This parasite is found especially in domestic, and wild ruminants in Europe, including Scotland, Australia, and North America, and has widespread (Mason 1989).

Parasites have a significant impact on wildlife populations, including reproduction and survival (Anderson 1978). Although there are many studies on the wild animals in Turkey (Uslu et al. 2008, Acıcı et al. 2012, Acıcı et al. 2017, Bolukbas et al. 2012, Girisgin et al. 2018, Dik and Kılınç 2015), studies on helminth infections that infect red deer are limited (Cengiz et al. 2019). So far, there has not been a study to determine the *Elaphostrongylus cervi* infection in red deer in Turkey.

With this first study in Turkey, it is aimed to determine the state of *E. cervi* infection in the red deer in Eskisehir and to contribute to the detection of the local fauna.

MATERIAL and METHODS

This study was carried out between June-July 2020 in Eskisehir (Figure 1). Based on the traces and signs of red deer in the Catacik region of Eskisehir, 32 fresh fecal samples belonging to animals from the places where the faeces density was found were taken into transparent nylon bags and recorded by numbering. The faeces obtained were brought to laboratory.

Figure 1. Location of Turkey and Eskişehir province (with black circles).

Stool samples were examined for cestode rings macroscopically, and microscopically by sedimentation, saturated salt flotation, and Baermann Wetzel methods. (Thienpont and et al. 1986). Light microscope (Olympus CX31-Olympus Imaging System LC30) was used for the identification of larvae obtained by Baerman technique. Total body length, maximum body width, tail length (from the anus to tail tip), tail extension length, and dorsal spine length were measured in a total of 50 dorsal spiny larvae in lactophenol solution.

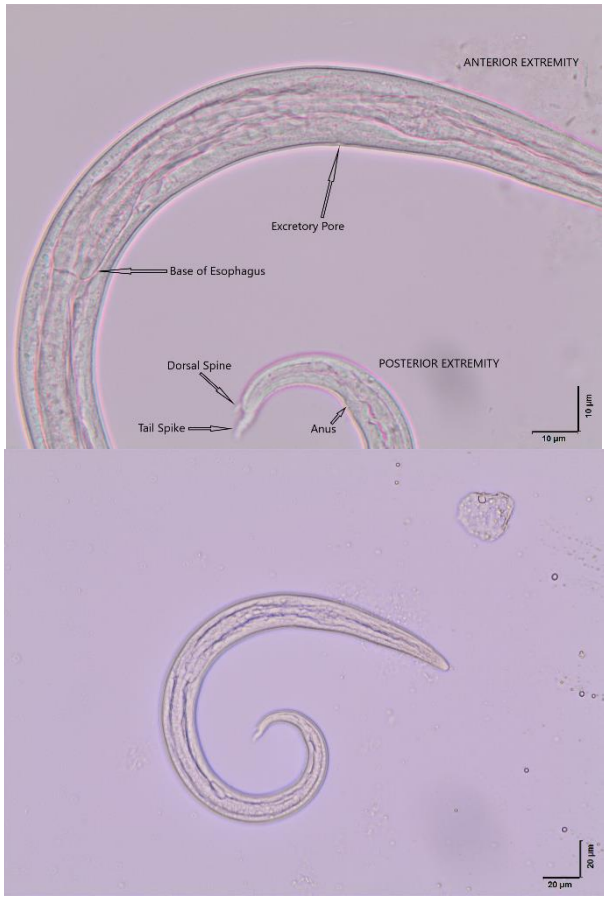
RESULTS

In the study, it was determined that the dorsal spiny larvae, whose morphometric measurements were made, belonged to the *E. cervi* species. Looking at the morphometric measurements of the dorsal-spined larvae, the average total length was 408 µm, the width was 20 µm, the tail was 38 µm, tail extension length was 9.23 µm, and dorsal spine was 2.69 µm. (Table 1).

Table 1. Measures (in μm) of dorsal-spined larvae recovered from red deer (*Cervus elaphus*).

References		This study	Baruš and Blazek 1973	Kutzer and Prosl 1975	English et al. 1985	Demiaszkiewicz 1986	Řezáč 1990	Vicente and Gortázar 2001	Morandi et al., 2006	Panayotova-Pencheva and Alexandrov 2008
Total length	Mean	408	-----	407	410	424	422	401	-----	423
	Min-Max	377-437	342-408	364-452	391-460	382-463	390-459	365-425	390-430	377-473
Width	Mean	20			18	19	19	-----		20
	Min-Max	16-26	18-20	17-21	16-21	16-24	16-23		18-20	18-24
Tail extension length	Mean	9,23	-----	-----	-----	-----	-----	-----	-----	-----
	Min-Max	7,81-9,98								
Dorsal Spine	Mean	2,69	-----	-----	-----	-----	-----	-----	-----	-----
	Min-Max	2,08-2,79								
Tail	Mean	38		43	42	-----	44	-----	------	37
	Min-Max	34-42	26-35	35-50	32-52		41-49			32-42

First stage *E. cervi* larvae have rhabditiform type esophagus. The excretory hole is in the anterior ventral of the body. Genital primordium is found in the last 1/3 of the body. The tail has a characteristic shape. They have a distinct triangular spine on the dorsal side parallel to the tail tip (Figure 2).



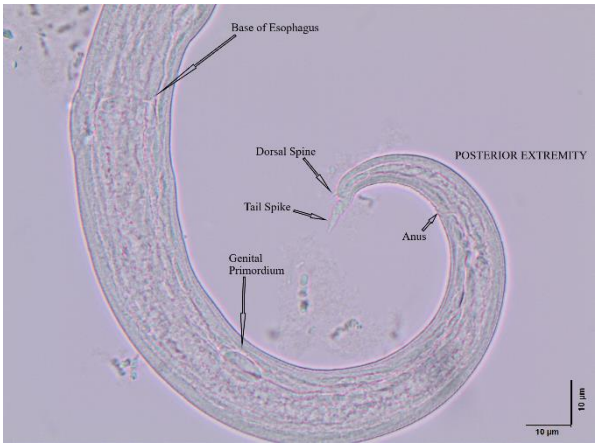


Figure 2. First stage *E. cervi* larva found in red deer (*C. elaphus* L.) from Turkey (original).

DISCUSSION and CONCLUSION

The red deer are ruminants that roam wildly in limited areas or are reared in certain protected environments in Turkey. Although there are many studies on the wild animals in Turkey (Uslu et al. 2008, Acıcı et al. 2012, Acıcı et al. 2017, Bolukbas et al. 2012, Girisgin et al. 2018, Dik and Kılınç 2015), studies on helminth infections that infect red deer are limited (Cengiz et al. 2019). So far, there has not been a study on *E. cervi* infection in Turkey.

This first study in Turkey reports the *E. cervi* infection in red deer in Eskisehir. First stage *E. cervi* larvae were found in all 32 stool samples. There are many studies abroad on *E. cervi* supported by both stool and necropsy examinations. (Hutsch et al. 2020, Panayotova-Pencheva and Alexandrov 2011, Alberti et al. 2011, Demiaszkiewicz et al. 2016, Sutherland 1976, Bregoli et al. 2006, Valcárcel and Garcia Romero 2002).

It was reported that 16.6% of the Red deer examined in Poland were infected with *Elaphostrongylus sp* (Hutsch et al. 2020). In another study, it was noted that 76.7% of the red deer were infected with *E. cervi* (Demiaszkiewicz et al. 2016).

In a study conducted in Italy, it was reported that 45.2% of the red deer were infected with *E. cervi* (Alberti et al., 2011). In the stool examination of 110 Red deer in Bulgaria, 75 (68%) of them were found to be infected with *E. cervi*. In the same study, *E. cervi* was found in the lung tissue of 5 deer by the necropsy (Panayotova-Pencheva and Alexandrov 2011).

In a study conducted on the red deer in New Zealand, it was reported that mature *E. cervi* were found in connective tissue associated with skeletal muscles and its larvae in the lungs, causing various pathological lesions in these tissues (Sutherland, 1976). In Italy, mature *E. cervi* were diagnosed in the submeningeal region for the first time in the red deer with neurological symptoms, and cerebral nematodiasis was reported (Bregoli et al. 2006).

In a study conducted in Spain, it was reported that 31.5% of the red deer were infected with mature *E. cervi* in the central nervous system, and *E. cervi* was recorded for the first time in the central nervous system of red deer in Spain (Valcárcel and Garcia to Romero. 2002)

Eskişehir province, where the study was conducted, is suitable for the survival of slugs that serve as an intermediate host to helminth infections, considering that it has a continental and humid climate under the influence of the Black Sea and Central Anatolia. The field where the study was conducted is not suitable for water slugs, but because of the dry areas suitable for land slugs, it only strengthens the possibility of *E. cervi* infections in deer. (Kuligowska and Demiaszkiewicz 2010). *E. cervi* also causes neurological lesions in small ruminates. Therefore, the grazing of domesticated small ruminates in areas where deer are present indicates that the disease with a high prevalence in deer may also transmit to small ruminates (Alberti et al. 2011, Handeland et al. 2000). The literature research has proven that this species has not been reported in Turkey before. *E. cervi*, diagnosed in Red deer with this study, is reported for the first time with this study. Therefore, this research will provide valuable data for the red deer in Turkey. More research is needed to determine the parasitic fauna of the red deer that live wildly in limited areas or are reared in certain protected environments in our country.

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Ethical statement: No ethical committee approval is required as the experimental animal is not used.

To carry out this research, the permission of the Ministry of Agriculture and Forestry dated 05/05/2020-E.19084 was obtained.

Conflict of interest : The authors declare that they have no conflict of interest.

REFERENCES

Acıcı M, Bolukbaş CS, Beyhan YE, Pekmezci GZ, Gurler AT, Umur Ş. Ectoparasites on roe deer (Capreolus capreolus) in Samsun, Turkey. Turk J Vet Anim Sci. 2012; 36(4): 456- 459. Doi:10.3906/vet-1009-471

Acıcı M, Demirtaş S, Umur Ş, Gurler AT, Bolukbaş CS. Infestations of flea species on small, wild mammals in the provinces of Aydın and Manisa in the Aegean Region, Turkey. Turk J Vet Anim Sci. 2017; 41(3): 449-452. Doi:10.3906/vet-1610-68

Alberti EG, Gioia G, Sironi G, Zanzani S. Elaphostrongylus cervi in a population of red deer (Cervus elaphus) and evidence of cerebrospinal nematodiasis in small ruminants in the province of Varese, Italy. Journal of Helminthology. 2011; 85(3), 313-318. Doi: 10.1017/S0022149X10000647

Anderson RM. The regulation of host population growth by parasitic species. Parasitology. 1978; 76, 119–57. Doi: 10.1017/S0031182000047739

Baruš V, Blažek K. Report on the finding of larval nematodes Elaphostrongylus cervi (Protostrongylidae) in the cranial cavity of a stag. Folia Parasitol (Praha). 1973; 20:279–280. Doi: 10.1007/s10344-008-0206-7

Bregoli M, Natale A, Cova M, Vascellari M, Pasolli C. Meningeal nematodiasis in a red deer (Cervus elaphus) in northeastern Italy - a case report. Vet. Arhiv. 2006; 76 (Suppl.), 287-293.

Bolukbas CS, Gurler AT, Beyhan YE, Acıcı M, Umur S. Helminths of roe deer (Capreolus capreolus) in the middle Black Sea region of Turkey. Parasitol Int. 2012: 61:729–730. Doi: 10.1016/j.parint.2012.06.008

Boden E. Black's Veterinary Dictionary 21. Ed. A&C Black Publishers Limited, 38 Soho Square, London. sy. 174. 2005

Cengiz G. Tenekeci GY. Bilgen N. Molecular and morphological characterization of Cysticercus tenuicollis in red deer (Cervus elaphus) from Turkey. Acta Parasitologica. 2019; 64:652–65. doi: 10.2478/s11686-019-00085-1. Doi: 10.2478/s11686-019-00085-1

Demiaszkiewicz AW. Laboratoryjna diagnostyka różnicowa Protostrongylidoz jeleniowatych. Med Welt, 1986; 42:660–663 in Polish

Demiaszkiewicz AW, Merta D, Kobielski J. Infection of red deer by parasites in South-Western Poland (Lower Silesian Wilderness). Med. Weter. 2016; 72 (5), 317-320.

Dik B, Kılınç ÖO. First case of Trichodectes pinguis (Phthiraptera: Ischnocera: Trichodectidae) on a Bear (Ursus arctos) in Turkey. Turkiye Parazitol Derg. 2015; 39, 313-315. Doi: 10.30782/uluvfd.405325

English A, Watt C, Corrigall W. Larvae of Elaphostrongylus cervi in the deer of Scotland. Vet Rec. 1985; 116:254–256

Girisgin AO, Çimenlikaya N, Bah SA, Aydın L, Girişgin O. Türkiye'de bazı yabani memelilerde bulunan dış parazit türlerinin ilk kayıtları. Uludag Univ Vet Fak Derg. 2018; 37(2), 133-136. Doi: 10.30782/uluvfd.405325

Handeland K, Gibbons LM, Skorping A. Experimental Elaphostrongylus cervi Infection in Sheep and Goats. Journal of Comparative Pathology. 2000; 123(4):248-57. Doi: 10.1053/jcpa.2000.0414

Hutsch KF, Czopowiczb M, Świsłockac M, Ratkiewiczc M, Borkowskac A, Kowalczykd R, Demiaszkiewicza AW. Patterns of parasite eggs, oocysts and larvae shedding by moose in the Biebrza marshland (NE Poland). IJP: Parasites and Wildlife. 2020; 11 191–197. doi: 10.1016/j.ijppaw.2020.02.007

Kuligowska I, Demiaszkiewicz AW. Infection of terrestial snails with larvae of Elaphostrongylus cervi (Nematoda, Protostrongylidae) in Białowieża National Park Helminthologia. 2010; 47, 1: 25 – 28. Doi:10.2478/s11687-010-0004-0

Kutzer E, Prosl H. Zur Kenntnis von Elaphostrongylus cervi Cameron, 1931. I. Morphologie und Diagnose. Wien Tierarztl Mschr. 1975; 62:258–266

Morandi F, Galuppi R, Nicoloso S, Benazzi C, Tampieri MP, Simoni P. Larvae of Elaphostrongylus cervi in a Population of Freeliving Red Deer in Italy. J Wildl Dis. 2006; 42:870-872 Doi: 10.1007/s10344-008-0206-7

Mason PC. Elaphostrongylus cervi-a review. Surveillance. 1989, 16(1): 3-10.

Panayotova-Pencheva M, Alexandrov M. Morphometric characteristics of first stage Elaphostrongylus cervi (Nematoda: Protostrongylidae) larvae from Bulgaria. Eur J Wildl Res 54:771–774 2008; DOI 10.1007/s10344-008-0206-7

Panayotova-Pencheva MS, Alexandrov MT. Etiopathological aspects of Elaphostrongylus cervi and Varestrongylus sagittatus infections in red deer in Bulgaria. Acta Vet Brno. 2011; 80(4): 349–352. Doi: 10.2754/avb201180040349

Řezáč P. Diferenciální diagnostika larev 1. stadia hlístic Varestrongylus sagittatus a Elaphostrongylus cervi. Veterinarstvi 1990; 40:311–313 in Czech. Doi: 0.1007/s10344-007-0143-x

Sutherland RJ. Elaphostrongylus cervi in Cervids in New Zealand. New Zealand Veterinary Journal. 1976; 24:11, 263-266. Doi: 10.1080/00480169.1976.34334

Thienpont D, Rochette F, Vanparijs OFJ. Diagnosing Helminthiasis by Coprological Examination. Second edition. Belgium: Janssen Research Foundation. 1986.

Uslu U, Dik B, Gokcen A. Ectoparasites of the ground squirrel (Citellus citellus (L.)) in Turkey. Turkiye Parazitol Derg. 2008; 32: 142-145.

Valcárcel F, Garcı´a Romero C. First report of Elaphostrongylus cervi in Spanish red deer (Cervus elaphus hispanicus). Journal of Helminthology. 2002; 76, 91–93. Doi: 10.1079/JOH2003232

Vicente J, Gortázar C. High prevalence of large spiny-tailed protostrongylid larvae in Iberian red deer. Vet Parasitol. 2001; 96:165–170 doi:10.1016/S0304-4017(00)00425-8 Doi: 10.1007/s10344-008-0206-7

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CASE REPORT

The Inbreeding Case of Bali Cattle (*Bos javanicus*) at Breeding Station

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ABSTRACT

Highly inbreeding level in the livestock had negative effect to productivity traits. This study was carried out to report first inbreeding case of Bali cattle (*Bos javanicus*) based on the records data from year 2013 to 2018 at breeding center of Indonesia. Two inbred calves (ID: 0991 and 0812) were born from the inbreeding mating between paternal halfsib parental (ID: 0874 and 0881). One inbred calf (ID: 0812) was dead one day after birth. The inbreeding coefficient (F_x) in both inbred calves were 0.125. It was seen that birth and adult performances in the inbred cattle were lower than their parents. It can be concluded that the level inbreeding 12.5% in Bali cattle had negative effect of low performance and calf's dead case.
Keywords: Bali cattle, Inbreeding coefficient, Inbred calves, Breeding center

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Bali Damızlık Sığır (*Bos javanicus*) Sürülerinde Akrabalık Durumu

ÖZ

Akrabalı yetiştirme çiftlik hayvanlarında verim özellikleri üzerinde olumsuz etkilere sahiptir. Bu çalışmada Endonezyada yetiştirme merkezinde (çiftliğindeki) yetiştirilen Bali (*Bos javanicus*) sığırlarının akrabalık durumu 2013 - 2018 yılları arasındaki kayıtlara dayalı olarak ortaya konmuştur. Üvey kardeş ebeveynlerin (ID: 0874 ve 0881) birleşmesinden 2 akrabalı yetişmiş buzağı (ID: 0991 ve 0812) elde edilmiştir. Böylece bir akrabalı yetişmıiş buzağı (ID: 0812) doğumdan bir gün sonra öldü. Böylece akrabalı yetişme katsayısı (F_x) her iki buzağıda da 0.125. Genel olarak akrabalı yetişmiş sığırların doğumda ve ergin yaştaki performansı ebeveynlerinden düşük olmaktadır.
Anahtar Kelimeler: Bali sığır, Akrabalı yetiştirme katsayısı, Akrabalı yetişmiş buzağı, Yetiştirme merkezi

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INTRODUCTION

The inbreeding commonly had negative effect to productive traits in livestock. Moreover, the inbreeding in the less number of animals population can be caused extinction (Julian et al. 2006; Theodorou and Couvet, 2006). However, many farm industries were managed the inbreeding to collect the desirable allele (gene). The inbreeding of livestock can be controled through inbreeding coefficient (F_x) value (Laws and Jamieson 2011). Alvarez et al. (2011) stated that the F_x value more than 0.30 can be increased the risk of mortality and disorder in offspring.

Bali cattle (*Bos javanicus*) are the one of Indonesian native cattle that originated from Bali island and was spread in other islands of Indonesia. Thus, two genetic markers in mtDNA of Bali cattle i.e. D-loop and Cytochrome Oxidase Sub unit 1 (COI) were similar to wild Banteng that spread in Southeast Asia (Wisesa et al. 2012; Wulandari et al. 2019). Bali cattle were declared as the Indonesian native cattle since year 2010 through decision of Indonesian Ministry of Agriculture No: 325/Kpts/OT.140/1/2010. Kaswati et al. (2013) reported that the average of weaning weight (205 days of age) and yearling weight (365 days of age) in Bali cattle at the breeding station (Bali Island) were 88.59±16.15 kg and 131.12±25.50 kg respectively. In addition, Priyanto et al. (2019) reported that the average of slaughter weight, carcass weight and dressing percentage in Bali cattle were 275.56±61.93 kg, 141.04±35.61 kg and 50.95±3.49% respectively.

The genetic improvement for Bali cattle can be conducted based on selection program. Thus, mostly the selection program of Bali cattle in Indonesia was performed with conventional method. The conventional selection to improve the productive traits can be performed through data and pedigree records of livestock. Despite, the data record is important to reduce the inbreeding risk in livestock. This study was aimed to report and evaluate the first inbreeding case in Bali cattle at Bureau of Artificial Insemination at Lelede (BAI Lelede), Indonesia. The results study is important for BAI Lelede to applied good breeding practices mainly in recording and mating systems in the future.

CASE HISTORY

The breeding program of Bali cattle at BAI Lelede was started at year 2013. The artificial insemination (AI) and natural mating (NM) methods were used in this breeding station. A Bali cow with service per conception (S/C) more than three will be mated with NM method. Bull ID: 0874 and cow ID: 0881 were born from AI method with similar sire (Straw ID: 11012/003) and different dam as presented in Figure 1. According to the herd records, both cattle were mated with NM method and had two calves. First calf (male) was born at 2017 with ID: 0812. Meanwhile, the second calf (female) was born at 2018 with ID: 0991. Unfortunately, calf ID: 0812 was dead one day after birth. Meanwhile, calf ID: 0991 still survive until in the present study (Figure 2). According to the data records, the birth performance (birth weight, heart girth, withers height and body length) in both calves were lower than those detected in both parents as presented in Table 1. In addition, the adult performance of cow ID: 0991 was lower than both parents as presented in Table 2. According to the farm manager, the inbreeding case in BAI Lelede was caused by human error, mainly in the breeding bull rotation controlling.

Table 1. The birth performance of inbred calves family at BAI Lelede

Cattle ID	Sex	Sire	Dam	Date of birth	Birth performances			
					BW (kg)	HG (cm)	WH (cm)	BL (cm)
0881	Female	11012/003	01179	15/07/2014	16	58	63	60
0874	Male	11012/003	05KYT	09/12/2014	15	60	59	60
0991	Female	0874	0881	07/09/2017	16	56	57	50
0812*	Male	0874	0881	14/08/2018	17	54	57	50

*dead; BW: birth weight; HG: heart girth; WH: withers height; BL: body length

Table 2. The performance of inbred calves family at adult years age in BAI Lelede

Cattle ID	Sex	Age (days)	Adult performances				Grade*
			AW (kg)	HG (cm)	WH (cm)	BL (cm)	
0881	Female	805	179.5	140	111	106	II
0874	Male	658	233	150	116	115	II
0991	Female	809	88.5	112	103	99	-

AW: adult weight; HG: heart girth; WH: withers height; BL: body length. *Grading of cattle based on National Standard of Bali cattle (SNI 7651.4:2015) with HG and WH measurements

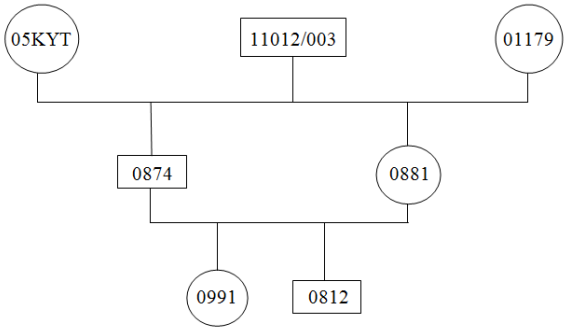

Figure 1. The inbreeding scheme in Bali cattle between two paternal halfsib parental (0874 and 0881) at BAI Lelede. □: male , ○: female

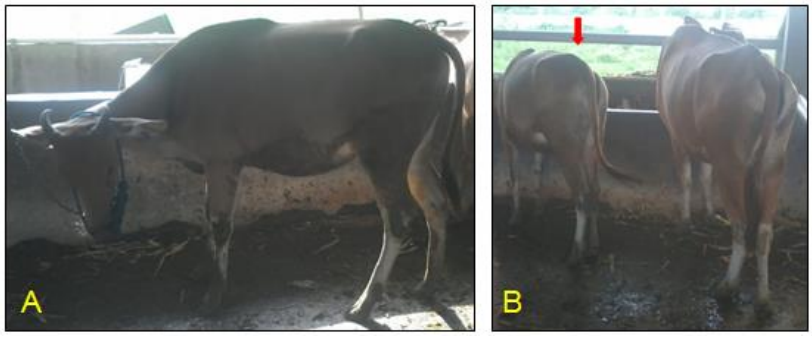

Figure 2. The inbred Bali cow ID: 0991 (A) had small body size (red arrow) compared with the other adult cow (B)

DISCUSSION

Two calfs of ID: 0812 and ID: 0991 were mentioned as the inbred calves. Thus, both calves were born from inbreeding between two paternal halfsib parents with inbreeding coefficient (F_x) value of 0.125. Sawitri et al. (2012) reported that the F_x value in Banteng (*Bos indicus*) at Surabaya zoo was 0.42 and higher than in this study. The inbred calf ID: 0991 was survived until adult age with lower body weight and body measurements. Barczak et al. (2009) reported that increasing 1% of inbreeding level in the sheep can be followed by reducing 9.50±2.30 gram of birth weight and 11.60±7.30 gram of fourth week's weight. Petrovic et al. (2013) reported Mis rams with F_x value 0.25 had negative impact to sperm DNA fragmentation level. Meanwhile, Analla et al. (1999) reported that increasing 10% of inbreeding level in the Merino sheep can be followed by reducing 0.21±0.01 kg of birth weight, 0.23±0.03 kg of 30th day's weight, 0.48±0.04 kg of 60th day's weight and 0.82±0.06 kg of 90th day's age. In addition, previous studies reported that the inbreeding in sheep had negative effect on reproductive traits (Selvaggi et al. 2010; Eteqadi et al. 2015), survival rate of lamb (Ceyhan et al. 2011) and body weight (Boujenane and Chami 1997; Mandal et al. 2002; Rzewuska et al. 2005; Norberg and Sorensen 2007). In dairy cattle, the inbreeding was reduced the milk production (Filho et al. 2015), survival rate (Thompson et al. 2000), conception rate (Hofmannova et al. 2019) and fertilization rate of *in vitro* embryo (Perez et al. 2017).

In the future, the recording and mating management systems in BAI Lelede must be improved to reduce the inbreeding case. Despite, nutrition feed control is important to increase the reproductive traits of cow. The efficiency reproduction can be increased using oestrus synchronization method with artificial insemination. Moreover, the NM system can be improved with bull rotation program. A breeding bull must be used along 2 - 3 years. The breeding bulls used for 3 years in the breeding station and these bulls can be distributed in the villager breeding center (VBC) which very hard to applied AI method. Hence, the inbreeding case can be reduced through bull rotation. The long distance between VBC, AI center or veterinary office can be caused late AI assessment and expensive cost. Furthermore, the inbred cow ID: 0991 can be culled from breeding station because of not in accordance with the Bali cattle breed standard. It can be concluded that level inbreeding 12.5% in Bali cattle had negative effect of low performance and calf's dead case.

KAYNAKLAR

Alvarez G, Quinteiro C, Ceballos FC. Inbreeding and genetic disorder. Adv Study Genet Dis. 2011; 2:21-44.

Analla M, Montilla JM, Serradilla JM. Study of the variability ofthe response to inbreeding for meat production in Merino sheep. J Anim Breed Genet. 1999; 116:481-488.

Barczak E, Wolc A, Wojtowski J, Slosarz P, Szwaczkowski T. Inbreeding and inbreeding depression on body weight in sheep. J Anim Feed Sci. 2009; 18:42-50.

Boujenane I, Chami A. Effects of inbreeding on reproduction, weights and survival of Sardi and Beni Guil sheep. Journal of Animal Breeding and Genetic. 1997; 114:23-31.

Ceyhan A, Kaygisiz A, Sezenler T. Effect of inbreeding on preweaning growth traits and survival rate in Sakiz sheep. J Anim Plant Sci. 2011; 21(1):1-4.

Eteqadi B, Hossein-Zadeh NG, Shadparvar AA. Inbreeding effect on reproductive traits in Iranian Guilan sheep. Trop Anim Health Prod. 2015; 47(3):533-539.

Filho JCR, Verneque RS, Torres RA, Lopes PS, Raidan FSS, Toral FLB. Inbreeding on productive and reproductive traits of dairy Gyr cattle. Rev Bras Zootec. 2015; 44(5):174-179.

Hofmannova M, Pribyl J, Krupa E, Pesek P. Estimation of inbreeding effect on conception in Czech Holstein. Czech J Anim Sci. 2019; 64(7):309-316.

Julian JOG, Barry WB, David HR, Jonathan DB, David WT, Richard F. Realistic levels of inbreeding depression strongly effect extinction risk in wild populations. Biol Conserv. 2006; 133:42-51.

Kaswati, Sumadi, Ngadiyono N. The heritability estimation of birth weight, weaning weight of Bali cattle at Balai Pembibitan Ternak Unggul Sapi Bali. Bullet Anim Sci. 2013; 37:74-78.

Laws RJ, Jamieson IG. Is lack of evidence of inbreeding depression in a threatened New Zealand robin indicative of reduced genetic load ?. Anim Conserv. 2011; 14:47-55.

Mandal AK, Pant KP, Rout PK, Singh SK, Roy R. Influence of inbreeding on growth traits of Muzaffarnagari sheep. Indian J Anim Sci. 2002; 72(11):988-990.

Norberg E, Sorensen AC. Inbreeding trend and inbreeding depression in the Danish, populations of Texel, Shropshire, and Oxford Down. J Anim Sci. 2007; 85(2):299-304.

Perez BC, Balieiro JCC, Ventura RV, Bruneli VAT. Inbreeding effects on *in vitro* embryo production traits in Guzera cattle. Animal. 2017; 11(11):1983-1990.

Petrovic VC, Maksimovic N, Petrovic MP, Petrovic MM, Ilic ZZ, Muslic DR. Effect of inbreeding on body growth traits and sperm DNA fragmentation in rams. Anim Sci Pap Rep. 2013; 31(1): 27-33.

Priyanto R, Nuraini H, Muladno, Ismail M, Wijayanto H. Slaughter, carcass and non-carcass characteristics of local cattle and buffalo in Indonesia. Pakistan J Nut. 2019; 18:117-124.

Rzewuska K, Klewiec J, Martyniuk E. Effect of inbred on reproduction and body weight of sheep in a closed Booroola flock. Anim Sci Pap Rep. 2005; 23(4):237-247.

Selvaggi M, Dario C, Peretti V, Ciotola F, Canicella D, Dario M. Inbreeding depression in Leccese sheep. Small Rum Res. 2010; 89(1): 42-46.

Sawitri R, Takandjandji M. Inbreeding population of Banteng (Bos javanicus d'Alton 1832) at Surabaya zoo. Bul Plas Nut. 2012; 18:84-94.

Theodorou K., Couvet D. On the expected relationship between inbreeding, fitness and extinction. Genet Sel Evol. 2006; 38:371-387.

Thompson JR, Everett RW, Wolfe CW. Effect of inbreeding on production and survival in Jerseys. J Dairy Sci. 2000; 83(9): 2131-2138.

Wisesa AANGD, Pemayun TGO, Mahardika IGNK. Analisis sekuen D-Loop DNA mitokondria sapi Bali dan Banteng dibandingkan dengan bangsa sapi lain di dunia. Indonesia Med Vet. 2012; 1(2):281-292.

Wulandari A, Nurgiartiningsih VMA, Kuswati, Susilorini TE, Agung PP. Filogeni beberapa sapi lokal Indonesia menggunakan DNA mitokondria COI (Cytochrome Oxidase Sub unit 1). JITPT. 2019; 6(2):278-282.

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