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Research Article

Serum and Saliva Sialic Acid and Oxidative Stress Parameters Changes in Bulls with Foot and Mouth Disease [1]

Erdoğan UZLU [1] Mahmut KARAPEHLİVAN [2] Hidayet Metin ERDOĞAN [1]
Şemistan KIZILTEPE [3] Ekin Emre ERKILIÇ [1] Hacı Ahmet DEVECİ [4]
Erhan GÖKÇE [1] İnan KAYA [5] Mehmet ÇİTİL [1]

[1] *This study was presented as poster proceeding in 11th Congress of the National Veterinary Internal Disease, May 21-24, 2015, Samsun/Türkiye*

[1] Kafkas Üniversitesi, Veteriner Fakültesi, İç Hastalıkları Anabilim Dalı, TR-36100 Kars - TÜRKİYE
[2] Kafkas Üniversitesi, Tıp Fakültesi, Tıbbi Biyokimya Anabilim Dalı, TR-36100 Kars - TÜRKİYE
[3] TC Gıda Tarım ve Hayvancılık Bakanlığı, İl Müdürlüğü, TR-36100 Kars - TÜRKİYE
[4] Gaziantep Üniversitesi, Islahiye Meslek Yüksekokulu, TR-27800 Islahiye, Gaziantep - TÜRKİYE
[5] Kafkas Üniversitesi, Fen Edebiyat Fakültesi, Biyoloji Bölümü, TR-36100 Kars - TÜRKİYE

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Abstract

The study comprised of 12 bulls, aged between 18-36 months, determined severe symptoms of Foot-and-mouth disease (FMD) and 10 clinically healthy bulls of similar age. Serum and saliva total sialic acid (SA), malondialdehyde (MDA), glutathione (GSH) and nitric oxide (NO) levels were measured. In this study were determined acute fever, anorexia, vesicular lesions in the mouth and feet of infected animals with consequent excessive salivation, lameness and reduced productivity as clinical signs. Mean serum SA, MDA, GSH and NO levels were 503.96±21.43 mg/L, 31.82±3.43 μmol/L, 63.43±2.92 mg/dL, and 6.49±0.36 nmol/L in healthy bulls and 862.01±17.35 mg/L, 82.49±9.90 μmol/L, 24.96±2.32 mg/dL, and 13.89±0.53 nmol/L in FMD cases, respectively. Mean saliva SA, MDA, GSH and NO levels were 75.98±10.25 mg/L, 1.06±0.17 μmol/L, 0.67±0.05 mg/dL, and 1.44±0.14 nmol/L in healthy bulls and 156.49±14.07 mg/L, 1.81±0.15 μmol/L, 0.34±0.03 mg/dL, and 2.44±0.16 nmol/L in FMD cases, respectively. The differences between the two groups were statistically significant (P<0.001 and P<0.01). Mean serum and saliva GSH level was lower in FMD while all other parameters were considerable high. As a result, showing signs of foot and mouth disease in bulls, serum and saliva in sialic acid and oxidative stress parameters are affected very significantly.

Keywords: *Bull, Malondialdehyde, Foot-and-mouth disease, Total sialic acid,* Glutathione, Nitric oxide

Şap Hastalıklı Boğalarda Serum ve Salya Sialik Asit ve Oksidatif Stres Parametrelerindeki Değişiklikler

Özet

Bu çalışmada 18-36 ay yaşları arasında klinik açıdan şiddetli şap belirtileri tespit edilen 12 ve aynı yaş aralığındaki sağlıklı 10 adet boğa değerlendirildi. Serum ve salyada total sialik asit (SA), malondialdehit (MDA), glutathione (GSH) ve nitrik oksit (NO) düzeyleri ölçüldü. Çalışmada klinik belirtiler olarak; akut ateş, iştahsızlık, ağızda çok yaygın veziküler lezyonlar ve buna bağlı aşırı salivasyon, şiddetli topallık ve verim düşüklüğü belirlendi. Sağlıklı boğalarda ortalama serum SA, MDA, GSH ve NO düzeyleri sırasıyla 503.96±21.43 mg/L, 31.82±3.43 μmol/L, 63.43±2.92 mg/dL ve 6.49±0.36 nmol/L, şaplı olarak değerlendirilen boğalarda ise 862.01±17.35 mg/L, 82.49±9.90 μmol/L, 24.96±2.32 mg/dL ve 13.89±0.53 nmol/L olarak belirlendi. Sağlıklı boğalardan elde edilen salyalarda ortalama SA, MDA, GSH ve NO düzeyleri sırasıyla 75.98±10.25 mg/L, 1.06±0.17 μmol/L, 0.67±0.05 mg/dL ve 1.44±0.14 nmol/L iken bu değerler şaplı olarak değerlendirilen boğalarda 156.49±14.07 mg/L, 1.81±0.15 μmol/L 0.34±0.03 mg/dL ve 2.44±0.16 nmol/L, olarak tespit edildi. İki grup arasındaki farkın istatistiksel olarak anlamlı olduğu belirlendi (P<0.001 ve P<0.01). Şaplı olarak değerlendirilen hayvanlarda serum ve salya GSH değerleri düşük, diğer parametreler ise yüksek olarak tespit edildi. Sonuç olarak şap belirtileri tespit edilen boğalardan elde edilen serum ve salyada sialik asit ve oksidatif stres parametrelerinin önemli derecede etkilendiği belirlendi.

Anahtar sözcükler: *Boğa, Şap hastalığı, Total sialik asit, Malondialdehit, Glutathione, Nitrik oksit*

İletişim (Correspondence)
+90 532 2757135
euzlu@hotmail.com

INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious and economically important disease caused by foot-and-mouth disease virus (FMDV). Animals that can be affected include cattle, buffaloes, sheep, goats, pigs and wild ruminants [1-3]. FMDV is a positive sense, single-stranded RNA virus (genus *Aphthovirus*, family *Picornaviridae*) occurring in seven serotypes, O, A, C, Asia 1, SAT 1, SAT 2 and SAT 3, each with a wide spectrum of antigenic and epidemiological distinct subtypes. The wide diversity is considered a consequence of the high mutation rate, quasi-species dynamics and recombination [4,5]. The disease spreads by contact between infected and domestic animals, by animal products (milk, meat and semen), by mechanical transfer on people, wild animals and birds, by vehicles and fomites and by the airborne route [2,6,7].

The clinical severity of FMD varies with the strain of virus, as well as the infecting dose, the species and individual susceptibility of the host. It is clinically most apparent in high-yielding dairy cattle and intensively reared pigs, in which the lesions can be severe and debilitating. In adult sheep and goats, FMD is frequently only a mild disease, with transitory clinical signs which can easily be missed by the stockman or veterinarian, or confused with other diseases presenting similar lesions [8,9]. However, even in some breeds of cattle, FMD can also be clinically difficult to recognize because of the mild appearance of the disease [10]. The disease is typically characterized by acute fever and the development of vesicular lesions in the mouth and feet of infected cloven-hoofed animals (principally cattle, pigs, sheep and goats) with consequent excessive salivation, anorexia, lameness, mortality of young animals and reduced productivity [10-13]. Foot-and-mouth disease usually has a high morbidity and low mortality, with mortality occurring mostly in young animals [6].

Sialic acid (SA), an acetylated derivative of neuroaminic acid, increases rapidly following the inflammatory and injury process [14,15]. Therefore the detection of SA particularly lipid bound sialic acid (LBSA) concentrations may be a valuable indicator of inflammatory diseases [16]. Previous studies already indicated increased serum SA concentrations during the course of many diseases including bovine leptospirosis [17-23].

The induction of lipid peroxidation gives rise to an increase in malondialdehyde (MDA) content. This procedure activates cell-protective antioxidant defense mechanisms such as glutathione, uric acid (UA) [24]. The measurement of UA, albumin, reduced glutathione (GSH) and MDA concentrations can therefore be used as indicators of oxidative stress in some diseases but not enough studies previously determined the oxidative stress in FMD [25,26]. In inflammatory conditions, nitric oxide (NO) production increases through stimulation of inducible nitric oxide synthase (iNOS) via activation of pro-inflammatory cytokines and causes NO mediated tissue injury by reacting with superoxide to generate peroxynitrite, a powerful [27].

This study was therefore designed to determine changes in SA, MDA, GSH and NO levels on plasma and saliva in cattle with FMD. And to evaluate usability of these markers obtained from body fluids by a non-invasive simple method for the first time.

MATERIAL and METHODS

The study comprised of 12 bulls, aged between 18-36 months, in all clinical symptoms of the disease is detected and concluded that clinically FMD and 10 clinically healthy bulls of similar age. All animals were from Kars district, Turkey and were subjected to similar management conditions. A complete physical examination was performed on each animal. Blood samples were collected from all animals via jugular vein into plain tubes and carried to laboratory immediately. Sera were collected by centrifugation at 3.000 g for 10 min at room temperature and kept frozen (-25°C) until analysis. All serum samples were analyzed within 15 days. Saliva samples directly taken from oral flowing clear saliva into sterile Eppendorf tubes which are closed after putting the samples. The samples are stored until the analysis phase on -25ºC.

Serum and saliva SA levels were measured calorimetrically according to the method detailed by Sydow [28]. Serum and saliva MDA concentrations were determined by the Thiobarbituric acid (TBA) reactivity method [29]. NO was determined according to the method of Miranda *et al.*[30]. The GSH content was measured according to the method of Beutler *et al.*[31]. Same procedures was performed during the measurement of the saliva and serum samples.

Statistical Analyses

Statistical analysis was performed using the SPSS statistical program. Normal distribution of the data was determined using Anderson-Darling Normality test. Values were expressed as mean ± standard error (SE). Independent t test was used to compare the parameters between the groups. Significant level was set at $P<0.05$.

RESULTS

Clinical Findings

In this study were determined acute fever, anorexia, vesicular lesions in the mouth and feet of infected animals with consequent excessive salivation, lameness and reduced productivity as clinical signs.

Serum and Saliva Biochemical Findings

The results of serum biochemical parameters examined for diseased and healthy animals are shown in *Table 1*. Mean SA, MDA, GSH and NO levels were 503.96±21.43 mg/L, 31.82±3.43 µmol/L, 63.43±2.92 mg/L, and 6.49±0.36 nmol/L in healthy bulls and 862.01±17.35 mg/L, 82.49±9.90 µmol/L, 24.96±2.32 mg/dL, and 13.89±0.53 nmol/L in FMD cases, respectively. The results of saliva biochemical parameters examined for diseased and healthy animals are shown in *Table 2*. Mean SA, MDA, GSH and NO levels were 75.98±10.25 mg/L, 1.06±.17 µmol/L, 0.67±0.05 mg/dL, and 1.44±0.14 nmol/L in healthy bulls and 156.49±14.07 mg/L, 1.81±0.15 µmol/L, 0.34±0.03 mg/dL, and 2.44±0.16 nmol/L in FMD cases, respectively. The differences between the two groups were statistically significant ($P<0.001$ and $P<0.01$). Mean GSH level was lower in FMD while all other parameters were considerable high *(Table 1, Table 2)*.

DISCUSSION

This study tried to disclose some indicators of oxidative stress and inflammation in natural cases of FMD in bulls. Although the pathogenesis of FMD is of complex nature and the underlying factors are not yet fully understood, several mechanisms have been studied; role of toxins released by the organism, viral attachment, inflammation and/or immune mediated organ dysfunction.

Clinical signs (acute fever, anorexia, vesicular lesions in the mouth and feet of infected animals with consequent excessive salivation, lameness and reduced productivity) determined in this study were in agreement with those reported for FMD [6,11-13].

Our study revealed a marked increase in SA in FMD cases as reported previously by some researchers in RPT, IBK and Leptospirosis [20-23]. Sialic acid is reported to increase in human and animals during a number of pathological situations where the contributory event is either of tissue damage, tissue proliferation or inflammation [15]. In these circumstances, rise in SA is attributed to liberation of sialic acid from cell membrane into circulation as SA is abundantly present in all biological membranes [15,18,32,33].

Another indicator of cellular damage during the course of FMD may be increased MDA, and decreased GSH pool, an indicator of lipid peroxidation. The MDA results of our study are similar to foot and mouth disease in recent years [34]. These findings may suggest the production of free radicals and of lipid peroxidation. This might have been the case in our study as FMD causes tissue damage in various organs via different mechanisms.

In our study, an important increase of NO levels was determined in serum and saliva samples obtained from FMD group therefore our study results indicate that picornavirus can induce the production of NO *in vivo*. It is known that NO plays an important role in the primary defense mechanism against several pathogens; bacteria, viruses and parasites and NO production to be induced by various viruses which inhibit virus replication *in vivo* and

Table 1. Changes in oxidative and biochemical parameters in serum, in bulls with FMD an control. Values are expressed as mean±SE

Tablo1. Şaplı ve sağlıklı boğaların serumlarındaki biyokimyasal ve oksidatif parametrelerdeki değişimler. Değerler ort.±standart hata olarak gösterilmiştir

Parameters	Control (n=10)	FMD (n=12)	P Values
SA mg/L	503.96±21.43 a	862.01±17.35 b	P<0.001
MDA µmol/L	31.82±3.43 a	82.49±9.90 b	P<0.001
GSH mg/dL	63.43±2.92 a	24.96±2.32 b	P<0.001
NO nmol/L	6.49±0.36 a	13.89±0.53 b	P<0.001

***a, b** refers to statistical significance between the groups (P<0.001)*
***SA:** Total sialic acid, **MDA:** Malondialdehyde, **GSH:** Glutathione, **NO:** Nitric oxide*

Table 2. Changes in oxidative and biochemical parameters in saliva, in bulls with FMD. Values are expressed as mean±SE

Tablo2. Şaplı ve sağlıklı boğaların salyalarındaki biyokimyasal ve oksidatif parametrelerdeki değişimler. Değerler ort. ± standart hata olarak gösterilmiştir

Parameters	Control (n=10)	FMD (n=12)	P Values
SA mg/L	75.98±10.25 a	156.49±14.07 b	P<0.001
MDA µmol/L	1.06±0.17 a	1.81±0.15 b	P<0.01
GSH mg/dL	0.67±0.05 a	0.34±0.03 b	P<0.001
NO nmol/L	1.44±0.14 a	2.44±0.16 b	P<0.001

***a, b** refers to statistical significance between the groups (P<0.01 and P<0.001)*
***SA:** Total sialic acid, **MDA:** Malondialdehyde, **GSH:** Glutathione, **NO:** Nitric oxide*

in vitro [35-37]. Increased NO, a gaseous free radical, in this study is in agreement with the study of cellular elements such as lipopolysaccharide and glycolipoprotein, have been reported to activate leukocytes and stimulate the production of pro-inflammatory cytokines which induces production of NO through activation of inducible nitric oxide synthase (iNOS) [38-42]. This finding may add credence to that NO may play role in the pathogenesis of FMD [37]. On the other hand, the protective or harmful effect of NO is suggested to be associated with the NO concentration [37].

According to the results obtained in this study some oxidative stress parameters can significantly increased in the saliva, as well as sera produced from diseases and oxidative damage to tissues along with other mechanisms might have taken part in the pathogenesis of FMD and further detailed studies at cellular level are needed to fully understand the pathogenesis and clinical expression of the disease in cattle, an important source of infection. It concluded that saliva could provide an appropriate quality of material for researchers in similar studies and the amount of these markers in other body fluids should be reviewed and evaluated in different diseases because saliva can be obtained by noninvasive method and, blood and saliva provide similar statistically significant results on the markers used to evaluate oxidative stress.

REFERENCES

1. Thompson D, Muriel P, Russell D, Osborne P, Bromley A, Rowland M, Creigh-Tyte S, Brown C: Economic costs of the foot and mouth disease outbreak in the United Kingdom in 2001. *Rev Sci Tech OffInt Epiz,* 21, 675-687, 2002.

2. Alexandersen S, Zhang Z, Donaldson AI, Garland AJ: The Pathogenesis and diagnosis of foot-and-mouth disease. *J Comp Pathol*, 129, 1-36, 2003. DOI: 10.1016/S0021-9975(03)00041-0

3. Alexandersen S, Mowat N: Foot-and-mouth disease: Host range and pathogenesis. *Curr Top Microbiol Immunol,* 288, 9-42, 2005. DOI: 10.1007/3-540-27109-0_2

4. Carrillo C, Tulman ER, Delhon G, Lu Z, Carreno A, Vagnozzi A, Kutish GF, Rock DL: Comparative genomics of foot-and-mouth disease virus. *J Virol*, 79, 6487-6504, 2005. DOI: 10.1128/JVI.79.10.6487-6504.2005

5. Domingo E, Pariente N, Airaksinen A, Gonzalez-Lopez C, Sierra S, Herrera M, Grande-Pérez A, Lowenstein PR, Manrubia SC, Lázaro E, Escarmís C: Foot-and-mouth disease virus evolution: Exploring pathways towards virus extinction. *Curr Top Microbiol Immunol,* 288, 149-173, 2005. DOI: 10.1007/3-540-27109-0_7

6. Grubman MJ, Baxt B: Foot-and-mouth disease. *Clin Microbiol Rev,* 17, 465-493, 2004. DOI: 10.1128/CMR.17.2.465-493.2004

7. Sellers R, Gloster J: Foot-and-mouth disease: A review of intranasal infection of cattle, sheep and pigs. *Vet J,* 177, 159-168, 2008. DOI: 10.1016/j.tvjl.2007.03.009

8. De la Rua R, Watkins GH, Watson PJ: Idiopathic mouth ulcers in sheep (letter). *Vet Rec,* 149, 30-31, 2001.

9. Watson P: The differential diagnosis of FMD in sheep in the UK in 2001. *State Vet J,* 20-24, 2002.

10. Kitching RP: Clinical variation in foot and mouth disease: Cattle. *Revue Sci Tech, Office Internat Epizoo*, 21, 499-504, 2002.

11. Watson P: Differential diagnosis of oral lesions and FMD in sheep. *In Pract*, 26, 182-191, 2004. DOI: 10.1136/inpract.26.4.182

12. Kitching RP, Hutber AM, Thrusfield MV: A review of foot-and-mouth disease with special consideration for the clinical and epidemiological factors relevant to predictive modelling of the disease. *Vet J,* 169, 197-209, 2005. DOI: 10.1016/j.tvjl.2004.06.001

13. Ryan E, Gloster J, Reid SM, Li Y, Ferris NP, Waters R, Juleff N, Charleston B, Bankowski B, Gubbins S, Wilesmith JW, King DP, Paton DJ: Clinical and laboratory investigations of the outbreaks of foot-and-mouth disease in southern England in 2007. *Vet Rec,* 163, 139-147, 2008. DOI: 10.1136/vr.163.5.139

14. Schauer R: Chemistry, metabolism and biological functions of sialic acid. *Carbohydrate Chem Biochem,* 40, 131-234, 1982.

15. Haq M, Haq S, Tutt P, Crook M: Serum total sialic acid and lipid-associated sialic acid in normal induvals patients with myocardial infarction and their relationship to acute phase proteins. *Ann Clin Biochem,* 30, 383-386, 1993. DOI: 10.1177/000456329303000406

16. Motoi Y, Kimura Y, Wakamatsu H, Shimbayashi K: Determination and clinical evaluation of sialic acid and mucoprotein in bovine blood. *J Jpn Vet Med Assoc,* 37, 643-649, 1984. DOI: 10.12935/jvma1951.37.643

17. Singh B, Choudhuri PC, Joshi HC: Serum mucoprotein and sialic acid enzootic bovine haematuria. *Zentr Veterinarmedizin Reihe A,* 27, 678-681, 1980. DOI: 10.1111/j.1439-0442.1980.tb01889.x

18. Stefenelli N, Klotz H, Engel A, Bauer P: Serum Sialic acid in malignant tumors, bacterial infections and chronic liver diseases. *J Cancer Res Clin Onco,* 109, 55-59, 1985.

19. Sydow G, Wittmann W, Bender E, Starick E: Der Sialinsäure gehalt im serum von mit bovine leukose virus infizierten Rindern. *Archiv Experim Vet,* 42, 194-197, 1988.

20. Çitil M, Güneş V, Karapehlivan M, Atalan G, Maraşlı Ş: Evaluation of serum sialic acid as an inflammation marker in cattle with traumatic reticulo peritonitis. *Revue Med Vet,* 155, 389-392, 2004.

21. Güneş V, Karapehlivan M, Çitil M, Atalan G, Maraşlı S: Relationship between serum sialic acid levels and eye lesions in calves with infectious bovine keratoconjunctivitis. *Revue Med Vet,* 155, 508-511, 2004.

22. Keleş İ, Ertekin A, Karaca M, Ekin S, Akkan HA: Sığırların leptospirozisinde serum sialic asit ve lipid bağlı sialic asit düzeyleri üzerine araştırma. *Yüzüncü Yıl Univ Vet Fak Derg,* 11, 121-122, 2000.

23. Erdoğan HM, Karapehlivan M, Çitil M, Atakişi O, Ünver A, Uzlu E: Serum sialic acid and oxidative stress parameters changes in cattle with leptospirosis, *Vet Res Commun,* 32, 333-339, 2008. DOI: 10.1007/s11259-008-9036-z

24. Frei B, Stocker R, Ames BN: Antioxidant defences and lipid peroxidation in human blood plasma. *Proceeding Nat Academy Sci*, 85, 9748-9752, 1988.

25. Kalaiselvi T, Panneersalvam C: Effect of L-carnitine on the status of lipid peroxidation and antioxidants in aging rats. *J Nutri Biochem*, 9, 575-581, 1998. DOI: 10.1016/S0955-2863(98)00052-7

26. Nath R, Prasad RI, Sarma S: Oxidative stress biomarkers in cross bred cows affected with Foot and Mouth Disease. *Indian J Anim Res,* 48, 628-632, 2014.

27. Carrillo-Vico A, Lardone PJ, Naji L, Fernandez-Santos JM, Martin-Lacave I, Guerrero JM, Calvo JR: Beneficial pleiotropic actions of melatonin in an experimental model of septic shock in mice: Regulation of pro-/anti-inflammatory cytokine network, protection against oxidative damage and anti-apoptotic effects. *J Pineal Res*, 39, 400-408, 2005. DOI: 10.1111/j.1600-079X.2005.00265.x

28. Sydow G: A simplified quick method for determination of sialic acid in serum. *Biomed Biochem Acta,* 44, 1721-1723, 1985.

29. Yoshoiko T, Kawada T: Lipid peroxidation in maternal and cord blood and protective mechanism against actived-oxygen toxicity in the blood. *Am J Obst Gyn*, 135, 372-376,1979.

30. Miranda KM, Espey MG, Wink DA: A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide,* 5, 62-71, 2001. DOI: 10.1006/niox.2000.0319

31. Beutler E, Duran O, Kelley BM: Improved method for determination of blood glutathione. *J Lab Clin Med,* 61, 882-888, 1963.

32. Taniuchi K, Chifu K, Hayashi N, Nakamachi Y, Yamaguchi N, Miyamoto Y, Doi K, Baba S, Uchida Y, Tsukada Y, Sugimori T: A new

enzymatic method for the determination of sialic acid in serum and its application for a marker of acute phase reactants. *Kobe J Med Sci,* 27, 91-102, 1981.

33. Thougaard AV, Hellmen E, Jensen AL: Total serum sialic acid is a general disease marker rather than a specific tumour marker in dogs. *J Vet Med A,* 45, 471-479, 1998. DOI: 10.1111/j.1439-0442.1998.tb00850.x

34. Mousa SA, Galal M KH: Alteration in clinical, hemobiochemical and oxidative stress parameters in Egyptian cattle infected with Foot and Mouth Disease. *J Anim Sci Adv,* 3, 485-491, 2013.

35. Croen KD: Evidence for an antiviral effect of nitricoxide. *J Clin Invest,* 91, 2446-2452, 1993. DOI: 10.1172/JCI116479

36. Schoedon G, Schneemann M, Walter R, Blau N, Hofer S, Schaffner A: Nitric oxide and infection: Another view. *Clin Infect Dis,* 2, 152-157, 1995. DOI: 10.1093/clinids/21.Supplement_2.S152

37. Bozukluhan K, Atakişi E, Atakişi O: Nitric Oxide levels, total antioxidant and oxidant capacity in cattle with Foot-and-Mouth-Disease. *Kafkas Univ Vet Fak Derg,* 19, 179-181, 2013. DOI: 10.9775/kvfd.2012.7244

38. Alves VAF, Gayotto LCC, Yasuda PH, Wakamatsu A, Kanamura CT, Brito T: Leptospiral antigens *(L. interrogans* sero group *ictero-haemorrhagiae)* in the kidney of experimentally infected guinea pigs and their relation to the pathogenesis of the renal injury. *Experim Pathol*, 42, 81-93, 1991. DOI: 10.1016/S0232-1513(11)80051-4

39. Werts C, Tapping RI, Mathison JC, Chuang TH, KravchenkoV, Saint Girons I, Haake DA, Godowski PJ, Hayashi F, Ozinsky A, Underhill DM, Kirschning CJ, Wagner H, Aderem A, Tobias PS, Ulevitch RJ: Leptospiral lipopolysaccharide activates cells through a TLR2-dependent mechanism. *Nature Immun,* 2, 346-352, 2001. DOI: 10.1038/86354

40. Yang CW, Wu MS, Pan MJ: Leptospirosis renal disease. *Neph Dial Transp,* 16 (Suppl. 5): 73-77, 2001. DOI: 10.1093/ndt/16.suppl_5.73

41. Diament D, Brunialti MK, Romero EC, Kallas EG, Salomao R: Peripheral blood mononuclear cell activation induced by *Leptospira interrogans* glycolipoprotein. *Infect Immunol,* 70, 1677-1683, 2002. DOI: 10.1128/IAI.70.4.1677-1683.2002

42. Marangoni A, Accardo S, Aldini R, Guardigli M, Cavrini F, Sambri V, Montagnani M, Roda A, Cevenini R: Production of reactive oxygen species and expression of inducible nitric oxide synthase in rat isolated Kupffer cells stimulated by *Leptospira interrogans* and *Borrelia burgdorferi*. *World J Gastroent,* 12, 3077-3081, 2006.

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Research Article

Prediction of Weights and Percentages of Retail Cuts in Holstein Bull Carcasses [1]

Hasan ÇİÇEK [1] Murat TANDOĞAN [1] Recep KARA [2]

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[1] Department of Animal Health Economics and Management, Afyon Kocatepe University, Faculty of Veterinary Medicine, TR-03200 Afyonkarahisar - TURKEY

[2] Department of Food Hygiene and Technology, Afyon Kocatepe University, Faculty of Veterinary Medicine, TR-03200 Afyonkarahisar - TURKEY

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Abstract

This study was made to predict the weights and percentages of retail cuts in Holstein bull carcasses. The study material were 47 Holstein bull (mean slaughter age: 20.57±0.606 months) carcasses. Hot carcass weight (HCW, kg), ribeye area (REA, cm^2) and fat thickness (FT, cm) were used to predict weights and percentages of retail cuts. Bull carcasses were divided into 12 retail cuts and grouped as total retail cuts (TRC), first degree retail cuts (FRC) and second degree retail cuts (SRC). Regression analysis was made (stepwise method) to predict weights and percentages of TRC, FRC and SRC. Slaughter weight (SW), dressing percentage (DP), HCW, REA and FT were determined as average 544.550±6.776 kg; 55.75±0.169%; 303.600±3.948 kg; 85.538±1.978 cm^2 and 0.310±0.023 cm, respectively. All predicted models (F) were found significantly (P<0.001; P<0.01) in the analysis. As a result, it was found that HCW is most important predictor in predicting the weights and percentages of retail cuts. HCW and REA explained for 94.5% (R^2) of the variation of dependent variable (Y_{TRC}) in the predicted model for weights of total retail cuts (TRC_{kg}). REA and HCW explained for 21.7% (R^2) of the variation of dependent variable (Y_{TRC}) in the predicted model for percentages of total retail cuts ($TRC_{\%}$).

Keywords: *Holstein bull, Carcass yield, Retail cuts, Regression analysis*

Holştayn Tosun Karkaslarında Perakende Parça Ağırlık ve Oranlarının Tahmini

Özet

Bu araştırma Holştayn tosun karkaslarında perakende parça ağırlık ve oranlarını tahmin etmek amacıyla yapılmıştır. Çalışmanın materyalini 47 adet Holştayn tosun (ortalama kesim yaşı: 20.57±0.606 ay) karkasları oluşturmuştur. Sıcak karkas ağırlığı (HCW, kg), kaburga gözü alanı (REA, cm^2) ve kabuk yağı kalınlığı (FT, cm) perakende parça ağırlık ve oranlarının tahmininde kullanılmıştır. Tosun karkasları 12 parçaya ayrılmış ve bu parçalar da toplam perakende parçalar (TRC), birinci derece perakende parçalar (FRC) ve ikinci derece perakende parçalar (SRC) olarak gruplandırılmıştır. TRC, FRC ve SRC ağırlık ve oranlarının tahmininde regresyon (stepwise yöntemi) analizi yapılmıştır. Kesim ağırlığı (SW), karkas randımanı (DP), HCW, REA ve FT sırasıyla ortalama 544.550±6.776 kg; 55.75±0.169%; 303.600±3.948 kg; 85.538±1.978 cm^2 ve 0.310±0.023 cm tespit edilmiştir. Yapılan analizde tüm tahmini modellerin (F) anlamlı (P<0.001; P<0.01) olduğu belirlenmiştir. HCW'nin perakende parça ağırlık ve oranların tahmininde en önemli belirleyici olduğu tespit edilmiştir. Toplam perakende parçaların ağırlıkları (TRC_{kg}) için tahmin edilen modelde HCW ve REA bağımlı değişkendeki (Y_{TRC}) varyasyonun 94.5% (R^2)'ini açıklamaktadır. Toplam perakende parçaların oranları ($TRC_{\%}$) için tahmin edilen modelde ise REA ve HCW bağımlı değişkendeki (Y_{TRC}) varyasyonun 21.7% (R^2)'sini açıklamaktadır.

Anahtar sözcükler: *Holştayn tosun, Karkas verimi, Perakende parçalar, Regresyon analizi*

INTRODUCTION

Carcass grading systems determine the economic value of a carcass in terms of a difference in yield and flavor characteristics. Nowadays, most carcass grading systems provide a visual evaluation in moving cutting chain that can easily measure parameters of carcasses or retail cuts [1]. The primary purpose of grading is identification of important qualities as commercially and facilitate of the carcass trade [2]. There are various advantages in terms of

İletişim (Correspondence)
+90 533 6304103
hascicek@hotmail.com

producers and consumers of these applications. Sale of graded carcasses' retail cuts offers an important choice factor for many consumers. In the same way, a graded carcass means the valued sales of a product for producers [3].

Carcass grading systems used around the world differ in terms of the applied techniques [4]. The method used in European Union is only based on visual evaluation and defines the carcass structure according to the 6 basic classification (S, E, U, R, O, P) and external fat level (from 1 to 5) [1]. Other grading methods include certain physical measurement and calculation in addition to the visual evaluation [4]. Example of the most established and widely known in this field is The United States Department of Agriculture (USDA) grading system. It is divided as Yield grades-YG (from 1 to 5) and Quality grades-QG (Prime, Choice, Select, Standard, Commercial, Utility, Cutter and Canner) [5]. USDA YG is based on the yield of boneless, closely trimmed, retail cuts and determined by some factors (fat thickness-FT, ribeye area-REA, hot carcass weight-HCW and percentage of kidney, pelvic and heart fat-KPH%). QG is based upon two factors: degree of marbling and degree of maturity [6].

The Japanese Meat Grading Association (JMGA), the Canadian and South Korean grading systems often have similar characteristics to the USDA grading system. It is observed the differences are in terms of some measurement and classification techniques. The classification method is used as in European Union in Australia (AUS-MEAT) and South Africa. Carcass grading method is applied with different techniques (Carcasses are classified according to yield and maturity status in the sex category) in South America countries (Brazil, Argentina, Uruguay and Chile) [5].

There are some researches about yield and quality grading of beef cattle carcasses. In these researches, FT, REA, HCW (or cold carcass weight-CCW) and KPH% were used to determine the carcass yield; marbling, meat color, fat color, texture, and maturity parameters were used in quality grading. Through these parameters, the weights and percentages of retail cuts were predicted, yield and quality characteristics of the carcasses were compared by type of animal, age and gender, and economic slaughter weight was determined [4,7-13].

There are some studies that are examined the weights and percentages of retail cuts [14] and that researched as economically in beef cattle carcasses in Turkey [15-17]. However, there are limited researches conducted on the carcass yield and quality [18-21]. At the same time, carcass weight and dressing percentage (DP) are taken into consideration in the beef market in Turkey; standard grading method is not applied. Therefore, the quality-price relationship is not established for producers.

In this study, yield of retail cuts was examined belonging to Holstein bull carcasses widely breeding in Turkey. HCW, REA and FT parameters were used to predict the weights and percentages of retail cuts.

MATERIAL and METHODS

Data Collection

Research materials are 47 (mean slaughter age: 20.57±0.606 months) Holstein bull carcasses which are fed in a private farm with the same ration (concentrated feed, alfalfa, maize silage and wheat straw) for approximately 6 months period.

Slaughter of the animals and cutting of carcasses is done in a private slaughterhouse in Ankara Province of Turkey (approved by Afyon Kocatepe University, the Local Ethics Committee on Animal Experiments, 23/05/2013, 49533702/331). Slaughter and cutting procedure was applied according to slaughter and cutting regulations of General Directorate of Meat and Dairy Board [22,23]. Slaughter weight-SW (kg) and HCW (kg) were determined. Carcasses were divided into two parts and chilled for 24 hours (between +2˚C and +4˚C) in cooling unit. HCW, REA and FT were used to predict weights and percentages of retail cuts. The left half of each carcass was cut between from 11-12th ribs and the REA (the longissimus muscle) and the FT (the subcutaneous fat) were measured. The longissimus muscle area was measured by plastic grid (with 1 cm x 1 cm). The carcasses are divided into 12 retail cuts at the cutting hall (between +8˚C and +12˚C). Total retail cuts-TRC were classified into first degree retail cuts-FRC (tenderloin, sirloin, rib roast, rump, knuckle, round eye and topside-outside flat) and second degree retail cuts-SRC (chuck, brisket, shoulder, flank and shank).

Each retail cut was weighed on precision scale and weights of retail cuts were determined. The percentages of TRC, FRC and SRC weights were calculated according to HCW [9].

Statistical Analysis

Descriptive statistics (mean, minimum and maximum values) were determined belonging to all variables. HCW, REA and FT parameters (independent variables) were used and multiple linear regression analysis was used to predict the weights and percentages belonging to TRC, FRC and SRC (dependent variables). Stepwise method was used in choosing the independent variables. Correlation analysis of all the variables that included in the model was done.

RESULTS

Descriptive statistics of Holstein bull carcasses are presented in *Table 1*. Approximately 56% of dressing percentage was obtained from animals, which are about 21 months old. Approximately 61% of total retail cuts (TRC) are provided from these carcasses (according to HCW).

Percentage of bones and crumbs (according to HCW) was detected 18.75% and 17.08%, respectively.

Predicted regression models for the weights and percentages of TRC, FRC and SRC are given in *Table 2* and *Table 3*. It was determined that all predicted models (*F*) are significant (*P*<0.001; *P*<0.01) which are using stepwise method in analysis.

It was determined that HCW and REA are the best predictor in predicted model (Y_{TRC}) for TRC_{kg} *(Table 2)*. HCW and REA explained for 94.5% (R^2) of the total variation in Y_{TRC}. According to the partial correlation coefficient (pr^2), HCW (94.3%) is most explanatory variable to REA (11.6%). HCW, FT and REA are predictor in predicted model (Y_{FRC}) for FRC_{kg}. These variables explained for 83.5% (R^2) of the total variation in Y_{FRC}. HCW has most explanatory power (pr^2: 81.2%) than other two variables. This was followed by the FT (16.4%) and REA (11.8%), respectively. It was found that FT's effect is significantly negative on Y_{FRC} in this model. It was determined that HCW was single explanatory variable in the Y_{SRC} model (R^2: 89.2).

It was found that REA and HCW are predictor (R^2: 21.7) in predicted model for $TRC_{\%}$ and it was determined that their variations explanatory power is in Y_{TRC} 11.6% and 10.8%, respectively *(Table 3)*. FT, HCW and REA explained for 44.5% of the total variation in $FRC_{\%}$ and HCW has higher partial correlation coefficient (pr^2: 16.2) according to other two variables. HCW (34.4%) was found single explanatory variable for $SRC_{\%}$.

Table 4 is seen that HCW effects positively to TRC_{kg}, FRC_{kg}, SRC_{kg}, $SRC_{\%}$ (*P*<0.01) and $TRC_{\%}$ (*P*<0.05). REA has only positive effect on the $TRC_{\%}$ (*P*<0.05). FT effects positively to TRC_{kg}, SRC_{kg} (*P*<0.01) and $SRC_{\%}$ (*P*<0.05).

DISCUSSION

In this study, HCW, REA and FT parameters were used to predict the weights and percentages of retail cuts of carcasses. There are some studies, which used by similar methods [7,9,10,12]. CCW was used instead of HCW in other research except Chen et al.[9]. REA and FT measurements

***Table 1.** Descriptive statistics of the carcass traits*
***Tablo 1.** Karkas özelliklerinin tanımlayıcı istatistikleri*

Parameters	n	Mean	S.E.	Min.	Max.
Slaughter age (month)	47	20.57	0.606	15	37
Slaughter weight-SW (kg)	47	544.550	6.776	420.069	685.114
Hot carcass weight-HCW (kg)	47	303.596	3.948	241.101	391.027
Dressing percentage-DP (%)	47	55.75	0.169	53.74	58.26
Rib eye area-REA (cm^2)	47	85.538	1.978	46.365	119.328
Fat tickness-FT (cm)	47	0.310	0.023	0.113	0.889
Total retail cuts-TRC (kg)	47	186.372	2.785	146.727	246.857
First degree retail cuts-FRC (kg)	47	73.958	0.823	61.502	88.841
Second degree retail cuts-SRC (kg)	47	112.413	2.088	80.902	158.016
Total retail cuts-TRC (%)	47	61.34	0.242	57.32	65.20
First degree retail cuts-FRC (%)	47	24.41	0.153	21.91	27.31
Second degree retail cuts-SRC (%)	47	36.93	0.272	33.57	41.66

***Table 2.** Predicted regression models for weight of retail cuts (stepwise method)*
***Tablo 2.** Karkas perakende parça ağırlıklarının tahmini regresyon modelleri (stepwise* yöntemi)

Models	Independent Variable	Intercept	Regression Coefficient	*SE*	*t*	*Sig.*	*F*	*Sig.*	R^2	pr^2
1 ($Y_{TRC, kg}$)		-29.714		8.408	-3.534	**0.001	378.965	*0.000	94.5	
	HCW		0.678	0.025	27.116	*0.000				94.3
	REA		0.120	0.050	2.397	***0.021				11.6
2 ($Y_{FRC, kg}$)		9.727		4.531	2.147	***0.037	72.405	*0.000	83.5	
	HCW		0.201	0.015	13.601	*0.000				81.2
	FT		-7.277	2.505	-2.905	**0.006				16.4
	REA		0.062	0.026	2.397	***0.021				11.8
3 ($Y_{SRC, kg}$)		-39.261		7.909	-4.964	*0.000	370.628	*0.000	89.2	
	HCW		0.500	0.026	19.252	*0.000				

***n:** 47, * P<0.001, ** P<0.01, *** P<0.05*

Table 3. *Predicted regression models for percentages of retail cuts (stepwise method)*
Tablo 3. *Karkas perakende parça oranlarının tahmini regresyon modelleri (stepwise yöntemi)*

Models	Independent Variable	Intercept	Regression Coefficient	*SE*	*t*	*Sig.*	*F*	*Sig.*	R^2	pr^2
1 ($Y_{TRC, \%}$)		52.181		2.764	18.877	*0.000	6.109	**0.005	21.7	
	REA		0.040	0.016	2.410	***0.020				11.6
	HCW		0.019	0.008	2.314	***0.025				10.8
2 ($Y_{FRC, \%}$)		27.482		1.542	17.818	*0.000	11.483	*0.000	44.5	
	FT		-2.168	0.853	-2.542	***0.015				13.0
	HCW		-0.015	0.005	-2.879	**0.006				16.2
	REA		0.023	0.009	2.648	***0.011				14.0
3 ($Y_{SRC, \%}$)		24.683		2.532	9.747	*0.000	23.582	*0.000	34.4	
	HCW		0.040	0.008	4.856	*0.000				

n: 47, * *P*<0.001, ** *P*<0.01, *** *P*<0.05

Table 4. *Pearson correlation coefficients of the carcass variables*
Tablo 4. *Karkas değişkenlerinin Pearson korelasyon katsayıları*

Variables	HCW	REA	FT	TRC_{kg}	FRC_{kg}	SRC_{kg}	$TRC_{\%}$	$FRC_{\%}$	$SRC_{\%}$
HCW	1.00								
REA	0.09	1.00							
FT	0.48**	-0.03	1.00						
TRC_{kg}	0.97**	0.17	0.45**	1.00					
FRC_{kg}	0.88**	0.24	0.25	0.89**	1.00				
SRC_{kg}	0.94**	0.13	0.49**	0.98**	0.79**	1.00			
$TRC_{\%}$	0.34*	0.35*	0.08	0.56**	0.44**	0.57**	1.00		
$FRC_{\%}$	-0.51**	0.28	-0.52**	-0.42**	-0.04	-0.54**	0.11	1.00	
$SRC_{\%}$	0.59**	0.16	0.36*	0.74**	0.42**	0.82**	0.83**	-0.46**	1.00

** *P*<0.01, * *P*<0.05

are usually carried out on between 12-13th ribs especially the USDA grading system in the world. In Turkey, in particular General Directorate of Meat and Dairy Board slaughterhouses, beef carcasses are quartered from between 11-12th ribs [16]. Thus, REA and FT measurements were made from between 11-12th ribs. In JMGA grading system, the measurement of these parameters is carried out from between 6-7th ribs [5].

In the study, regression analysis with stepwise method was used to examine the quantity and the direction of the relationship between the HCW, REA and FT (independent variables) and the weights and percentages of TRC, FRC and SRC (dependent variables). It is reported long of decades that this method is most popular method used to determine carcass composition [7].

It was found that HCW is most important predictor to predict of the weights and percentages of retail cuts of the carcasses. In addition, HCW affects positive all variables except the $FRC_{\%}$. High correlation was found between HCW and the weights of TRC (0.97), FRC (0.88) and SRC (0.94) *(Table 4)*. In a previous study, it was reported that high correlation between (0.96) HCW and weights of total retail cuts [9]. In another study, it was detected high correlation (0.94) between CCW and weights of total retail cuts [10]. It was determined that increase in HCW or CCW, increases REA and FT linearly [13].

REA is most important predictor after HCW in prediction of weights and percentages of the TRC and FRC. REA was found insignificant prediction of weights and percentages of SRC. REA affects positively all variables except the FT. Chen et al.[9] found a similar situation in their research. Carcass weight, sex, nutritional status and measurement location (11-12th rib section) may have been affected the average value determined for the REA (85.538±1.978 cm²) which differ from some research results. In some research, REA was reported between 68.13-84.3 cm² [9-12]. In studies from Turkey, REA values belonging to Brown Swiss, Charolais x Brown Swiss, Charolais and Eastern Anatolian Red steers, Friesian and Friesian Crossbreeds were measured between 64.73-101.15 cm² [18-21].

FT has only been predictor in the prediction of weights and percentages of FRC. FT explained alone (pr^2) for 16.4%

and 13.0% of the variation of $Y_{FRC, kg}$ and $Y_{FRC, \%}$ respectively. However, FT has negative impact both on the weights and percentages of FRC. The determined average values for FT (0.301±0.023 cm), as in the REA which can be expected to differ depending on the results of other research depending on carcass weight, sex, nutritional status and place of measurement (11-12th ribs section). In studies which done with carcasses belonging to different animal breeds, FT was reported between 0.58 - 0.96 cm [9,10,12]. In the study conducted in Turkey FT was measured between 0.29 - 0.93 cm [18-20].

In Japanese Black steers carcasses, which measurements carried out in the 6-7th ribs, REA and FT were determined 42.7 cm^2 and 2.6 cm, respectively. In the same study, age, slaughter weight and CCW were reported 27.3 months, 635.5 kg and 402.6 kg, respectively [7].

As a result; in this study, regression models were predicted for weights of TRC ($Y_{TRC, kg}$ = -29.714 + 0.678HCW + 0.120REA), and percentages of TRC ($Y_{TRC, \%}$ = 52.181 + 0.040REA + 0.019HCW) in Holstein bull carcasses. Total yield of retail cuts can be predicted for Holstein bull carcasses by these models which using HCW and REA values. Likewise, regression models were predicted for weights of FRC ($Y_{FRC, kg}$ = 9.727 + 0.201HCW - 7.277FT + 0.062REA), and percentages of FRC ($Y_{FRC, \%}$ = 27.482 - 2.168FT - 0.015HCW + 0.023REA). First degree retail cuts yield can be calculated which using HCW, REA and FT values. HCW is just enough to know for second degree retail cuts yield. It should be noted that predicted regression coefficients changed if there is a difference in the number of carcass, animal breed, slaughter age, sex, nutrition, carcass weight (HCW or CCW) and measurement techniques (for REA and FT).

The regression coefficients can be determined for HCW, REA and FT in beef carcasses belonging to different breeds in Turkey. More research needs to be done on the subject in the future. In this study, a survey was conducted for the yield level of the beef carcasses. Carcass grading methods has been improved both for yield and quality in the world. Turkey is located in a major shortcoming in this subject; meat standards should be improved at the national level for the different breed of cattle carcasses with future studies.

REFERENCES

1. Beriain MJ, Indurain G, Carr TR, Insausti K, Sarries V, Purroy A: Contrasting appraisals of quality and value of beef carcasses in Spain and the United States. *Revue Med Vet,* 164 (7): 337-342, 2013.

2. Price MA: Development of carcass grading and classification systems (chapter 8). **In,** Morgan Jones SD (Ed): Quality and Grading of Carcasses of Meat Animals. 173-200, CRC Press, Inc., USA, 1995.

3. Tatum D: Beef Grading. 4, National Cattlemen's Beef Association. USA, 2007.

4. Strydom PE, Smith MF: Predicting yields of high priced trimmed beef cuts by means of carcass weight and visual assessments of fat cover and conformation. *S Afr J Anim Sci,* 35, 195-205, 2005. DOI: 10.4314/sajas.v35i3.4059

5. Polkinghorne RJ, Thompson JM: Meat standards and grading A world view. *Meat Sci,* 86, 227-235, 2010. DOI: 10.1016/j.meatsci.2010.05.010

6. Boggs DL, Merkel RA, Doumit ME, Bruns K: Livestock and carcasses: An integrated approach to evaluation, grading and selection. 6th ed., 111-141, Kendall/Hunt Publishing Company, Iowa, USA, 2006.

7. Maeno H, Oishi K, Mitsuhashi T, Kumagai H, Hirooka H: Prediction of carcass composition and individual carcass cuts of Japanese Black steers. *Meat Sci,* 96, 1365-1370, 2014. DOI: 10.1016/j.meatsci.2013.11.017

8. Lawrence TE, Elam NA, Miller MF, Brooks JC, Hilton GG, VanOverbeke DL, McKeith FK, Killefer J, Montgomery TH, Allen DM, Griffin DB, Delmore RJ, Nichols WT, Streeter MN, Yates DA, Hutcheson JP: Predicting red meat yields in carcasses from beef-type and calf fed Holstein steers using the United States Department of Agriculture calculated yield grade. *J Anim Sci,* 88, 2139-2142, 2010. DOI: 10.2527/jas.2009-2739

9. Chen Y, Li C, Liu L, Zhou G, Xu X, Gao F: Prediction of yield of retail cuts for native and crossbred Chinese Yellow cattle. *Anim Sci J,* 78, 440-444, 2007. DOI: 10.1111/j.1740-0929.2007.00459.x

10. Choy YH, Choi SB, Jeon GJ, Kim HC, Chung HJ, Lee JM, Park BY, Lee SH: Prediction of retail beef yield using parameters based on Korean Beef Carcass Grading Standards. *Korean J Food Sci Anim Resour,* 30, 905-909, 2010. DOI: 10.5851/kosfa.2010.30.6.905

11. Lee JM, Yoo YM, Park BY, Chae HS, Hwang IH, Choi YI: A research note on predicting the carcass yield of Korean native cattle (Hanwoo). *Meat Sci,* 69, 583-587, 2005. DOI: 10.1016/j.meatsci.2004.09.004

12. Park GB, Moon SS, Ko YD, Ha JK, Lee JG, Chang HH, Joo ST: Influence of slaughter weight and sex on yield and quality grades of Hanwoo (Korean native cattle) carcasses. *J Anim Sci,* 80, 129-136, 2002.

13. Moon SS, Hwang IH, Jin SK, Lee JG, Joo ST, Park GB: Carcass traits determining quality and yield grades of Hanwoo Steers. *Asian-Aust J Anim Sci,* 16, 1049-1054, 2003. DOI: 10.5713/ajas.2003.1049

14. Yücesan A, Ergün Ö: Çeşitli sığır ırklarımıza ait karkaslarda değerli et preparatlarının tespiti ve karkaslara oranları üzerine araştırmalar. *İstanbul Üniv Vet Fak Derg,* 26 (2): 345-352, 2000.

15. Sarıözkan S, Akçay A, Bayram D: Zavot ırkı sığırlarda karkas özellikleri ve karkas parçalamanın ekonomik yönü. *Ankara Üniv Vet Fak Derg,* 60, 257-262, 2013. DOI: 10.1501/Vetfak_0000002589

16. Kale MC: Et ve Balık Ürünleri Anonim Şirketi kombinalarında sığır etinin karkas veya parçalanmış et olarak sürümünün işletme gelirine etkisi. *Doktora tezi,* Ankara Üniv. Sağlık Bil. Enst., 2008.

17. Kale MC, Aral Y, Aydın E, Cevger Y, Sakarya E, Güloğlu SC: Determination of by-product economic values for slaughtered cattle and sheep. *Kafkas Univ Vet Fak Derg,* 17, 551-556, 2011. DOI: 10.9775/kvfd.2010.3945

18. Özlütürk A, Tüzemen N, Yanar M, Esenbuga N, Dursun E: Fattening performance, carcass traits and meat quality characteristics of calves sired by Charolais, Simmental and Eastern Anatolian Red sires mated to Eastern Anatolian Red dams. *Meat Sci,* 67, 463-470, 2004. DOI: 10.1016/j.meatsci.2003.11.022

19. Özlütürk A, Esenbuğa N, Yanar M, Ünlü N, Macit M, Kopuzlu S: The effect of duration of finishing period on the performance, slaughter, carcass, and beef quality characteristics of Eastern Anatolian Red bulls. *Turk J Vet Anim Sci,* 32 (6): 441-448, 2008.

20. Sağsöz Y, Çoban Ö, Laçin E, Sabuncuoğlu N, Yıldız A: Esmer ve Şarole x Esmer danaların besi performansı ve karkas özellikleri. *Atatürk Üniv Ziraat Fak Derg,* 36 (2): 163-169, 2005.

21. Güngör M, Alçiçek A, Tümer S, Önenç A: Siyah Alaca ve farklı etçi ırk melezlerinin besi performanslarının araştırılması. *J Aegean Agri Res Inst,* 14 (1): 27-40, 2004.

22. GDMMBa: General Directory of Meat and Milk Board, Slaughter Regulation (No: 37), 20, Ankara, Turkey, 2012.

23. GDMMBb: General Directory of Meat and Milk Board. Cutting and Management Regulation, No: 204, 2000.

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Research Article

Immunohistochemical Distribituon of COX-1, COX-2, and TGFβ-1 in the Duodenum of Rats Treated with Capsaicin

[1] Department of Histology - Embryology, Faculty of Veterinary Medicine, Namik Kemal University, TR-59000 Tekirdag - TURKEY
[2] School of Health Sciences, Kafkas University, TR-36040 Kars - TURKEY
[3] Department of Histology - Embryology, Faculty of Veterinary Medicine, Kafkas University, TR-36040 Kars - TURKEY
[4] Department of Histology - Embryology, Faculty of Medicine, Mugla Sıtkı Kocman University, TR-48000 Mugla - TURKEY

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Abstract

The purpose of this study was to investigate the effects of capsaicin on the duodenal distribution of cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2) and Transforming growth factor β-1 (TGFβ-1) in rats. Rats were divided into two groups (n =10 in each group). Experimental group treated with capsaicin (1 mg/kg, subcutaneously) was injected in 10% ethanol, 1% Tween and 80% distilled water everyday for a period of one week and in not injected control group. Localisations of COX-1, COX-2 and TGFβ-1 were observed in villus and crypt ephitelial cells, and smooth muscle cells. In conclusion, in this study, capsaicin administration was found to increase the release of COX-1 and COX-2, and TGFβ-1.

Keywords: *Capsaicin, Cyclooxygenase, Duodenum, Transforming growth factor β-1*

Capsaicin Uygulanan Sıçanların Duodenumda COX-1, COX-2 ve TGFβ-1'in İmmünohistokimyasal Dağılımı

Özet

Bu çalışmada capsaicin uygulanan ratların duodenum dokusunda siklooksijenaz-1 (COX-1), siklooksijenaz-2 (COX-2) ve transforme edici büyüme faktörü β-1 (TGFβ-1)'in dağılımını araştırmak amaçlandı. Ratlar iki gruba ayrıldı (her grupta n=10). Capsaicin uygulanan deneme grubuna (1 mg/kg, subkutan yolla) %10 ethanol, %1 tween, %80 distile su karışımı bir haftalık periyot boyunca hergün enjekte edildi. COX-1, COX-2 ve TGFβ-1'in dağılımı villus ve kript epitel hücreleri ve düz kas hücrelerinde tespit edildi. Sonuçta, bu çalışmada capsaicin uygulamasının COX-1 ve COX-2 ve TGFβ-1 salınımını arttırdığı tespit edildi.

Anahtar sözcükler: *Capsaicin, Siklooksijenaz, Duodenum, Transforme edici büyüme faktörü β-1*

INTRODUCTION

Capsaicin is an active component of chili peppers, which are plants belonging to the genus Capsicum annuum [1,2]. In alternative medicine, it is used for relieving pains such as neuropathic pains, arthritis, pains after chemotherapy [3]. And it has analgesic effect [4,5]. It is also noted that it has effect on growth and development by increasing the release of growth factors [2,6-8].

It has been known for years that non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, ibuprofen, and acetaminophen, provide relief from fever, pains, and inflammation through their actions on cyclooxygenase (COX) enzymes [9]. COX is the enzyme that catalyzes the first two steps in the biosynthesis of the prostaglandins from the substrate arachidonic acid [10].

Two COX isozymes, COX-1 and COX-2, have been identified. Although both enzymes have similar functions, their temporal and spatial expression patterns are very different. COX-1 is constitutively expressed in many tissues, including the gatrointestinal mucosa [11]. In contrast, COX-2, is normally undetectable in most tissues; however, increased expression of COX-2 express inflammatory conditions of the gastrointestinal tract (e.g., inflammatory bowel

İletişim (Correspondence)
+90 282 2504715
buhal@hotmail.com

disease) [12]. This simple distinction, implying that COX-1 is constitutive and COX-2 inducible in nature, has been questioned on the basis of evidence about the constitutive expression of COX-2 in normal tissues, such as brain, kidney and blood vessel [13].

Transforming growth factor-β (TGF-β) is a growth factor which release from almost every tissue in mammals [14]. It consist of three members (TGF-β_1, TGF-β_2 ve TGF-β_3) [15]. This family regulate numerous biological activities, including cell proliferation, differentiation, adhesion, and apoptosis, extracellular matrix (ECM) production, and early embryo development and immunity [16]. It was demonstrated that TGF-β localisated in villus and crypt epithelial cells in small intesine [17,18].

With this study, it was aimed to investigate the effects of capsaicin, which was found to benefits such as have analgesic [3] and growth promoting [6,9], on the secretion of COX-1 that is protective for physiological functions of duodenal tissues [20], COX-2 that is suppressor in pathological situations [21] and TGF-β1 that acts as regulatory as well as serving growth and development functions [16].

MATERIAL and METHODS

Animals and Experimental Design

Tissue samples were collected in compliance with an approved Kafkas University Institutional Animal Care and Use Committee Protocol (KAU-HADYEK/2014-033).

Twenty 50 days old male Spraque Dawley rats were randomly divided into two groups as; experimental (n=10) and control (n=10). Rats were housed in a continuously ventilated room at a mean temperature of 22±2°C with a lighting period of 12 h dark and 12 light. Animals were fed standard rodent chow (Bayramoğlu, Erzurum, Turkey) and water ad libitum. The amount of capsaicin used in our study was based on studies conducted by Moran et al.[22] and Tütüncü [23]. For the rats in experimental group, 1 mg/kg of capsaicin (cat no. M 2028, Sigma - Aldrich, Germany) was dissolved in 10% ethanol and mixed with 1% Tween (cat no. M 8170772100, Merck, USA) and 80% distilled water. Capsaicin solution was freshly prepared according to daily body weights of the rats and injected subcutaneously with an insulin injector at the same time every day for a week. For the rats in control group had no application.

After one week, all rats were sacrified by cervical dislocation method under diethylether anaesthesia and duodenum samples were taken.

Immunohistochemical Procedure

For immunohistochemistry, the sections were incubated in 3% H_2O_2 for 10 min to inhibit endogenous peroxidase activity after undergoing deparaffinization and rehydration. Maximum heat was applied in a microwave for 10 min in citrate buffer solution to reveal antigens, after being washed with PBS (Phosphate Buffer Saline). Sections were then incubated with primary antibodies anti-COX-1 (5F6/F4, ab695, abcam), anti-COX-2 (M-19, sc-1747, Santa Cruz) diluted to 1 : 200 in PBS at room temperature for one hour and polyclonal goat anti-TGFβ-1 (sc-90, SantaCruz, USA) diluted 1 : 100 in PBS. Only PBS was dripped on the tissues of the negative control group. Streptavidin - biotin peroxidase technique was used after primary antibody incubation. Streptavidin - HRP (Horse Radish Peroxidase) (Invitrogen Histostain plus Broad Spectrum (AEC) Ref. 85.9943) was dripped on the sections, and then incubated at room temperature for 15 min. 3,3′-Diaminobenzidine tetrahydrochloride (0.5 mg/mL; Dako Corp.) was used as chromogen followed by hematoxylin counterstaining. Sections were mounted with immunoMount and examined by light microscope (Olympus BX51, Shinjuku, Tokyo Japan). Scoring was conducted using a semi-quantitative method [6] on an area of the sections, based on criteria of the percentage of stained cells and the degree of staining. The degree of staining was evaluated as follows; no reaction (-), slight (+), moderate (++) and intensive (+++).

RESULTS

It was observed normal histological structure in two groups.

COX-1 Immunoreactivity in Duodenum

While intensive cytoplasmic reaction was observed in villus epithelial cells and in crypt epithelial cells of experimental group, slight cytoplasmic reaction was observed in villus epithelial cells and in crypt epithelial cells of control group. No reaction was observed in goblet cells of all group. Intensive cytoplasmic reaction was present in smooth muscle cells of experimental group and slight cytoplasmic reaction was present in smooth muscle cells of control group *(Fig. 1, Table 1)*.

COX-2 Immunoreactivity in Duodenum

Moderate cytoplasmic reaction was observed in villus epithelial cells and in crypt epithelial cells of experimental group. Slight cytoplasmic reaction was present in villus epithelial cells and in crypt epithelial cells of control group. No reaction was determined in goblet cells of all groups and intensive reaction was remerkable in connective tissue

Table 1. *Comparison of COX-1 immunoreactivity's degree among groups*
Tablo 1. *Gruplar arasında COX-1 immünoreaktivitesinin karşılaştırılması*

Duodenum (COX - 1)	Experimental Group	Control Group
Villus ephitelial cells	+++	+
Cyript ephitelial cells	+++	+
Goblet cells	-	-
Smooth muscle cells	+++	+

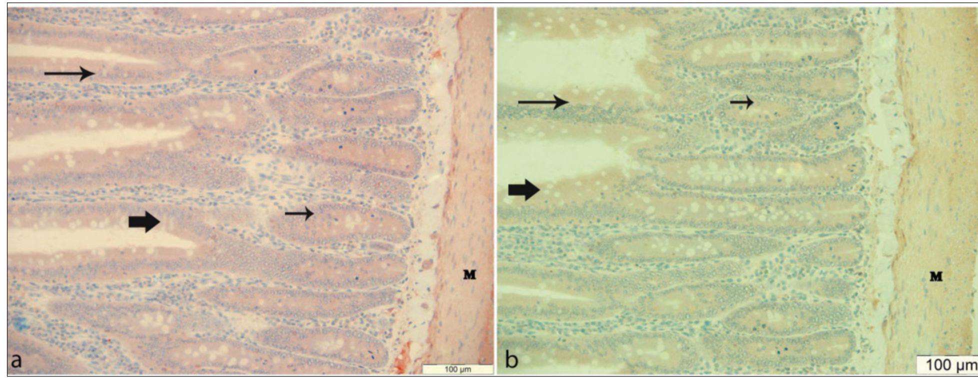

Fig 1. COX-1 immunoreactivity in rat duodenum. **a:** Experimental group, **b:** control group, Villus epithelial cell *(long arrow)*, goblet cell *(thick arrows)*, crypt epithelial cell *(short arrow)*, smooth muscle cell (M). Immunohistochemistry, Bar: 100 µm

Şekil 1. Rat duodenumunda COX-1 immünoreaktivitesi. **a:** Deney grubu, **b:** kontrol grubu, Villus epitel hücresi *(uzun ok)*, goblet hücresi *(kalın ok)*, kript epitel hücresi *(kısa ok)*, düz kas hücresi (M). İmmünohistokimya, Bar: 100 µm

***Table 2.** Comparison of COX-2 immunoreactivity's degree among groups*
***Tablo 2.** Gruplar arasında COX-2 immünoreaktivitesinin karşılaştırılması*

Duodenum (COX-2)	Experimental Group	Control Group
Villus ephitelial cells	++	+
Cyript ephitelial cells	++	+
Goblet cells	-	-
Connective tissue cell	+++	++
Smooth muscle cells	++	+

***Table 3.** Comparison of TGFβ-1 immunoreactivity's degree among groups*
***Tablo 3.** Gruplar arasında TGFβ-1 immünoreaktivitesinin karşılaştırılması*

Duodenum (TGFβ - 1)	Experimental Group	Control Group
Villus ephitelial cells	+++	+
Cyript ephitelial cells	+++	+
Goblet cells	-	-
Smooth muscle cells	++	+

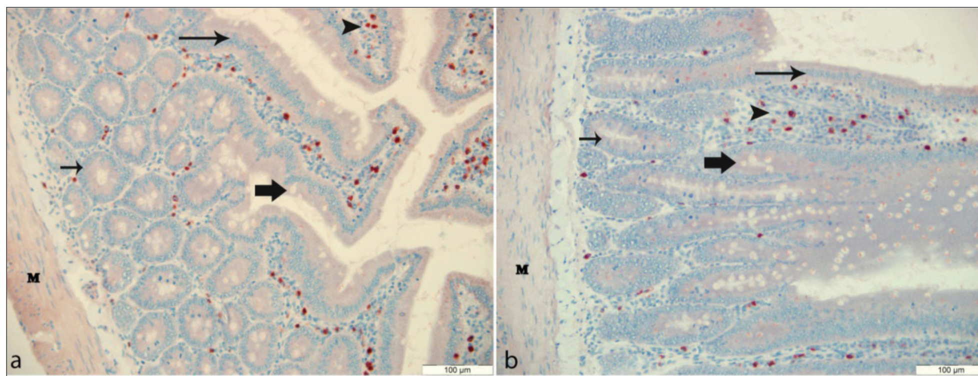

Fig 2. COX-2 immunoreactivity in rat duodenum. **a:** Experimental group, **b:** control group, Villus epithelial cell *(long arrow)*, goblet cell *(thick arrows)*, crypt epithelial cell *(short arrow)*, smooth muscle cell (M), connective tissue cell *(arrow head)*. Immunohistochemistry, Bar: 100 µm

Şekil 2. Rat duodenumunda COX-2 immünoreaktivitesi. **a:** Deney grubu, **b:** kontrol grubu, Villus epitel hücresi *(uzun ok)*, goblet hücresi *(kalın ok)*, kript epitel hücresi *(kısa ok)*, düz kas hücresi (M). İmmünohistokimya, Bar: 100 µm

cells of experimental group and moderate reaction was remerkable in connective tissue cells of control group. While moderate reaction was observed in smooth muscle cells in experimental groups, slight reaction was determined in smooth muscle cells in control group *(Fig. 2, Table 2)*.

TGFβ-1 Immunoreactivity in Duodenum

While intensive cytoplasmic reaction was observed in villus epithelial cells and in crypt epitelial cells of experimental group, slight cytoplasmic reaction was observed in villus epithelial cells and in crypt epitelial cells of control group. No reaction was determined goblet cells of all groups. While moderate reaction was determined in smooth muscle cells of experimental group, slight reaction was observed in smooth muscle cells of control group *(Fig. 3, Table 3)*.

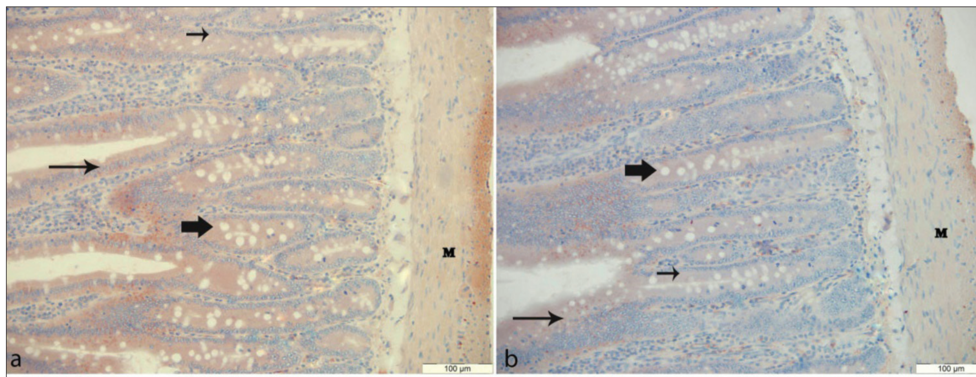

Fig 3. TGFβ-1 immunoreactivity in rat duodenum. **a:** Experimental group, **b:** control group, Villus epithelial cell *(long arrow)*, goblet cell *(thick arrows)*, crypt epithelial cell *(short arrow)*, smooth muscle cell (M). Immunohistochemistry, Bar: 100 µm

Şekil 3. Rat duodenumunda TGFβ-1 immünoreaktivitesi. **a:** Deney grubu, **b:** kontrol grubu, Villus epitel hücresi *(uzun ok)*, goblet hücresi *(kalın ok)*, kript epitel hücresi *(kısa ok)*, düz kas hücresi (M). İmmünohistokimya, Bar: 100 µm

DISCUSSION

Pain is the body's message that something is wrong in the system. These issues of pain are not diseases of themselves, but are symptoms of a dysfunction in associated structures [24]. Capsaicin belongs to group of analgesic substances [4,5,25]. This effect of capsaicin was found to function by preventing substance P, which is expressed in non-myelinated nerve fibers involved in carrying pain sensation from periphery to the center, from reaching the brain [26]. Because of this feature of capsaicin, it was predicted to be used in the treatment of arthritis, mild pains, as well as neuropathic pains following chemotherapy and sclerosis application [3].

COX expression has focused on pathological samples [3]. COX-1 is a constitutive enzyme and has a homeostatic role in gastrointestinal mucosa protection. COX-2 isoenzyme, frequently undetectable in most normal tissues, but quickly induced by inflammatory factors [13,27,28]. COX-2 selective agents were first approved for the treatment of acute and chronic pains and indications exist for the treatment of osteoarthritis, rheumatoid arthritis and a variety of musculoskeletal disorders [29,30].

In our study, COX-1 and COX-2 secretion was found to increase in capsaicin treated group. The findings support the view that capsaicin has a role in healing and reducing pains by both preventing pains sense from reaching the brain and increasing the release of COX-1 and COX-2.

In contrast to COX-1, it is generally believed that COX-2 is not present in most normal tissues, with the exaception of the brain [31], kidney [32], and female reproductive system [33]. However, some previous studies have detected COX-2 in other normal tissues [34-41]. In our study, in addition to the aforementioned studies, the presence of COX-2 expression was detected in the duodenal tissue in both the control group and capsaicin treated group.

Expression of COX-1 was found mostly in blood vessels, connective tissue cells, smooth muscle cells, platelets and only rarely in parenchymal cells. In contrast, COX-2 was found predominantly in parenchymal cells, and only occasionally in resident inflammatory cells, connective tissue cells, endothelial cells and smooth muscle cells. Also, expression of COX-1 was greater than the expression of COX-2 in the small intestine [40]. In our study, COX-1 and COX-2 secretion was detected in similar cells (villus and crypt epithelial cells, smooth muscle cells). In addition, COX-2 was also identified in connective tissue cells unlike COX-1. In the study, COX-1 reaction intensity was seen to be more compared to COX-2 in cells with similar [40] reactions.

TGF-β is a multifunctional growth factor that influences growth and differentiation in many cell types [42] and modulation of cell growth, apoptosis and differentiation of intestinal epithelial cells [43,44]. TGF-β protein has been demonstrated villus and crypt epithelial cells in small intesine [17,18,45]. Besides the studies mentioned in our study, TGFβ-1 secretion was found in villus and crypt epithelial cells in addition muscle tissue cells.

Capsaicin application has been expressed to increase TGFβ release in various tissues [7,8]. However, no study was found in the literature regarding the effect of capsaicin application on TGFβ-1 release in the duodenum. Bakir and Sarı [6] stated that capsaicin application increases the release of PDGF-C and PDGFR-α in villus, crypt epithelial cells, goblet cells and muscle cells of the duodenum. In our study, capsaicin application was also determined to increase the release of TGFβ-1 in villus and crypt epithelial cells, and muscle tissue cells in duodenum.

As a result of our study in which we immunohistochemically investigated COX-1, COX-2 and TGFβ-1 secretion in duodenum tissue of capsaicin treated rats, it was seen that capsaicin application increases the release of COX-1 that has a regulatory role in the gastrointestinal

tract, COX-2 that has regulatory role and functions in pathological and inflammatory conditions and TGFβ-1 that has positive effects on growth and development.

REFERENCES

1. Lembeck F: Columbus, capsicum and capsaicin: Past, present and future. *Acta Physiol Hung,* 69, 265-273, 1987.

2. Szalasi A, Blumberg PM: Vanilliod (capsaicin) receptors and mechanisms. *Pharmacol Rev*, 51, 159-211, 1999.

3. Başak S, Dikicioglu E, Turgutanıt S, Sarıerler M: Early and late effects of capsaicin pretreatment in otitis media with effusion. *Otol Neurotol,* 26, 344-350, 2005.

4. Caterina MJ, Leffler A, Malmberg AB, Martin WJ, Trafton J, Petersen - Zeitz KR, Kdtzenburg M, Basbaum AI, Julius D: Impaired nociception and pain sensation in mice lacking the capsaicin receptor. *Science,* 288, 306-313, 2000. DOI: 10.1126/science.288.5464.306

5. Erdost H, Ozfiliz N, Ozgüden OS, Ilhan T, Ozer A: Expression of a capsaicin reseptor (VRI) in the testis of mice after an application of capsaicin. *Bull Vet Inst Pulaway*, 51, 649-653, 2007.

6. Bakır B, Sarı EK: Immunohistochemical distribution of platelet derived growth factor-c and platelet derived growth factor receptor-alpha in small intestine of rats treated with capsaicin. *Turk J Vet Anim Sci*, 39, 160-167, 2015. DOI: 10.3906/vet-1405-24

7. Nur G, Nazlı M, Yıldız SE: Immunohistochemical localization of transforming growth factor beta 2 and gene expression using real-time PCR in capsaicin-administered rat testis during puberty. *Turk J Vet Anim Sci*, 38, 377-382, 2014. DOI: 10.3906/vet-1302-16

8. Yıldız SE, Nazlı M, Nur G: Immunohistochemical distribution and gene expression of transforming growth factor alpha in ovarian tissue of rats treated with capsaicin in puberty. *Turk J Med Sci*, 43, 326-332, 2013. DOI: 10.3906/sag-1205-120

9. Smith WL, Garavito RM, Dewitt DL: Prostaglandin endoperoxide H synthases (Cyclooxygenases) -1 and -2. *J Biol Chem*, 271, 331-357, 1996. DOI: 10.1074/jbc.271.52.33157

10. Vane JR, Bakhlel YS, Botting RM: Cyclooxygenases-1 and -2. *Annu Rev Pharmacol Toxicol*, 38, 97-120, 1998. DOI: 10.1146/annurev.pharmtox.38.1.97

11. Williams CS, Dubois RN: Prostaglandin endoperoxide synthase: Why two isoforms? *Am J Physiol*, 270, 393-400, 1996.

12. Singer II, Kawka DW, Schloemann S: Cyclooxygenase-2 is induced in colonic epithelial cells in inflammatory bowel disease. *Gastroenterology*, 115, 297-306, 1998. DOI: 10.1016/S0016-5085(98)70196-9

13. Warner T, Mitchell JA: Cyclooxygenases: New forms, new inhibitors, and lessons from the clinic. *FASEB J*, 18, 790-804, 2004.

14. Wang F, Shi R, Zhao L: Expression and significance of TGF-β1 and VEGF in formation of new blood vessels after rabbit corneal suture. *Recent Adv Ophthalmol*, 28, 96-99, 2008.

15. Ohtomo K, Ebihara N, Matsuda A, Tokura T, Funaki T, Murakami A: Role of TGF-β1 in tissue eosinophilia associated with vernal kerato-conjunctivitis. *Experimental Eye Res*, 91, 748-754, 2010. DOI: 10.1016/j.exer.2010.08.025

16. Powell DW, Mifflin RC, Valentich JD, Crowe SE, Saade JI, West AB: Myofibroblasts, paracrine cells important in health and disease. *Am J Physiol*, 277, C1-C19, 1999.

17. Barnard JA, Beauchamp RD, Coffey RJ, Moses HL: Regulation of intestinal epithelial cell growth by transforming growth factor type β. *Proc Natl Acad Sci,* 86, 1578-1582, 1989.

18. Barnard JA, Warwick GJ, Gold LI: Localization of transforming growth factor β isoforms in the normal murine small intestine and colon. *Gastroenterology,* 105, 67-73, 1993.

19. Harada N, Okajima, Arai M, Kurihara H, Nagata N: Administration of capsaicin and isoflavone promotes hair growth by increasing insulin-l ike growth factor-I production in mice and in humans with alopecia. *Growth horm IGF res,* 17, 408-415, 2007.

20. Habib A, Bernard M, Lebret C, Creminon B, Esposito A, Tedgui J, Maclouf J: Regulation of the expression of cyclooxygenase-2 by nitric oxide in rat peritoneal macrophages. *J Immunol,* 158, 38-45, 1997.

21. Hoffman C: COX-2 in brain and spinal cord-implications for therepeutic use. *Curr Med Chem*, 7, 1113-1120, 2000.

22. Moran C, Morales L, Razo RS, Apolonio J, Quiroz U, Chavira R, Dominguez R: Effects of sensorial denervation induced by capsaicin injection at birth or on day three of life, on puberty, induced ovulation and pregnancy. *Life Sci*, 73, 2113-2125, 2003. DOI: 10.1016/S0024-3205(03)00598-8

23. Tütüncü S, Özfiliz N: Distribution of the vanilloid (capsaicin) receptor type 1 in the capsaicin treated rat ovaries on different sexual development periods. *Rev Méd Vét,* 162, 460-467, 2011.

24. Eccleston C: Role of psychology in pain management. *Br J Anaesth*, 87, 144-152, 2001. DOI: 10.1093/bja/87.1.144

25. Yoshimura M, Yonehara N, Ito T, Kawai Y, Tamura T: Effects of topically applied capsaicin cream on neurogenic imflammation and thermal sensitivity in rats. *Jpn J Pharmacol,* 82, 116-121, 2000.

26. Shure D, Senior RM, Griffin GL, Deuel TF: PDGF-AA homodimers are potent chemoattractants for fibro blast and neutrophils, and for monocytes activated by lymphocytes or cytokines. *Biochem Biophys Res Commun*, 186, 1510-1514, 1994. DOI: 10.1016/S0006-291X(05)81577-3

27. Dannenberg AJ, Altorki NK, Boyle JO, Dang C, Howe LR, Weskler BB, Subbaramaıah K: Cyclooxygenase-2: A pharmacological target for the prevention of cancer. *Lancet Oncol,* 2, 544-551, 2001. DOI: 10.1016/S1470-2045(01)00488-0

28. Van Rees BP, Rıstımaki A: Cyclooxygenase-2 in carcinogenesis of the gastrointestinal tract. *Scand J Gastroenterol*, 36, 897-903, 2001.

29. Flower RJ: Drugs which inhibit prostaglandin biosythesis. *Pharmacol Rev,* 26, 33-67, 1974.

30. Sinatra R: Role of COX-2 inhibitors in the evolution of acute pain management. *J Pain Symptom Manage*, 24, S18-S27, 2002. DOI: 10.1016/S0885-3924(02)00410-4

31. Yermakowa A, O'banion MK: Cyclooxygenases in the central nervous system: Implications for treatment of neurological disorders. *Curr Pharm Des*, 6, 1755-1776, 2000. DOI: 10.2174/1381612003398672

32. Harris RC, Beyer MD: Physiological regulation of cyclooxygenase-2 in the kidney. *Am J Physiol Renal Physiol,* 281, 1-11, 2001.

33. Sirois J, Sayasith K, Brown KA, Stock AE, Bouchard N, Dore M: Cyclooxygenase-2 and its role in ovulation: A 2004 account. *Human Reprod Update*, 17, 373-385, 2006. DOI: 10.1093/humupd/dmh032

34. Asano K, Lilly CM, Drazen JM: Prostaglandin G/H synthase-2 is the constitutive and dominant isoform in cultured human lung epithelial cells. *Am J Physiol*, 271, L126-L131, 1996.

35. Fornai M, Blandizzi C, Colucco R: Role of cyclooxygenase-1 and -2 in the modulation of neuromuscular functions in the distal colon of humans and mice. *Gut*, 54, 608-616, 2005.

36. O'neil GP, Ford-Hudchinson AW: Expression of mRNA for cyclo-oxygenase-1 and cyclooxygenase-2 in human tissues. *FEBS Lett*, 330, 156-160, 1993. DOI: 10.1016/0014-5793(93)80263-T

37. Robertson RP: Dominance of cyclooxygenase-2 in the regulation of pancreatic islet prostaglandin synthesis. *Diabetes*, 47, 1379-1383, 1998. DOI: 10.2337/diabetes.47.9.1379

38. Sano H, Kawahito Y, Wilder RL: Expression of cyclooxygenase-1 and -2 in human colorectal cancer. *Cancer Res*, 55, 3785-3789, 1995.

39. Yasojima K, Schwab C, Mcgeer EG, Mcgeer PL: Distribution of cyclooxygenase-1 and cyclooxygenase-2 mRNAs and proteins in human brain and peripheral organs. *Brain Res*, 830, 226-236, 1998. DOI: 10.1016/S0006-8993(99)01389-X

40. Zidar N, Odar K, Glavak D, Jer'se M, Zupanc T, Stajer D: Cyclo-oxygenase in normal human tissues - is COX-1 really a constitutive isoform, and COX-2 an inducible isoform? *J Cell Mol Med*, 13, 3753-3763, 2009. DOI: 10.1111/j.1582-4934.2008.00430.x

41. Zimmerman KC, Sarbia M, Schror K, Weber AA: Constitutive cyclooxygenase-2 expression in healthy human and rabbit gastric mucosa. *Mol Pharmacol,* 54, 536-534, 1998. DOI: 10.1124/mol.54.3.536

42. Miller DA, Lee A, Matsui Y, Chen EY, Moses HL, Derynck R: Complementary DNA cloning of the murine transforming growth factor-β3 (TGF-β3) precursor and the comparative expression of TGF-β3 and TGF-β1 messenger RNA in murine embryos and adult tissues. *Mol Endocrinol*, 3, 1926-1934, 1989. DOI: 10.1210/mend-3-12-1926

43. Fujiawara T, Stolker JM, Watanabe T, Rashid A, Longo P, Eshleman JR, Booker S, Lynch HT, Jass JR, Green JS, Kim H, Jen J, Vogelstein B, Hamilton SR: Accumulated clonal genetic alterations in familial and sporadic colorectal carcinomas with widespread instability in microsatellite sequences. *Am J Pathol,* 153, 1063-1078, 1998. DOI: 10.1016/S0002-9440(10)65651-9

44. Markowitzs D, Roberts B: Tumor suppressor activity of the TGF-beta pathway in human cancers. *Cytokine Growth Factor Rev,* 7, 93-102, 1997. DOI: 10.1016/1359-6101(96)00001-9

45. Koyama S, Podolsky DK: Differential expression of transforming growth factors α and β in rat intestinal epithelial cells. *J Clin Invest*, 83, 1768-1773, 1989. DOI: 10.1172/JCI114080

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Research Article

MHC-DRB1/DQB1 Genes Polymorphism and Its Association with Resistance to *Cystic Echinococcosis* in Chinese Merino Sheep

Hong SHEN [1,†] Zhitao WANG [1,†] Xuhai WANG [1] Yongsheng ZHANG [1]
Song JIANG [1] Xin LI [1] Bin JIA [1]

[1] College of Animal Science and Technology, Shihezi University, Shihezi, Xinjiang, CHINA
† Hong Shen and Zhitao Wang contributed equally to this article and should be considered as co-first authors

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Abstract

In this study, we used the single strand conformation polymorphism (SSCP) method to analyze the associations between polymorphisms of exon 2 of the DRB1 and DQB1 genes and Cystic *Echinococcosis* (CE) in Chinese Merino sheep. We examined 96 CE positive sheep and 115 negative sheep in this study. The results showed thatthere were 53 genotypes controlled by 22 alleles in the DRB1 gene and 47 genotypes controlled by 17 alleles in the DQB1 gene. The comparison of allele frequencies in the CE positive and negative animals revealed that DRB1 alleles k (P<0.05), q , t (P<0.01) and alleles a , l (P<0.01). These results indicate a strong association between these alleles k,q,t and CE resistance, the alleles a,l are associated with CE susceptibility. We analyzed the DRB1 genotype frequencies and found that the genotypes KK and TT (P<0.05) are associated with CE resistance, while AA, LL (P<0.01), and AL (P<0.05) were related to CE susceptibility. In DQB1 exon 2 the d and e alleles (P<0.01) were related to CE resistance, while c and k (P<0.01) were significantly related to CE susceptibility. We analyzed the DQB1 genotype frequencies and found that DD and EG (P<0.01) were associated with CE resistance, while genotypes CC, KK, and CK (P<0.01) were associated with CE susceptibility. Using haplotype analysis and artificially infection tests with *Echinococcus granulosus* (EG), we found that the DRB1-TT/DQB1-EE was a CE resistant haplotype in Chinese Merino sheep.

Keywords: *DRB1, DQB1Echinococcosis Resistance, Susceptibility*

MHC-DRB1/DQB1 Gen Polimorfizmi ve Çin Merinos Koyununda Kistik Echinococcosise Dirençle İlişkisi

Özet

Bu çalışmada tek sarmal konformasyon polimorfizm (SSCP) metodu kullanılarak DRB1 ve DQB1 genlerinin ekzon 2'leri ile Çin Merinos koyununda Kistik *Echinococcosis* (CE) polimorfizmleri arasındaki ilişki araştırıldı. Çalışmada 96 CE pozitif ve 115 negatif koyun kullanıldı. DRB1 geninde 22 allel tarafından kontrol edilen 53 genotip ile DQB1 geninde 17 allel tarafından kontrol edilen 47 genotip bulunduğu tespit edildi. CE pozitif ve negatif hayvanlarda allel frekansları karşılaştırıldığında DRB1 allel k (P<0.05), q, t (P<0.01) ve a, l (P<0.01). Elde edilen bulgular doğrultusunda CE direnci ile k, q, t allelleri arasında güçlü ilişki olduğu ve a, 1 allellerinin CE duyarlılığı ile ilgili olduğu belirlendi. DRB1 genotip frekansları analiz edildiğinde KK ve TT genotiplerinin (P<0.05) CE direnci ile ilişkili olduğu, AA, LL (P<0.01) ve AL (P<0.05)'nin ise CE duyarlılığı ile ilişkili olduğu tespit edildi. DQB1 ekzon 2'de d ve e allelleri (P<0.01) CE direnci ile ilişkili iken c ve k (P<0.01) anlamlı derecede CE duyarlılığı ile ilişkiliydi. DQB1 genotip frekansları analiz edildiğinde DD ve EG (P<0.01) CE direnci ile ilişkili iken CC, KK ve CK (P<0.01) genotipleri CE duyarlılığı ile ilişkili bulundu. Haplotip analizi ve *Echinococcus granulosus* (EG) ile deneysel enfeksiyon testi kullanılarak DRB1-TT/DQB1-EE Çin Merinos koyununda CE dirençli haplotip olarak belirlendi.

Anahtar sözcükler: *DRB1, DQB1Echinococcosis Direnç, Duyarlılık*

INTRODUCTION

The major histocompatibility complex (MHC) is a tightly linked cluster of genes and is the most highly polymorphic set of genes in vertebrate genomes. In 2001, it was proposed that MHC genes may be candidate genetic markers for disease resistance [1]. MHC genes play a central role in vertebrate immunity because they code for proteins that present peptides to T cells [2]. The immune response is triggered when MHC non-self-peptide complexes are recognized by T cells [3]. MHC class I molecules present epitopes of proteins synthesized inside the cell and initiate

İletişim (Correspondence)
+86 135 79451966
jiabin@shzu.edu.cn

CD8 cell responses [4]. The class II molecules present foreign peptides that are obtained by phagocytosis and processed within the host cells. The class II molecules initiate responses by CD4+ cells [5,6].

As in other vertebrate species, the MHC genes of *Ovis aries* (Ovar) include two major subfamilies: class I and class II genes. There is a high degree of polymorphism in the class II genes and most of the class II gene polymorphic sites are located in exon 2. The interaction between host and parasite drives a variety of biological processes [7]. Co-evolution may be mediated at the genetic level via the host recognition of parasite antigens and the consequent alteration of virulence genes [8]. In terms of immune recognition and reaction, MHC is the most important genetic element of the mammalian immune system [9,10]. A variety of studies have been performed in many fields as a result of the highly polymorphic character of the MHC genes. Currently, the research for correlation between Ovar polymorphism and disease resistance/susceptibility is mainly concentrated on class II genes [11-17]. However, many studies examining the associations between MHC and hydatidosis have focused on humans [18-22]. This experimental research content mainly investigating the associations between Ovar polymorphism and resistance or susceptibility to the hydatidosis.

Cystic *Echinococcosis* (CE) is also called hydatidosis and is a cosmopolitan zoonotic parasitic disease caused by the larval stage (metacestode stage) of the tapeworm Echinococcus granulosus (EG). The parasite cycles between canines as definitive hosts and various herbivores as intermediate hosts. In the intermediate hosts and humans, the larvae develop into hydatid cysts in various organs, including the liver and lungs. CE is associated with severe morbidity and disability, especially in pastoral areas of Northwestern China, where the overall prevalence rate of hydatidosis is 38.89-61.25% [23]. The prevalence of hydatidosis decreases livestock production and reduces human lifequality. Chinese Merino sheep is well known sheep breed for wool production, which is beneficial to local sheep husbandry. However, thisbreed is more susceptible to the hydatidosis. Thus, we investigated the causes of disease susceptibility.

In this study, we examined the association between Ovar polymorphisms and resistance or susceptibility to CE by using single strand conformation polymorphism (SSCP). This research mainly explore the correlation between genetic markers and the resistance to CE.

MATERIAL and METHODS

Ethics Statement: This research was approved by the Ethical Committee of Animal Experiments of the Institute of Zoology, Chinese Academy of Sciences. This committee does not issue a number to any animal study. All sheep care and use were conducted in strict accordance with the Animal Research Committee guidelines of the Institute of Zoology, Chinese Academy of Sciences. All surgery was performed under anesthesia, and all efforts were made to minimize suffering.

Study Areas and Sample Preparation: All of the animals included in this study were adult Chinese Merino sheep. The samples were obtained from the Yili district in Xinjiang Uygur Autonomous Region, China. This region has CE disease incidence. We used ELISA kits to divide the Chinese Merino sheep into CE negative and CE positive groups. The livers and lungs were macroscopically evaluated to confirm the presence of lesions characteristic of CE disease. Of the 211 sheep selected for this study, 96 had lesions from CE, and 115 had no lesions. Genomic DNA was extracted from 211 blood samples and stored at -20°C until analysis.

Primer Design: DRB1 gene primer synthesis references reported sequences of primers [24,25], by the Shanghai sangon biotech synthesis, the primer sequence were as follows:

OLA-ERB1 (GC): 5'-CCG GAA TTC CCG TCT CTG CAG CAC ATT TCT T-3'; HL031: 5'-TTT AAA TTC GCG CTC ACC TCG CCG CT-3'; OLA-XRBI: 5'-AGC TCG AGC GCT GCA CAG TGA AAC TC-3'.

With reference to the goat reported a DQBI MHC gene exon 2 of the results of the study [26,27]. According to the Genbank database of sheep MHC-DQB sequence accession NO. Z28523, synthetic primers DQB/FW and DQB/REV, the specific primer sequences are as follows:

DQB/FW: 5'-CCC CGC AGA GGA TTT CGT G-3'; DQB/REV: 5'-ACC TCG CCG CTG CCA GGT-3'.

Polymerase Chain Reaction Amplification: Exon 2 of DRB1 was amplified by PCR in two stages. The first round of PCR was performed with primers ERB1 and HL031. Genomic DNA (100 ng) was amplified in a total volume of 20 µl and included 1.5 mmol MgCl and 120 µmol dNTPs. Primers were added at a concentration of 0.2 mmol, and 1.5 U of Taq polymerase was used in each reaction. The reactions were performed in a thermocycler using the following conditions: one cycle of incubation for 5 min at 94°C, followed by 15 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 60 s. A final extension was performed at 72°C for 10 min. We used 3 µl of the resulting mixture and the ERB1 and XRBI primers for the second round of PCR [24]. The conditions for the second round of PCR were as follows: one cycle for 5 min at 94°C, followed by 30 cycles of 94°C for 30 s, 63°C for 30 s, and 72°C for 60 s. A final extension was performed at 72°C for 10 min.

DQB1 exon 2 was amplified with primers FW and REV [25]. The PCR was performed in 50 µl reaction volumes with 150 ng DNA, 1.5 mmol MgCl, 100 µmol dNTPs, 0.2 mmol of each primer, and 2 U Taq polymerase. The PCR

was conducted using the following cycling conditions: 5 min at 95°C, followed by 33 cycles of 94°C for 30 s, 67°C for 30 s and 72°C for 45 s; a final extension was performed at 72°C for 10 min.

Single Stranded Conformation Polymorphism: One microliter aliquots of the PCR products were mixed with 7 μl denaturing solution (95% formamide, 25 mM EDTA, 0.025% xylene-cyanole and 0.025% bromophenol blue) and then incubated at 98°C for 10 min and chilled on ice for 5 min. The denatured DNA was loaded on an 8% PAGE gel in 0.5× TBE buffer and the DNA was separated at a constant voltage of 180 V for 4 h. The gel was then stained with 0.1% silver nitrate solution. The configuration of the bands was visualized by silver staining. Samples with similar banding patterns were rearranged and run again on neighboring lanes to enable genotyping.

Cloning and Sequencing: We selected resistant susceptible alleles of the DRB1 and DQB1 genes to clone and sequence. The purified PCR products were cloned in the pGEM-T vector. The inserts were amplified and chosen for sequencing by running the amplifications on an SSCP gel. Only those clones that presented exactly the same SSCP patterns as the genomic SSCP were sequenced. At least three subclones from each individual were sequenced.

Artificial Infection Experiment: Sixteen two year-old Chinese Merino sheep tested as negative for hydatidosis by ELISA were chosen to conduct artificial infection experiments with EG. Eight sheep with the resistant haplotype were chosen as a test group. The other eight animals had haplotypes that were not associated with either CE resistance or CE susceptibility. Each sheep was fed on ten adult cestodes with fertilized egg proglottides by mouth. These sixteen sheep were bred under the same conditions.

Statistical Analysis: The allelic and genotypic frequencies were estimated with t-tests to evaluate the relationship between genotypic polymorphisms and CE infection. The chi-square test was used to analyze the relationship between the different haplotypes and CE resistance. The CE infection rates of the test group and the control group after artificial infection were compared using Fisher's exact test. The sequence alignments were performed using DNAman and Mega4 software [28].

RESULTS

PCR Amplification and SSCP Analysis: We used the DRB1 and DQB1 primers, and the amplified fragment lengths were 296 bp and 280 bp, respectively. The SSCP analysis of the amplified products was performed. Under the established conditions, 53 different SSCP patterns were detected in the DRB1 gene, which was controlled by 22 alleles. The alleles were named 'a', 'b', 'c', 'd', 'e', 'f', 'g', 'h', 'i', 'j', 'k', 'l', 'm', 'n', 'o', 'p', 'q', 'r', 's', 't', 'x', and 'y' *(Fig. 1)*. There were 47 genotypes detected in the DQB1 gene, which was controlled by 17 alleles. The alleles were named 'a', 'b', 'c', 'd', 'e', 'f', 'g', 'h', 'i', 'j', 'k', 'l', 'm', 'n', 'o', 'p', and 'q' *(Fig. 2)*.

Association between MHC Polymorphism and CE Resistance/Susceptibility: The allele frequency of exon 2 of the DRB1 gene in CE positive and CE negative animals was analyzed. The results showed that the k ($P<0.05$), q and t ($P<0.01$) alleles were significantly more common in the CE negative animals than in the CE positive animals. These results indicated that k, q and t were related to CE resistance. The frequencies of the a and l ($P<0.01$) alleles in the CE positive animals were significantly higher than in the CE negative animals. These results suggested that a and l were related to CE susceptibility *(Table1)*.

Additional analysis of the genotype frequencies found that KK and TT ($P<0.05$) were resistant to CE, while AA, LL ($P<0.01$), and AL ($P<0.05$) were susceptible to CE *(Table 2)*. The results for DQB1 exon 2 showed that d and e ($P<0.01$) were CE resistant alleles, and the c and k ($P<0.01$) alleles were susceptible to CE *(Table 3)*.

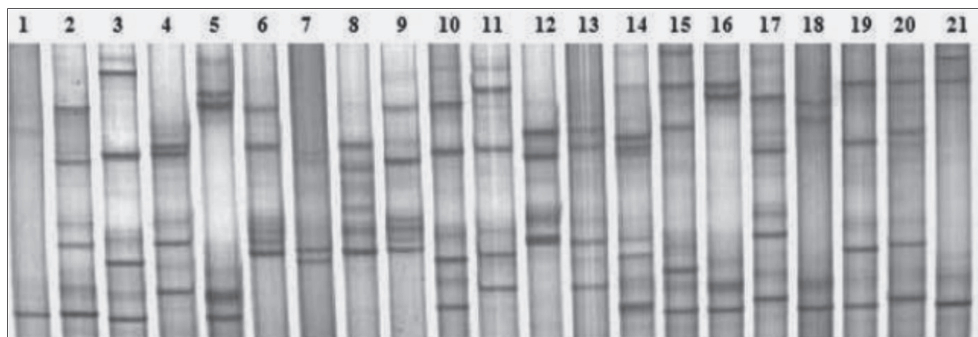

Fig 1. SSCP patterns of DRB1 exon 2 after silver staining in Chinese merino sheep. The alleles are 'a' (lane 1), 'b' (lane 2), 'l' (lane 3), 'i' (lane 4), 'q' (lane 5), 'm' (lane 6), '0' (lane 7), 'n' (lane 8), 'x' (lane 9), 'x' (lane 9), 'c' (lane 10), 'd' (lane 11), 'k' (lane 12/22), 'e' (lane 13), 'j' (lane 14), 'f' (lane 15), 'g' (lane 16), 'h' (lane 17), 's' (lane 18), 'r' (lane 19), 'y' (lane 20), 'p' (lane 21), and 't' (lane 23). The alleles k, q and t were significantly associated with CE resistance, while alleles a and l were associated with CE susceptibility

Şekil 1. Çin merinos koyununda gümüş boyama sonrasında DRB1 ekzon 2'nin SSCP görüntüsü. Alleller; 'a' (şerit 1), 'b' (şerit 2), 'l' (şerit 3), 'i' (şerit 4), 'q' (şerit 5), 'm' (şerit 6), '0' (şerit 7), 'n' (şerit 8), 'x' (şerit 9), 'x' (şerit 9), 'c' (şerit 10), 'd' (şerit 11), 'k' (şerit 12/22), 'e' (şerit 13), 'j' (şerit 14), 'f' (şerit 15), 'g' (şerit 16), 'h' (şerit 17), 's' (şerit 18), 'r' (şerit 19), 'y' (şerit 20), 'p' (şerit 21) ve 't' (şerit 23). k, q ve t CE direnci ile ilişkili iken a ve l allelleri CE duyarlılığı ile ilişkilidir

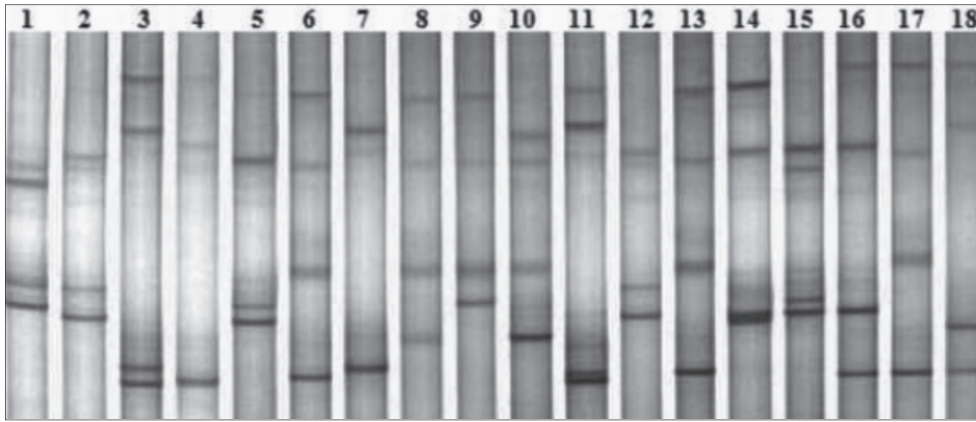

Fig 2. SSCP patterns of DQB1 exon 2 after silver staining in Chinese merino sheep. The alleles are 'd' (lane 1), 'e' (lane 2), 'a' (lane 3), 'h' (lane 4), 'l' (lane 5), 'k' (lane 6/13), 'i' (lane 7), 'p' (lane 8), 'q' (lane 9), 'o' (lane 10), 'c' (lane 11), 'n' (lane 12), 'b' (lane 14), 'j (lane 15), 'm' (lane 16), 'f' (lane 17), and 'g' (lane 18). The alleles d and e were related to CE resistance, while c and k were significantly susceptible to CE

Şekil 2. Çin merinos koyununda gümüş boyama sonrasında DQB1 ekzon 2'nin SSCP görüntüsü. Alleller; 'd' (şerit 1), 'e' (şerit 2), 'a' (şerit 3), 'h' (şerit 4), 'l' (şerit 5), 'k' (şerit 6/13), 'i' (şerit 7), 'p' (şerit 8), 'q' (şerit 9), 'o' (şerit 10), 'c' (şerit 11), 'n' (şerit 12), 'b' (şerit 14), 'j (şerit 15), 'm' (şerit 16), 'f' (şerit 17) ve 'g' (şerit 18). d ve e CE direnci ile ilişkili iken c ve k anlamlı derecede CE duyarlılığı ile ilişkilidir

***Table 1.** The allele frequency of DRB1 exon 2 in CE negative and positive sheep*
***Tablo 1.** CE negatif ve pozitif koyunlarda DRB1 ekzon 2'nin allel frekansları*

CE Negative (N = 104)			CE Positive (N = 93)		
Allele	**Number**	**Frequency**	**Allele**	**Number**	**Frequency**
a	5	0.024038	a	36	0.193548**
b	10	0.048076	b	9	0.048387
c	10	0.048076	c	15	0.080645
d	11	0.052885	d	9	0.048387
e	5	0.024038	e	6	0.032258
f	3	0.014423	f	4	0.021505
g	2	0.009615	g	4	0.021505
h	4	0.019231	h	3	0.016129
i	6	0.028846	i	2	0.010753
j	12	0.057692	j	8	0.043011
k	29	0.139423*	k	12	0.064516
l	7	0.033654	l	28	0.150538**
m	7	0.033654	m	9	0.048387
n	6	0.028846	n	2	0.010753
o	3	0.014423	o	8	0.043011
p	6	0.028846	p	2	0.010753
q	27	0.129808**	q	10	0.053763
r	8	0.038462	r	2	0.010753
s	14	0.067308	s	6	0.032258
t	23	0.110577**	t	3	0.016129
x	4	0.019231	x	4	0.021505
y	6	0.028846	y	4	0.021505

** represents P<0.05, ** represents P<0.01*

The genotypes DD and EG (P<0.01) were associated with resistance to C.E, while CC, KK, and CK (P<0.01) were associated with CE susceptibility *(Table 4)*. To efficiently analyze the polymorphisms of the DRB1/DQB1 genes and CE the relationships between different haplotypes and CE resistance/susceptibility were examined. The results found that haplotypes DRB1-TT/DQB1-EE (P<0.05) were resistant to CE, while DRB1-LL/DQB1-CK and DRB1-AA/DQB1-CC (P<0.01) were susceptible to CE *(Table 5)*.

Sequence Comparison of Resistant and Susceptible Alleles: The sequence analysis revealed that the resistant and susceptible alleles were typical multiple mutations. We compared the resistant DRB1 exon 2 alleles k, q, and t to the susceptible alleles a and l and found the alleles to be significantly different *(Fig. 3)*. To detect whether the nucleotide mutation resulted in amino acid variation, we also examined the amino acid sequence. These data are shown in *Fig. 4*.

We also found the resistant and susceptible DQB1 alleles typically included multiple mutations *(Fig. 5)*. Many of the nucleotide mutations resulted in amino acid variations *(Fig. 6)*, which may be a reason for the different alleles in CE resistance or susceptibility, as amino acid sequence changes mightlead to changes in the function of the encoded protein. It is unclear how the functional protein controls resistance or susceptibility, and further studies are required to elucidate the mechanism.

Artificial Infection Experiment: We found the DRB1-TT/DQB1-EE haplotype to be resistant to infection and this result was verified using artificial infection tests. We sacrificed 16 sheep that were artificially infected with mature EG for pathological autopsy 60 days after infection. A positive infection was determined based on visible protruding EG cysts on the liver or lung surface and the presence of hydatid sand by microscopic examination of the cyst fluid. The results showed 3 positive infections in the test group and 6 positive infections in the control group. Thus, the infection rate in the test group was significantly lower than in the control group (P<0.05). These data confirmed that DRB1-TT/DQB1-EE was a resistant haplotype in Chinese Merino sheep.

DISCUSSION

The extensive diversity at many MHC loci provides a valuable source of genetic markers for examining the relationship between host and disease resistance or

Table 2. *The genotype frequency of DRB1 exon 2 in CE negative and positive sheep*
Tablo 2. *CE negatif ve pozitif koyunlarda DRB1 ekzon 2'nin genotip frekansları*

CE Negative (N = 104)			CE Positive (N = 93)		
Genotype	**Number**	**Frequency**	**Genotype**	**Number**	**Frequency**
AA	1	0.009615	AA	12	0.129032**
BB	3	0.028846	BB	3	0.032258
CC	3	0.028846	CC	5	0.053763
DD	2	0.019231	DD	2	0.021505
EE	1	0.009615	EE	2	0.021505
FF	1	0.009615	FF	2	0.021505
GG	1	0.009615	GG	2	0.021505
HH	2	0.019231	HH	1	0.010753
II	3	0.028846	II	1	0.010753
JJ	3	0.028846	JJ	2	0.021505
KK	8	0.076923*	KK	1	0.010753
LL	1	0.009615	LL	8	0.086022**
MM	3	0.028846	MM	4	0.043011
NN	2	0.019231	NN	1	0.010753
OO	1	0.009615	OO	4	0.043011
PP	3	0.028846	PP	1	0.010753
QQ	9	0.086538	QQ	3	0.032258
RR	3	0.028846	RR	1	0.010753
SS	7	0.067308	SS	3	0.032258
TT	9	0.086538*	TT	1	0.010753
XX	2	0.019231	XX	1	0.010753
YY	3	0.028846	YY	2	0.021505
AB	1	0.009615	AB	1	0.010753
AC	1	0.009615	AC	2	0.021505
AL	1	0.009615	AL	7	0.075269*
AJ	0	0	AJ	1	0.010753
AK	0	0	AK	1	0.010753
BD	1	0.009615	BD	0	0
BK	2	0.019231	BK	2	0.021505
CD	2	0.019231	CD	0	0
CE	1	0.009615	CE	0	0
CL	0	0	CL	1	0.010753
CK	0	0	CK	2	0.021505
DL	0	0	DL	2	0.021505
DJ	0	0	DJ	1	0.010753
DK	2	0.019231	DK	2	0.021505
EK	0	0	EK	1	0.010753
ER	1	0.009615	ER	0	0
EQ	0	0	EQ	1	0.010753
FJ	1	0.009615	FJ	0	0
FR	1	0.009615	FR	0	0
KQ	4	0.038462	KQ	0	0
HQ	0	0	HQ	1	0.010753
KT	2	0.019231	KT	1	0.010753
LK	3	0.028846	LK	1	0.010753
LJ	0	0	LJ	1	0.010753
LQ	1	0.009615	LQ	0	0
MJ	1	0.009615	MJ	1	0.010753
XQ	0	0	XQ	2	0.021505
NJ	1	0.009615	NJ	0	0
NQ	1	0.009615	NQ	0	0
QJ	1	0.009615	QJ	0	0
QT	3	0.028846	QT	0	0

** represents P<0.05, ** represents P<0.01*

susceptibility [26]. Many researchers have examined genetic markers associated with resistance or susceptibility to parasites. These prior studies have improved the diagnosis and selection of desirable genotypes. Many studies have examined hydatidosis resistance/susceptibility and MHC polymorphisms. However, these studies focused on humans [19,20,29,30] and mice [31-33]. For example, Al-Ghoury determined that HLA-DR1, 8 and DR-52 are associated with resistance and that HLA-DR 16 is associated with susceptibility to EG infection in Yemeni patients [34]. Li reported that susceptibility to alveolar *Echinococcosis* (A.E) was significantly associated with HLA-DR4 and that the DR7 allele might confer protection against A.E in humans [35,36].

Table 3. *The allele frequency of DQB1 exon 2 in CE negative and positive sheep*
Tablo 3. *CE negatif ve pozitif koyunlarda DQB1 ekzon 2'nin allel frekansları*

CE Negative (N = 115)			CE Positive (N = 96)		
Allele	**Number**	**Frequency**	**Allele**	**Number**	**Frequency**
a	17	0.073913	a	13	0.067708
b	8	0.034783	b	5	0.026042
c	6	0.026087	c	45	0.234375**
d	47	0.204348**	d	18	0.093750
e	54	0.234783**	e	15	0.078125
f	10	0.043478	f	6	0.031250
g	27	0.117391	g	11	0.057292
h	13	0.056522	h	8	0.041667
i	14	0.060870	i	7	0.036458
j	4	0.017391	j	3	0.015625
k	6	0.026087	k	34	0.177083**
l	6	0.026087	l	10	0.052083
m	5	0.021739	m	6	0.031250
n	6	0.026087	n	7	0.036458
o	3	0.013043	o	2	0.010417
p	2	0.008696	p	0	0
q	2	0.008696	q	2	0.010417

*** represents P<0.01*

In this study, we found that there were many polymorphisms in DRB1 exon 2 and in DQB1 by screening genetic markers of CE resistance in Chinese Merino sheep. We also confirmed that the DRB1-TT/DQB1-EE haplotype was resistant to CE by artificial infection. Li investigated the association between the polymorphism of DRB1 exon 2 and CE resistance in Hazakh sheep. They found a strong association between DRB1 polymorphisms and CE resistance and confirmed that Mvalbc-SacIIab-Hin1Iab was the resistant haplotype of CE in Hazakh sheep [37]. These results were similar to our findings in this study. We also found that several alleles and genotypes of DRB1 exon 2 were associated with CE resistance or susceptibility in Hazakh sheep. These results indicated that alleles H and F and genotypes FF and GH exhibited a correlation with CE resistance. However, alleles K and G and the genotype KK had a significant predisposition to CE infection. Shen reported that the DQB1 gene had a significant association with resistance to CE in Dolang sheep and Chinese Merino sheep [38,39]. Similarly, Yu suggested that the DRB1 gene was associated with CE resistance in Dolang sheep [40]. These results proved that Ovar polymorphisms were associated with resistance/susceptibility to CE in sheep. However, there has been a discrepancy among different populations that might be attributable to difficulties associated with the MHC typing methods used by the majority of these investigations. Additionally, there are ethnic differences in the distribution of MHC alleles in different populations.

In vertebrates, MHC plays a central role in foreign antigen recognition and immune response to pathogens [41].

Table 4. *The genotype frequency of DQB1 exon 2 in CE negative and positive sheep*

Tablo 4. *CE negatif ve pozitif koyunlarda DQB1 ekzon 2'nin genotip frekansları*

CE Negative (N = 115)			CE Positive (N = 96)		
Genotype	**Number**	**Frequency**	**Genotype**	**Number**	**Frequency**
AA	4	0.034783	AA	3	0.031250
BB	2	0.017391	BB	1	0.010417
CC	1	0.008696	CC	12	0.125000**
DD	18	0.156522**	DD	4	0.041667
EE	11	0.095652*	EE	4	0.041667
FF	2	0.017391	FF	1	0.010417
GG	7	0.060870	GG	4	0.041667
HH	3	0.026087	HH	3	0.031250
II	3	0.026087	II	1	0.010417
JJ	2	0.017391	JJ	1	0.010417
KK	1	0.008696	KK	9	0.093750**
LL	3	0.026087	LL	4	0.041667
MM	2	0.017391	MM	3	0.031250
NN	3	0.026087	NN	3	0.031250
OO	1	0.008696	OO	1	0.010417
PP	1	0.008696	PP	0	0
QQ	1	0.008696	QQ	1	0.010417
AB	1	0.008696	AB	0	0
AC	1	0.008696	AC	4	0.041667
AD	1	0.008696**	AD	0	0
AE	4	0.034783	AE	0	0
AK	1	0.008696	AK	1	0.010417
AI	1	0.008696	AI	1	0.010417
AH	0	0	AH	1	0.010417
BC	1	0.008696	BC	2	0.020833
BE	2	0.017391	BE	0	0
BL	0	0	BL	1	0.010417
CD	0	0	CD	3	0.031250
CE	0	0	CE	2	0.020833
CF	1	0.008696	CF	1	0.010417
CK	1	0.008696	CK	9	0.093750**
DE	2	0.017391	DE	1	0.010417
DF	2	0.017391	DF	0	0
DG	1	0.008696	DG	0	0
DH	2	0.017391	DH	0	0
DI	2	0.017391	DI	2	0.020833
DM	1	0.008696	DM	0	0
DK	0	0	DK	4	0.010417
EF	3	0.026087	EF	3	0.031250
EG	11	0.095652**	EG	1	0.010417
EH	5	0.043478	EH	0	0
EI	5	0.043478	EI	0	0
GK	1	0.008696	GK	2	0.020833
OK	1	0.008696	OK	0	0
HI	0	0	HI	1	0.010417
IJ	0	0	IJ	1	0.010417
LN	0	0	LN	1	0.010417

* *represents P<0.05, ** represents P<0.01*

There may be several MHC alleles that are better suited to display antigens to certain diseases and thus generate better immunity through an improved T-cell response repertoire. However, there were many other unknown host genetic factors that could play roles in initial CE infection.

To verify whether the DRB1-TT/DQB1-EE genotypes were resistant genetic markers we used artificial infection tests. Zheng collected the cyst vesicle fluid from the diseased livers of the artificially infected sheep and then injected the fluid into healthy sheep in the peritoneum [42]. An infection model using EG was established using this method. In our study, adult cestodes with fertilized egg proglottides were fed orally to sheep. The objective was to imitate natural infection with hydatids. The result indicated that the haplotype DRB1-TT/DQB1-EE was resistant to CE Therefore, the DRB1-TT/DQB1-EE haplotype could be used as a genetic marker of CE resistance. Haplotype analysis may lead to the identification of more significant associations and improve our understanding of the role of MHC and antigens in CE resistance.In future breeding and treatment studies, greater consideration should be given to genetic markers of resistance/susceptibility.

In conclusion, the results of this study suggest that MHC polymorphisms maybe used in linkage and association research on CE resistance in Chinese Merino sheep. The identification of MHC haplotypes composed of such polymorphisms is a powerful tool for analysing the associations between MHC and immunity to infectious diseases.

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REFERENCES

1. Palti Y, Nichols KM, Waller KI, Parsons JE, Thorgaard GH: Association between DNA polymorphisms tightly linked to MHC class II genes and IHN virus resistance in backcrosses of rainbow and cutthroat trout. *Aquaculture,* 194, 283-289, 2001. DOI: 10.1016/S0044-8486(00)00526-3

2. Oppelt C, Starkloff A, Rausch P, Von Holst D, Rodel HG: Major histocompatibility complex variation and age-specific endoparasite load in subadult European rabbits. *Molecular Ecology,* 19, 4155-4167, 2010. DOI: 10.1111/j.1365-294X.2010.04766.x

3. Janeway CA, Travers P, Walport M, Shlomchik M: Immunobiology. The Immune System in Health and Disease. Grand Science, New York, 2001.

4. Rothbard JB, Gefter ML: Interactions between immunogenic peptides and MHC proteins. *Annu Rev Immunol,* 9, 527-565, 1991. DOI: 10.1146/annurev.iy.09.040191.002523

5. Lanzavecchia A: Receptor-mediated antigen uptake and its effect on antigen presentation to class II-restricted T lymphocytes. *Annu Rev Immunol*, 8, 773-793, 1990. DOI: 10.1146/annurev.immunol.8.1.773

6. Konig R, Houng LY, Germain RN: MHC class II interaction with CD4 mediated by a region analogous to the MHC class I binding site for CD8. *Nature,* 356, 796-798, 1992. DOI: 10.1038/356796a0

7. Summers K1, McKeon S, Sellars J, Keusenkothen M, Morris J: Parasitic exploitation as an engine of diversity. *Biol Rev,* 78, 639-675, 2003. DOI: 10.1017/S146479310300616X

8. Bergelson J, Kreitman M, Stahl EA, Tian D: Evolutionary dynamics of plant R-genes. *Science,* 292, 2281-2285, 2001. DOI: 10.1126/science.1061337

9. Hedrick PW, Kim TJ: Genetics of complex polymorphisms, parasites and maintenance of the major histocompatibility complex variation. **In,** Singh RS, Krimbas CB (Eds): Evolutionary Genetics: From Molecules to Morphology. 204-234, Cambridge, Cambridge University Press, 2000.

10. Penn DJ, Damijanovich K, Potts WK: MHC heterozygosity confers a selective advantage against multiple-strain infections. *Proc the Natl Acad Sci USA,* 99, 11260-11264, 2002. **DOI:** 10.1073/pnas.162006499

11. Dukkipati VSR, Blair HT, Garrick DJ, Lopez-Villalobos N, Whittington RJ: Association of microsatellite polymorphisms with immune responses to a killed *Mycobacterium avium* subsp. *paratuberculosis* vaccine in Merino sheep. *New Zealand Vet J*, 58, 237-245, 2010. DOI: 10.1080/

Table 5. *The part haplotypes in CE negative and positive sheep*

Tablo 5. *CE negatif ve pozitif koyunlarda parça haplotipler*

Haplotype	CE Negative (104)		CE Positive (93)		χ^2
	Number	Frequency	Number	Frequency	
DRB1-TT/DQB1-EE	8	0.076923	0.010753	1	4.523688*
DRB1-KK/DQB1-EG	5	0.048077	0.010753	1	1.521913
DRB1-SS/DQB1-DD	6	0.057692	0.032258	3	0.853513
DRB1-QQ/DQB1-GG	7	0.067308	0.032258	3	1.650508
DRB1-KQ/DQB1-EH	3	0.028846	0	0	1.138309
DRB1-LL/DQ1-CK	0	0	0.075269	7	6.848920**
DRB1-AA/DQB1-CC	1	0.009615	0.086022	8	6.764010**
DRB1-AL/DQB1-KK	1	0.09615	0.043011	4	1.487790
DRB1-CC/DQB1-DK	0	0	0.032258	3	1.845730

*①$\chi^2>\chi^2_{0.01\text{-}1}$ = 6.63, P<0.01, $\chi^2>\chi^2_{0.05\text{-}1}$ = 3.84, P<0.05, $\chi^2<\chi^2_{0.05\text{-}1}$ = 3.84, P>0.05; * P<0.05, ** P<0.01. ②There are only part of the haplotypes,the others because of no significant difference, so there is no list*

Fig 3. Nucleotide sequences of DRB1 exon2 in Chinese Merino sheep. BLAST results of resistant and susceptible alleles. The alleles k, q and t are resistant to CE, while the alleles a and l are susceptible to CE. Their nucleotide sequences exhibit typical multiple mutations

Şekil 3. Çin Merinos koyununda DRB1 ekzon 2'nin nükleotid sekansı. Dirençli ve duyarlı allellerin BLAST sonuçları. k, q ve t alleleri CE'ye dirençli iken a ve l allelleri CE'ye duyarlıdır. Nükleotid sekansları tipik çoklu mutasyonlar göstermektedir

Fig 4. Amino acid sequences encoded by DRB1 exon 2 in Chinese Merino sheep. Comparison result of resistant and susceptible alleles. The alleles k, q and t are resistant to CE, while a and l are susceptible to CE. The amino acid variations resulting from nucleotide mutations are shown

Şekil 4. Çin Merinos koyununda DRB1 ekzon 2 tarafından kodlanan amino asit sekansları. Dirençli ve duyarlı allellerin karşılaştırma sonuçları. k, q ve t allelleri CE'ye dirençli iken a ve l CE'ye duyarlıdır. Nükleotid mutasyonlarından kaynaklanan amino asit varyasyonları gösterilmektedir

Fig 5. Nucleotide sequences of DQB1 exon 2 in Chinese Merino sheep. BLAST result of resistant and susceptible alleles. The alleles d and e are related to CE resistance, while c and k are susceptible to CE. These nucleotide sequences also exhibit typical multiple mutations

Şekil 5. Çin Merinos koyununda DQB1 ekzon 2'nin nükleotid sekansı. Dirençli ve duyarlı allellerin BLAST sonuçları. d ve e alleleri CE'ye dirençli iken c ve k allelleri CE'ye duyarlıdır. Nükleotid sekansları tipik çoklu mutasyonlar göstermektedir

```
allele c  PRRGFRGPV.VPLLLHQRDGAGAVRDQIHLQPGGVRALRQRLGRVPRGDAAGAAASRVLEQPEGLPGADAGRGGHGVQKQLPGVCPLHLAAAR  92
allele d  ------vs-yg-v----------ece--------------------g------------q------------------t----gsr-------  93
allele e  ------vs-yg-v----------ec--v------------------g-------vr--------h-------------t----g---------  93
allele k  ---------.g-v----------ece------------------a-g--p----gr----------------------t----gsr-------  92
```

Fig 6. Amino acid sequences encoded by DQB1 exon2 in Chinese Merino sheep. Comparison result of resistant and susceptible alleles. The alleles d and e are related to CE resistance, while c and k are susceptible to CE. The nucleotide mutations change the amino acid composition

Şekil 6. Çin Merinos koyununda DQB1 ekzon 2 tarafından kodlanan amino asit sekansları. Dirençli ve duyarlı allellerin karşılaştırma sonuçları. d ve e allelleri CE'ye dirençli iken c ve k CE'ye duyarlıdır. Nükleotid mutasyonları amino asit kompozisyonunu değiştirmektedir

00480169.2010.69154

12. Dukkipati VSR, Blair HT, Garrick DJ, Murray A: Ovar-Mhc--ovine major histocompatibility complex: role in genetic resistance to diseases. *New Zealand Vet J,* 54, 153-160, 2006. DOI: 10.1080/00480169.2006.36689

13. Krawczyk A, Slota E: Genetic markers to gastrointestinal nematode resistance in sheep: A review. *Helminthologia,* 46, 3-8, 2009. DOI: 10.2478/s11687-009-0001-3

14. Stear MJ, Belch A, Donskow-Schmelter K, Fitton LA, Innocent GT: Detection of genes with moderate effects on disease resistance using ovine MHC and resistance to nematodes as an example. *Vet Immunol Immunopathol,* 120, 3-9, 2007. DOI: 10.1016/j.vetimm.2007.07.012

15. Sayers G, Good B, Hanrahan JP, Ryan M, Angles JM: Major histocompatibility complex DRB1 gene: Its role in nematode resistance in Suffolk and Texel sheep breeds. *Parasitology,* 131, 403-409, 2005. DOI: 10.1017/S0031182005007778

16. Paterson S, Wilson K, Pemberton JM: Major histocompatibility complex variation associated with juvenile survival and parasite resistance in a large unmanaged ungulate population *(Ovis aries* L.). *Proc Natl Acad Sci USA,* 95, 3714-3719, 1998. DOI: 10.1073/pnas.95.7.3714

17. Schwaiger FW, Gostomski D, Stear MJ, Duncan JL, McKellar QA: An ovine major histocompatibility complex DRB1 allele is associated with low faecal egg counts following natural, predominantly *Ostertagia circumcincta* infection. *Int J Parasitol,* 25, 815-822, 1995. DOI: 10.1016/0020-7519(94)00216-B

18. Vuitton DA, Mantion G, Bartholomot B, Giraudoux P, Bresson-Hadni S: Parasite-host relationships and treatment. *Bulletin De L Academie Nationale De Medecine Med,* 192 (6): 1103-1116, 2008.

19. Azab ME, Bishara SA, Helmy H, Oteifa NM, El-Hoseiny LM: Association of some HLA-DRB1 antigens with *Echinococcus granulosus* specific humoral immune response. *J Egyptian Soc Parasitol,* 34 (1): 183-196, 2004.

20. Azab ME, Bishara SA, Ramzy RMR, Oteifa NM, El-Hoseiny LM: The evaluation of HLA-DRB1 antigens as susceptibility markers for unilocular cystic echinococcosis in Egyptian patients. *Parasitol Res,* 92, 473-477, 2004. DOI: 10.1007/s00436-004-1073-0

21. Harraga S, Godot V, Bresson-Hadni S, Mantion G, Vuitton DA: Profile of cytokine production within the periparasitic granuloma in human alveolar echinococcosis. *Acta Tropica,* 85, 231-236, 2003. DOI: 10.1016/S0001-706X(02)00218-8

22. Godot V, Harraga S, Beurton I, Tiberghien P, Sarciron E: Resistance/susceptibility to *Echinococcus multilocularis* infection and cytokine profile in human. II. Influence of HLA B8, DR3, DQ2 haplotype. *Clin Exp Immunol,*121, 491-498, 2000. DOI: 10.1046/j.1365-2249.2000.01309.x

23. Li Y, Yan JL, Li J, Li Z, Zhang LA: Investigation and analysis of epidemic conditions of hydatid disease of sheep in Xinjiang. *J Shihezi Univ (Natural Sci),* 23, 60-63, 2005.

24. Konnai S, Nagaoka Y, Takesima S, Onuma M, Aida Y: Technical note: DNA typing for ovine MHC DRB1 using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). *J Dairy Sci,* 86, 3362-3365, 2003. DOI: 10.3168/jds.S0022-0302(03)73939-3

25. Ballingall KT, Fardoe K, McKeever DJ: Genomic organisation and allelic diversity within coding and non-coding regions of the Ovar-DRB1 locus. *Immunogenetics,* 60, 95-103, 2008. DOI: 10.1007/s00251-008-0278-2

26. Amills M, Sulas C, Sanchez A, Bertoni G, Zanoni R: Structural characterization of the caprine major histocompatibility complex class II DQB1 (Cahi-DQB1) Gene. *Mol Immunol,* 41, 843-846, 2005. DOI: 10.1016/j.molimm.2004.05.002

27. Almawi WY, Busson M, Tamim H, Al-Harbi EM, Finan RR: HLA class II profile and distribution of HLA-DRB1 and HLA-DQB1 alleles and haplotypes among Lebanese and Bahraini Arabs. *Clin Diagn Lab Immunol,* 11, 770-774, 2004. DOI: 10.1128/CDLI.11.4.770-774.2004

28. Abdallah KS, Cao Y, Wei DJ: Epidemiologic investigation of extra-intestinal pathogenic *E. coli* (ExPEC) based on PCR phylogenetic group and fimH single nucleotide polymorphisms (SNPs) in China. *Int J Mol Epidemiol Genetics,* 2 (4): 339-353, 2011.

29. Aydinli B, Pirim I, Polat KY, Gursan N, Atamanalp SS: Association between hepatic alveolar echinococcosis and frequency of human leukocyte antigen class I and II alleles in Turkish patients. *Hepatol Res,* 37, 806-810, 2004. DOI: 10.1111/j.1872-034X.2007.00137.x

30. Abdelnoor AM, Chakhtoura M, Al-Awar G: HLA associations, antibody titers and circulating immune complexes in patients with hydatid disease. *Tissue Antigens,* 69 (5): 373-532, 2007.

31. Wedekind C, Walker M, Little TJ: The separate and combined effects of MHC genotype, parasite clone, and host gender on the course of malaria in mice. *BMC Genetics,* 7, 55, 2006. DOI: 10.1186/1471-2156-7-55

32. Zhang WB, You H, Li J, Zhang ZZ, Turson G: Immunoglobulin profiles in a murine intermediate host model of resistance for *Echinococcus granulosus* infection. *Parasite Immunol,* 25, 161-168, 2003. DOI: 10.1046/j.1365-3024.2003.00622.x

33. Dai WJ, Waldvogel A, Jungi T, Stettler M, Gottstein B: Inducible nitric oxide synthase deficiency in mice increases resistance to chronic infection with *Echinococcus multilocularis. Immunology,* 108, 238-244, 2003. DOI: 10.1046/j.1365-2567.2003.01567.x

34. Al-Ghoury A-BA, El-Hamshary EE, Azazy AA, Hussein EM, Rayan HZ: HLA class II alleles: Susceptibility or resistance to cystic echinococcosis in Yemeni patients. *Parasitol Res,* 107, 355-361, 2010. DOI: 10.1007/s00436-010-1868-0

35. Li FR, Shi YE, Shi DZ, Vuitton DA, Craig PS: HLA-DRB1 allele in 35 patients with alveolar echinococcosis in Gansu province of China. *Chinese Med J (Engl),* 116 (10): 1557-1560, 2003.

36. Li RY, Jia B, Zhang WJ, Zhao ZS, Shi GQ: Analysis of the relationship between MHC-DRB1 gene polymorphism and hydatidosis in Kazakh sheep. Asian-Austral J Anim Scie, 23, 1145-1151, 2010. DOI: 10.5713/ajas.2010.90480

37. Wen QN, Jia B, Shen H, Bai S, Du YC: Relationship between polymorphism of MHC-DRB1 gene and hydatidosis resistance in Kazak sheep. *Chinese J Zoonoses,* 26 (9): 805-809, 2010.

38. Shen H, Du YC, Jia B, Chen YL, Peng LZ: Analysis of polymorphism of MHC-DQB 1 gene and resistance of hydatidosis in Dolang sheep. *Chinese J Zoonoses,* 25 (1): 17-22, 2009.

39. Shen H, Jia B, Chen YL, Du YC, Zeng XC: Polymorphism of MHC-DQB1 gene and resistance to hydatidos is in Chinese Merino sheep. *Chinese J Prev Vet Med,* 30 (9): 682-688,2008.

40. Yu ZY, Li H, Jia B, Jiang WS, Peng LZ: Analysis of polymorphism of MHC-DRB1 gene and resistance of hydatidosis in Dolang sheep. *Chinese J Anim Vet Sci,* 38 (11): 1149-1153, 2007.

41. Piertney SB, Oliver MK: The evolutionary ecology of the major histocompatibility complex. *Heredity,* 96, 7-21, 2006. DOI: 10.1038/sj.hdy.6800724

42. Zheng H, Xu ZX, Wen H: Initial establishment of sheep model with *Echinococcus granulosus* infection. *Endemic Dis Bull,* 15, 15-16, 2000.

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Research Article

Effects of Dietary Soapwort Extract Supplementation on Laying Performance, Blood Biochemical Parameters, Fatty Acid Profile of Breast Meat and Antioxidative Potential of Liver and Heart Tissues in Cold Stressed Laying Japanese Quail

Mehmet ÇİFTÇİ [1] Bestami DALKILIÇ [2] Ülkü Gülcihan ŞİMŞEK [3]
Mehmet Ali AZMAN [1] Zeki ERİŞİR [3] Mehtap ÖZÇELİK [4] Ökkeş YILMAZ [5]
Seda İFLAZOĞLU MUTLU [1] Fatma TERLEMEZ [1] Muammer BAHŞİ [6]

[1] Dept. of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Med., University of Firat, TR-23119 Elazig - TURKEY
[2] Vocational School of Technical Sciences, University of Gaziantep, TR- 27310 Gaziantep - TURKEY
[3] Department of Animal Science, Faculty of Veterinary Medicine, University of Firat, TR-23119 Elazig - TURKEY
[4] Vocational School of Health Services, University of Firat, TR-23119 Elazig - TURKEY
[5] Department of Biology, Faculty of Arts and Sciences, University of Firat, TR-23119 Elazig - TURKEY
[6] Department of Primary School Education, Faculty of Education, University of Firat, TR-23119 Elazig - TURKEY

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Abstract

The purpose of this study was to evaluate the effects of dietary Soapwort Extract (SE) supplementation on laying performance, egg quality, some offal weights, some serum parameters, antioxidant status of liver and heart tissues and fatty acid composition of breast meat in chronic cold stressed Japanese quails. A total of sixty three 45-day-old Japanese quails were divided into three groups with three replicate. One group was fed with the corn-soybean based diet alone (control group) and the others were fed with the basal diet supplemented by 50 and 100 ppm SE. Cold stress was applied every night between 22.00 to 06.00 h as 7±1°C. SE supplementation had no significant effect on laying performance, offal weights and egg quality parameters with the exception of egg and albumen weights. Serum glucose, triglyceride, uric acid and aspartate aminotransferase (AST) levels were reduced in SE-50 group. While supplementation of 100 ppm SE decreased both liver and heart MDA levels, both amounts of SE increased the liver GSH-Px enzyme activity. Supplementation of 100 ppm SE decreased the levels of oleic acid and MUFA in breast meat, and it significantly increased the linoleic, linolenic and arachidonic acid, PUFA, total omega 6 and the ratio of omega 6/omega 3. It is displayed that SE supplementation to diets of laying quails exposed to cold stress can alleviate the detrimental effects of oxidative stress with no affecting on performance parameters.

Keywords: *Antioxidant, Cold stress, Fatty acid, Laying performance, Saponin, Soapwort extract*

Soğuk Stresine Maruz Bırakılan Japon Bıldırcınlarında Diyete İlave Edilen Çöven Ekstraktının Yumurtlama Performansı, Kan Biyokimyasal Parametreleri, Göğüs Eti Yağ Asidi Profili İle Karaciğer ve Kalp Dokusu Antioksidatif Potansiyeli Üzerine Etkisi

Özet

Bu çalışmada, karma yeme ilave edilen çöven ekstraktının (SE), kronik soğuk stresine maruz bırakılan Japon bıldırcınlarında performans, yumurta kalitesi, bazı organ ağırlıkları, bazı serum parametreleri, karaciğer ve kalp dokularının antioksidan durumları ve göğüs eti yağ asidi profili üzerine etkileri araştırılmıştır. Toplam olarak 63 adet 45 günlük bıldırcın, 3 tekerrürlü 3 gruba ayrılmıştır. Gruplardan biri mısır-soya küspesine dayalı temel yem ile (kontrol grubu), diğer gruplar temel yeme 50 ve 100 ppm SE ilave edilen yemle beslenmişlerdir. Soğuk stresi her akşam 22.00-06.00 saatleri arasında kümes ısısı 7±1°C'ye düşürülerek uygulanmıştır. SE ilavesi performans, bazı organ ağırlıkları ile yumurta ve albümün ağırlığı hariç yumurta kalite parametrelerini etkilememiştir. Serum glikoz, trigliserit, ürik asit ve AST düzeyleri SE-50 grubunda düşmüştür. 100 ppm SE ilavesi karaciğer ve kalp dokularında MDA düzeylerini düşürürken, karaciğer GSH-Px enzim aktivitesi her iki SE grubunda artmıştır. 100 ppm SE ilavesi göğüs etinde oleik asit ve MUFA düzeylerini düşürürken, linoleik, linolenik ve araşidonik asit, PUFA, toplam omega 6 ile omega 6/omega 3 düzeylerini önemli ölçüde yükseltmiştir. Sonuç olarak; soğuğa maruz kalan yumurtacı bıldırcınlarda diyete ilave edilen SE'nin performans parametrelerini etkilemeden oksidatif stresin zararlı etkilerini azaltabileceği ortaya koyulmuştur.

Anahtar sözcükler: *Antioksidan, Soğuk stres, Yağ asidi, Yumurtlama performansı, Saponin, Çöven ekstraktı*

İletişim (Correspondence)
+90 424 2370000/3920
mciftci@firat.edu.tr

INTRODUCTION

During winter, the ambient temperature ranges from –5 to +5°C in many regions of the world, including Turkey. Such cold conditions negatively influence the performance of laying quails, increasing feed intake while decreasing egg production, egg weight, eggshell thickness and haugh unit [1]. Additionally, stressful conditions can provoke the creation of lipid peroxidation products especially in membranes and end up tissue damage [2].

Several plants have a worth value role in physiological and biochemical activities and reactions with the phyto-chemical contents of them. Therefore nowadays, contents of chemical matters of plants and their protective effects on disease are investigated [3]. Saponins which are found in many plants are glycosides containing a steroid or triterpenoid nucleus with one or more side chain of carbohydrates. Saponins have hemolytic effect when given intravenously. They have bitter taste and are distributed through the bark, leaves, stems, roots and flowers of the plants [4]. Recent years, the saponins containing plants and their effects on human and animal health are one of the most studied area. Several pharmacological properties are attributed to saponins such as immunological adjuvant [5], anticarcinogenic [6], hypocholesterolaemic [7], antifungal [8], anti-inflammatory and antioxidant [9,10]. Additionally, they are supplemented to poultry diets for decreasing the excrete amonium [11].

Saponin-rich and economically important taxa of *Gypsophylia sp. is a* perennial, herbaceous plant from Caryophyllaceae family which named as "Çöven" by public and are used to make "Tahini Halvah", "Foam Halvah", "Turkish Delight", "Herbal Cheese" and "Bread of Çöven", also to product detergent as fabrication, fire extinguisher, liquor, soap and to obtain commercial saponin [12,13]. These plants notified as diuretic, expectorant, acne remover are used to polish gold at jewellery sector and exported to abroad [13]. Saponins are organic compounds which are water soluble carbohydrates [14].

It is well known that use of plant materials are due to the antioxidant, antimicrobial, carminative substances content that activate the organism from the detrimental effects of stressful conditions. The effects of SE on laying performance, egg quality, carcass parameters, fatty acid profile of breast meat, some biochemical parameters and antioxidant status of liver and heart tissues of laying quails reared at low ambient temperature was studied.

MATERIAL and METHODS

Sixty three, 45-day-old and 199 g mean body weight Japanese quail *(Coturnix Coturnix Japonica)* were obtained from a commercial seller in Elazig province of Turkey. The experiment was conducted at the Poultry Unit of Veterinary Faculty of Firat University, after the local ethic committee approval (Official form date and number: 25.02.2015 and 2015/08). The quails were divided into three groups of 21 female each with three replicate. Animals were adapted to experimental conditions until they were the hen-day 5%. Cold stress was applied every night between 22.00 to 06.00 h as 7±1°C. During the day, 20±2°C thermo-neutral ambient temperature was provided. The relative humidity of the environment was 60-65%. One group was fed with the basal diet alone (control group) and the others were fed with the basal diet supplemented by 50 and 100 ppm Soapwort Extract (SE-50 and SE-100 groups respectively). Chemical composition of feed ingredients and Soapwort extract were analyzed according to the Association of Official Analytical Chemists [15] procedures and crude fiber was determined by the methods of Crampton and Maynard [16]. The carbohydrate level in the Soapwort extract was determined by the method of Lane and Eynon [17]. The metabolisable energy (ME, kcal/kg) was calculated according to Carpenter and Clegg [18] = 53+38 B formula [B= (crude protein %) + (2.25) (ether extract %) + (1.1) (starch %) + (sugar %)]. The amount of saponin within Soapwort extract was determined using the method described by Lalitha et al.[19]. The chemical composition of the SE used in the experiment is shown in *Table 1*. The quails were fed with isonitrogenic and isocaloric diets according to the National Research Council [20] recommendations are given at *Table 2*. Diets and fresh water was provided for *ad libitum*. A photoperiod of 16 h/day was maintained. All birds were kept under standard laying cages with 7 birds per cage under the same environmental conditions. The experiment was continued 42 days.

Feed intake, feed conversion ratios (FCR) of the groups were weekly determined. Egg production was daily determined and all eggs were weighted individually. Additionally, for determining the egg quality parameters, eggs were collected once a week after 2nd week of the experiment. The next day, the collected eggs were evaluated for the weights of egg, shell, albumen and egg yolk as well as shell thickness, yolk color and shape index. Egg shells were washed under tap water gently and dried in the air after 24 h and then evaluated. Approximately, 360 eggs were evaluated at each dietary treatment for egg quality parameters.

At the end of the experiment, 6 quails from each group, were randomly selected and slaughtered with decapitation in order to collect blood samples. Serum was separated and stored at -20°C until analysis. Following slaughtering, offal weights were evaluated in accordance with Institute of Turkish Standards Rules [21]. Liver, heart and spleen percentages were calculated from whole body (slaughter weight). *M. pectoralis profundus* of breast were obtained and stored (-20°C) for fatty acid composition analyses. Blood samples were centrifuged at 4.000 rpm for 10 min, the serums were separated. Serum glucose,

Table 1. *The chemical composition of the soapwort extract, %*
Tablo 1. Çöven ekstraktının kimyasal bileşeni, %

Analysis	Result, %
Dry matter	90.73
Crude protein	2.23
Ether extract	1.22
Crude cellulose	0.83
Ash	6.10
Carbohydrates	36.35
Saponin	44.00

Table 2. *Ingredients and chemical composition of standard diet*
Tablo 2. *Bazal diyetin kompozisyonu ve bileşimi*

Feed Ingredients (g/kg)		Chemical Composition (g/kg)	
Maize	528.0	Dry matter	907.0
Soybean meal (48% CP)	190.0	Crude protein	200.0
Sunflower meal (36% CP)	115.0	Ether extract	63.0
Fullfat soybean	60.0	Crude fiber	52.5
Vegetable oil	34.0	Crude ash	92.2
Dicalcium phosphate	15.10	Sugar	49.0
Calcium carbonate	53.3	Starch	335.0
Salt	2.4	Calcium**	25.0
Sodium bicarbonate	1.0	Available Phosphorus**	8.4
DL-Methionine	0.1	Sodium**	1.8
L-Lysine	0.5	Methionine+Cystine**	7.0
L-Threonine	0.3	Lysine**	10.0
Vitamin-Mineral Premix*	0.3	Threonine**	7.7
		Tryptophan**	2.5
		ME, kcal/kg***	2938
Total	1000.0		

** Provided per kg of diet: retinol, 2.64 mg; cholecalciferol, 0.04 mg; dl-a-tocopherol-acetate, 11 mg; riboflavin, 9.0 mg; pantothenic acid, 11.0 mg; vitamin B_{12}, 0.013 mg; niacin, 26 mg; choline, 900 mg; vitamin K, 1.5 mg; folic acid, 1.5 mg; biotin, 0.25 mg; iron, 30 mg; zinc, 40 mg; manganese, 60 mg; copper, 8 mg; selenium, 0.2 mg; ** Calculated; **** Calculated, ME (kcal/kg) = 53+38 B used formula. B= (Crude protein, %) + (2.25) (Ether extract, %) + (1.1) (Starch, %) + (Sugar, %)

triglyceride, total, LDL and HDL cholesterol, uric acid and AST concentrations were measured using a biochemical analyzer (Olimpus AU-600) at University of Firat, Faculty of Medicine, Department of Biochemistry. Malondialdehyde (MDA) level of liver and heart was spectrophotometrically measured with the procedures described by Placer et al.[22]. SOD activity of liver and heart was measured using xanthine and xanthine oxidases to generate superoxide radicals which react with nitroblue tetrazolium (NBT) by the methods of Sun et al.[23]. The GSH-Px activity was determined according to Lawrence and Burk [24]. The GSH content of the liver and heart was measured at 412 nm by the method of Sedlak and Lindsay [25].

Extraction of lipids from the breast meat was performed according to the method of Hara and Radin [26]. For the preparation of methyl esters, lipid extract in a hexane: isopropanol phase was placed in 30-mL experiment tubes. Five milliliters of 2% methanolic sulfuric acid was added and the mixture was vortexed. This mixture was left to methylate in a 50°C incubation for 15 h. It was then cooled at room temperature, and 5 mL of 5% sodium chloride was added and mixed. The fatty acid methyl esters that were produced were extracted with 5 mL of hexane. The hexane phase was then removed with a pipette and treated with 5 mL of 2% $KHCO_3$. The solvent in the methyl ester-containing mixture was evaporated at 45°C with a nitrogen flow and dissolved with 1 mL of hexane. All the mixture containing the solvent and hexane was then placed in 2- mL closed autosampler vials and analyzed [27].

All data were analyzed by analysis of variance procedures and significant differences were further subjected to Tukey HSD multiple range tests by using SPSS 11.5 for Windows [28]. The results were considered as significant when P<0.05, P<0.01 and P<0.001.

RESULTS

Supplementation of 50 ppm SE significantly decreased (P<0.05) egg weight in fourth week and 100 ppm SE significantly decreased the feed intake in third week when compared with control group *(Table 3)*. Addition of 100 ppm SE caused significant reductions in egg weight (P<0.01) and albumen weight (P<0.001) when compared with control group *(Table 4)*. Soapwort supplementation had no effect on the offal weights and percentages *(Table 5)*.

Serum total cholesterol, HDL and LDL cholesterol levels were similar among groups *(Table 6)*. However, dietary supplementation of 50 ppm SE reduced the serum glucose, triglyceride, uric acid and AST levels (P<0.05) in comparison with the control group. In addition, 100 ppm SE significantly reduced the only AST level when compared with the control group.

The essential effect of SE was seen on antioxidant status of liver and heart tissue as shown at *Table 7*. The supplementation of 100 ppm SE significantly decreased both liver and heart MDA levels (P<0.05), and both amounts increased the liver GSH-Px enzyme activity (P<0.01) as compared with control group.

The other essential data was obtained on fatty acid profile of breast meat of quails subjected to cold stress as shown at *Table 8*. The essential fatty acid deposition was significantly increased by SE supplementation. Although oleic acid (C18:1 ω9) and MUFA were decreased, linoleic acid (C18:2 ω6), alfa-linolenic acid (C18:3 ω3) and arachidonic acid (C20:4 ω6) which called essential fatty acids, PUFA, total omega 6 and total omega 6/omega 3 were increased by 100 ppm SE supplementation.

Table 3. *Effect of soapwort extract supplementation on laying performance of laying quails reared under low ambient condition*

Tablo 3. Çöven ekstraktı ilavesinin soğuk şartlarda yetiştirilen yumurtacı bıldırcınlarda yumurtlama performansına etkisi

Weeks	Control	SE-50	SE-100	SEM	P
Egg Weight, g					
1	11.27	11.60	10.72	0.37	NS
2	12.29	12.33	11.53	0.34	NS
3	12.56	11.80	11.99	0.20	NS
4	14.01^{a}	12.90^{b}	13.33^{ab}	0.21	*
5	13.88	13.34	12.92	0.20	NS
6	14.04	13.83	13.44	0.14	NS
0-6	13.01	12.63	12.32	0.17	NS
Feed Intake, g/bird/day					
1	31.82	28.77	30.06	0.88	NS
2	35.03	33.84	32.31	1.15	NS
3	41.42^{a}	37.84^{ab}	34.79^{b}	1.24	*
4	37.62	33.77	34.45	1.57	NS
5	38.53	37.67	34.79	1.22	NS
6	39.92	41.05	40.04	0.55	NS
0-6	37.39	35.49	34.41	0.86	NS
Egg Production, %					
1	29.36	32.53	30.16	1.22	NS
2	36.05	40.81	40.13	3.43	NS
3	61.79	57.82	60.88	5.45	NS
4	68.48	67.12	70.06	4.14	NS
5	74.49	77.32	81.63	2.90	NS
6	82.31	84.69	92.51	2.92	NS
0-6	58.75	60.05	62.56	2.90	NS
Feed Conversion Ratio, g feed intake x female number/egg production x egg weight					
1	9.61	7.62	9.29	0.53	NS
2	7.90	6.72	6.98	0.58	NS
3	5.33	5.54	4.76	0.53	NS
4	3.92	3.90	3.68	0.21	NS
5	3.71	3.65	3.29	0.15	NS
6	3.45	3.50	3.21	0.09	NS
0-6	4.89	4.67	4.46	0.17	NS

SE-50: *Supplemented 50 ppm Soapwort Extract (SE);* ***SE-100:*** *Supplemented 100 ppm SE;* ***P:*** *Statistical significance,* ***SEM:*** *Standard error mean,* ***NS:*** *No significant, * P<0.05, ^{a,b} Mean values with different superscripts within a row differ significantly*

Table 4. *Effect of soapwort extract supplementation on egg quality collected once a week for the last four weeks of laying quails reared under low ambient condition*

Tablo 4. Çöven ekstraktı ilavesinin soğuk şartlarda yetiştirilen yumurtacı bıldırcınlarda denemenin son dört haftası boyunca haftada bir kez toplanan yumurtaların kalitesine etkisi

Weeks	Control	SE-50	SE-100	SEM	P
Examined egg weight, g	13.67^{a}	13.39^{ab}	13.17^{b}	0.06	**
Shell weight, g	1.07	1.05	1.04	0.01	NS
Shell thickness, mm	2.64	2.57	2.63	0.01	NS
Yolk color	9.43	9.41	9.69	0.05	NS
Egg yolk weight, g	4.27	4.15	4.17	0.03	NS
Albumen weight, g	7.01^{a}	6.98^{a}	6.68^{b}	0.03	***
Shape index, %	77.15	77.62	76.69	0.24	NS

SE-50: *Supplemented 50 ppm Soapwort Extract (SE);* ***SE-100:*** *Supplemented 100 ppm SE;* ***P:*** *Statistical significance,* ***SEM:*** *Standard error mean,* ***NS:*** *No significant, ** P<0.01, *** P<0.001, ^{a,b} Mean values with different superscripts within a row differ significantly*

Table 5. *Effect of soapwort extract supplementation on some offal weights and percentages of laying quails reared under low ambient condition*

Tablo 5. Çöven ekstraktı ilavesinin soğuk şartlarda yetiştirilen yumurtacı bıldırcınların bazı organ ağırlıklarına ve oranlarına etkisi

Traits	Control	SE-50	SE-100	SEM	P
Slaughter Weight, g	268.00	260.33	260.00	2.52	NS
Liver Weight, g	7.71	8.61	7.58	0.28	NS
Liver Ratio, %	2.87	3.30	2.91	0.10	NS
Heart Weight, g	2.23	2.06	2.28	0.08	NS
Heart Ratio, %	0.83	0.79	0.88	0.03	NS
Spleen Weight, g	0.12	0.12	0.12	0.01	NS
Spleen Ratio, %	0.04	0.04	0.05	0.00	NS

SE-50: *Supplemented 50 ppm Soapwort Extract (SE);* ***SE-100:*** *Supplemented 100 ppm SE*
P: *Statistical significance,* ***SEM:*** *Standard error mean,* ***NS:*** *No significant*

DISCUSSION

Previously, saponin-rich plant species are recognized as antinutritional feed which should be processed before using as a feed supplement [29]. Nowadays saponins are considered as health beneficial natural food components due to their anticarcinogenic [6], hypocholesterolemic [7,30], hepatoprotective adjuvant [4,5], antiviral, antifungal [8], antiinflamatory and antioxidant [9,10] properties which are shown *in vitro* and *in vivo* animal tests. Saponins are used as a feed supplement in livestock for reducing the emission of ammonia from animal excreta due to their surfactant activity [11]. Preliminary studies have shown that saponin supplements have variable effects on performance of studied animals. Rowland et al.[11] have obtained that supplementing 31 and 155 ppm saponin yucca powder to the diets of laying hens increased the egg production performance and lowered the house ammonia amount while supplementing 465 ppm decreased egg production performance. Likewise, Aslan et al.[31] reported that dietary supplementation of laying hens by 100 ppm Deodorase induced positive effects on egg production, enhanced antioxidant capacities and decreased glycemia and cholesterolemia. However, Kutlu et al.[30] have obtained that supplementing 30, 60 and 120 ppm saponin yucca extract into the laying hen diets had no effects on feed intake, egg production rate, feed conversion ratio, daily body

Table 6. *Effect of soapwort extract supplementation on some serum metabolites of laying quails reared under low ambient condition*

Tablo 6. Çöven ekstraktı ilavesinin soğuk şartlarda yetiştirilen yumurtacı bıldırcınlarda *bazı serum metabolitlerine etkisi*

Traits	Control	SE-50	SE-100	SEM	P
Glucose, mg/dL	251.00^{a}	198.67^{b}	243.67^{a}	8.79	*
Triglyceride, mg/dL	107.67^{a}	77.00^{b}	97.83^{ab}	5.06	*
Total Cholesterol, mg/dL	176.00	182.17	173.67	5.07	NS
HDL Cholesterol, mg/dL	90.83	103.83	92.83	6.77	NS
LDL Cholesterol, mg/dL	66.67	56.33	62.50	2.15	NS
Uric Acid, mg/dL	5.10^{a}	3.76^{b}	4.80^{ab}	0.33	*
AST, U/L	272.50^{a}	212.50^{b}	222.83^{b}	8.18	*

SE-50: *Supplemented 50 ppm Soapwort Extract (SE);* ***SE-100:*** *Supplemented 100 ppm SE;* ***AST:*** *Aspartate aminotransferase;* ***P:*** *Statistical significance,* ***SEM:*** *Standard error mean, * P<0.05, a,b Mean values with different superscripts within a row differ significantly*

Table 7. *Effect of soapwort extract supplementation on antioxidant status of liver and heart tissues in laying quails reared under low ambient condition*

Tablo 7. Çöven ekstraktı ilavesinin soğuk şartlarda yetiştirilen yumurtacı bıldırcınlarda *karaciğer ve kalp dokusu antioksidan durumuna etkisi*

Traits	Control	SE-50	SE-100	SEM	P
MDA (nmol/g protein)					
Liver	4.92^{a}	4.12^{ab}	3.43^{b}	0.23	*
Heart	4.59^{a}	3.62^{ab}	2.46^{b}	0.31	*
GSH (nmol/g protein)					
Liver	0.07	0.09	0.07	0.00	NS
Heart	0.10	0.10	0.10	0.00	NS
GSH-Px (U/g protein)					
Liver	0.09^{b}	0.11^{a}	0.12^{a}	0.01	**
Heart	0.12	0.13	0.12	0.00	NS
SOD (U/g protein/mL)					
Liver	30.02	28.97	28.09	0.83	NS
Heart	70.04	66.88	68.43	1.86	NS

SE-50: *Supplemented 50 ppm Soapwort Extract (SE);* ***SE-100:*** *Supplemented 100 ppm SE;* ***MDA:*** *Malondialdehyde;* ***GSH:*** *Glutatione;* ***GSH-Px:*** *Glutathione peroxidase;* ***SOD:*** *Superoxide dismutase;* ***P:*** *Statistical significance,* ***SEM:*** *Standard error mean,* ***NS:*** *No significant,* P<0.05, ** P<0.01, a,b Mean values with different superscripts within a row differ significantly*

Table 8. *Effect of soapwort extract supplementation on fatty acid profile of breast meat in laying quails reared under low ambient condition*

Tablo 8. Çöven ekstraktı ilavesinin soğuk şartlarda yetiştirilen yumurtacı bıldırcınlarda *göğüs eti yağ asidi profiline etkisi*

Fatty Acids	Control	SE-50	SE-100	SEM	P
C16:0	21.74	20.97	19.30	0.55	NS
C16:1 ω7	3.27	2.71	3.16	0.18	NS
C18:0	17.29	17.67	18.43	0.83	NS
C18:1 ω9	33.15^{a}	35.01^{a}	27.52^{b}	1.34	*
C18:2 ω6	16.49^{b}	16.09^{b}	20.18^{a}	0.57	*
C18:3 ω3	0.35^{b}	0.31^{b}	0.49^{a}	0.03	*
C18:3 ω6	0.95	0.88	0.91	0.03	NS
C20:4 ω6	3.85^{b}	3.44^{b}	6.92^{a}	0.53	*
C24:1	0.55	0.57	0.66	0.07	NS
C22:6 ω3	2.36	2.35	2.43	0.25	NS
ΣSFA	39.03	38.64	37.73	0.35	NS
ΣMUFA	36.97^{a}	38.29^{a}	31.34^{b}	0.35	***
ΣPUFA	24.00^{b}	23.07^{b}	30.93^{a}	1.05	***
Σ ω-6	21.29^{b}	20.41^{b}	28.01^{a}	0.90	***
Σ ω-3	2.71	2.66	2.92	0.14	NS
Σ ω-6/ω-3	7.86^{b}	7.67^{b}	9.59^{a}	0.50	*

SE-50: *Supplemented 50 ppm Soapwort Extract (SE);* ***SE-100:*** *Supplemented 100 ppm SE;* ***C16:0:*** *Palmitic acid;* ***C16:1 ω7:*** *Palmitoleic acid;* ***C18:0:*** *Stearic acid;* ***C18:1 ω9:*** *Oleic acid;* ***C18:2 ω6:*** *Linoleic acid;* ***C18:3 ω3:*** *α-Linolenic acid;* ***C18:3 ω6:*** *γ-Linolenic acid;* ***C20:4 ω6:*** *Arachidonic acid;* ***C24:1 ω9:*** *Nervonic acid;* ***C22:6 ω3:*** *Docosa hexaenoic acid;* ***SFA:*** *Saturated Fatty Acid;* ***MUFA:*** *Monounsaturated Fatty Acid;* ***PUFA:*** *Polyunsaturated Fatty Acid;* ***P:*** *Statistical significance,* ***SEM:*** *Standard error mean,* ***NS:*** *No significant, * P<0.05, *** P<0.001 a,b Mean values with different superscripts within a row differ significantly*

weight gain, white and yolk weights, shell thickness and shape index but reduced yolk cholesterol content in a dose related. In the present study SE supplementation had no effect on performance parameters except reductions in feed intake at the third week and egg weight at the fourth week. The reduced egg weights within four week may depend on reduced feed intake seen at the previous week. The exciting data was obtained from the collected eggs for determining the external and internal egg quality parameters through the last four week of the experiment. Addition of 100 ppm SE caused significant reductions in egg weight and albumen weight when compared with control group. There were no differences in examined offal weights and percentages in the present study. Similarly to present study, in some studies carried out with saponin extract supplementation to livestock diets indicated no differences were determined in performance parameters such as feed intake, growth or laying performance while in some studies either adverse effects or positive effects were observed. So it can be concluded that variable effects may be seen by saponin feeding experiments. Heywang and Bird [32] and Anderson [33] who reported that supplementing alfalfa saponin over 0.15% and 0.1%, respectively to diets of broilers had negative effects on growth performance. In the same study of Anderson [33] reported that 0.3% saponins to layer hens diet caused a reduction on egg production but this reduction was temporary and by the time egg production has returned to the normal/or higher levels. Similarly, in laying hens, Heywang et al.[34] obtained that adding 0.29 and 0.40% saponins from alfalfa to diets caused a reduction on feed intake and egg production without any effect on egg weight. In another study with growing chicks, Jenkins and Atwal [35] reported that supplementing different saponin sources with different amounts as 0.1% to 0.9%, they obtained that gypsophila saponins and quillaja saponins reduced growth

performance and feed consumption but sarsaponin had no effect on same parameters. Whitehead et al.[36] reported that the effect of supplementing saponin to diets on the performance of laying hens was dose dependent and no effects were seen with 0.1% saponin while negative effects were seen with 0.4-0.5% saponin on performance and liver lipid concentrations, and egg production rate. Cheeke et al.[37] reported that the depressed feed intake by the bitter taste of saponin is caused the negative effects on performance. Additionally, saponins has interaction with some vitamins and trace minerals such as zinc and this influences the metabolic and digestive enzymes [35,38,39].

In the present study, serum total cholesterol, HDL and LDL cholesterol levels were similar among groups. However, dietary supplementation of 50 ppm SE reduced the serum glucose, triglyceride, uric acid and AST levels in comparison with the control group. In addition, 100 ppm SE significantly reduced the only AST level when compared with the control group. Inconsistent with the present study, the lowering ability of saponins on serum cholesterol has also been reported for both human and animals [7,39,40]. Consistent with this study, Yang et al.[41] observed that the green tea saponins had no effect on the serum cholesterol levels of broilers. Also, Whitehead et al.[36] reported that dietary saponins reduced the liver cholesterol and plasma triglyceride levels without any effect on plasma cholesterol (HDL, LDL, total) levels in broilers. Similarly to our results, in a study conducted in rats, steroidal saponins contained Yucca schidigera supplementation reduced the plasma triglyceride, uric acid and glucose levels [42]. Increased serum uric acid, AST, glucose levels of control group in the present study could be due to cold conditions. Under stress condition, some metabolic alterations occur because of the subsequent secretion of corticosterone (CS). CS promotes gluconeogenesis for endogenous glucose production from glycogen stores, or by synthesis *in vivo* from gluconeogenic precursors such as amino acids and the others. Increasing serum glucose level of the quail under cold condition may be associated with effects of CS on carbohydrate metabolism [43]. On the other hand, CS induces the catabolism of structural protein to free amino acids. This action causes to uric acid excretion [44]. Prolonged stress also induces lipolysis and causes to increase in free fatty acid concentration and serum triglycerides [43]. Increased triglyceride level of the study could be clarified with this metabolism. Likewise, thermal stress influences blood chemistry values of northern Bobwhite quails [45].

The body also has the regular antioxidant system, but additional exogenous antioxidants have synergetic and beneficial effects to body defense system. Most of the plant bioactive compounds have antioxidant properties. Soapwort saponins have *in vitro* antioxidant activity also proved in a study carried out by Arslan and Celik [9]. Kucukkurt et al.[46] reported that saponin contained *Agrostemma githago* L. and *Saponaria officinalis* L. extracts enhanced the antioxidant status and decreased the incidence of lipid peroxidation in blood samples of rats exposed to X-radiation. Sur et al.[10] investigated that antioxidant mechanism of tea saponins was occurred by xanthine and xanthine oxidase pathway in rats. Consistent with above findings, the supplementation of 100 ppm SE significantly decreased both liver and heart MDA levels, and both amounts increased the liver GSH-Px enzyme activity as compared with control group.

It is well known that poultry meat is particularly preferred due to its relatively high content of polyunsaturated fatty acids with its low content of cholesterol, but meat chemical compositions differ among poultry species and influence by nutritional factors [41]. In the present study the essential fatty acid deposition of breast meat was significantly increased by 100 ppm SE supplementation. It is the worth value that arachidonic acid, alfa-linolenic acid and linoleic acid ratio of breast meat were 1.8, 1.4 and 1.2 fold much more deposited respectively in SE 100 group than control group. Although oleic acid (C18:1 ω9) and MUFA were decreased, PUFA, total omega 6 and total omega 6/omega 3 were increased by 100 ppm SE supplementation. The fat metabolism altering effects of saponins from different sources were previously studied. Recently, Rohaida et al.[47] reported that saponin contained Candle Nut Kernel Meal supplementation to broiler diets at the rate of 2% were increased linolenic acid (LNA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DPA) of breast meat, whereas linoleic acid (LA) and the ratio of ω-6:ω-3 fatty acids were decreased when compared to control. Supporting to our results, in a lamb study, Brogna et al.[48] observed that supplementing Quillaja saponin increased arachidonic acid (C20:4 ω6) and ω-3:ω-6 (LNA/LA) level of longissimus dorsi muscle but they did not observe any difference on SFA, MUFA and PUFA levels. Afrose et al.[40] also, reported that Karaya saponin supplementation reduced saturated fatty acid levels due to the decrease in palmitic (C16:0) and stearic (C18:0) acids levels in the thigh and breast muscle of broilers. However they observed the total MUFA as well as PUFA were improved in response to treatment with Karaya saponin was primarily caused by the increase in oleic (C18:1) and linoleic (C18:1) acids levels in thigh and breast muscle. The researches connected these results that adding karaya saponin to the diet of broilers, caused a significant reduction in the content of cholesterol and triglycerides in the broiler meat.

The results of this study demonstrated that both amounts of SE supplementation, especially 100 ppm, to diets of laying quails exposed to cold stress prevented the detrimental effects of oxidative stress without any negative effect on performance. Furthermore, SE supplementation increased the essential fatty acid deposition of breast meat and the altering effect of SE on fatty acid profile of deposited fats may be needed to a detailed study in unstressed birds.

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REFERENCES

1. Sahin N, Sahin K, Onderci M: Vitamin E and selenium supplementation to alleviate cold-stress-associated deterioration in egg quality and egg yolk mineral concentrations of Japanese quails. *Biol Trace Elem Res,* 96, 179-189, 2003. DOI: 10.1385/BTER:96:1-3:179

2. Kovacs P, Juranek I, Stankovicova T, Svec P: Lipid peroxidation during acute stress. *Pharmazie*, 51, 51-53 1996.

3. Wallace RJ, Oleszek W, Franz C, Hahn I, Baser KH, Mathe A, Teichmann K: Dietary plant bioactives for poultry health and productivity. *Br Poult Sci*, 51, 461-487, 2010. DOI: 10.1080/00071668.2010.506908

4. Moghimipour E, Handali S: Saponin: Properties, methods of evaluation and applications. *Ann Res Rev Biol,* 5, 207-220, 2015. DOI: 10.9734/ARRB/2015/11674

5. Fleck JD, Kauffmann C, Spilki F, Lencina CL, Roehe PM, Gosmann G: Adjuvant activity of *Quillaja brasiliensis* saponins on the immune responses to bovine herpesvirus type 1 in mice. *Vaccine*, 24, 7129-7134, 2006. DOI: 10.1016/j.vaccine.2006.06.059

6. Xiao X, Bai P, Bui Nguyen TM, Xiao J, Liu S, Yang G, Hu L, Chen X, Zhang X, Liu J, Wang H: The antitumoral effect of Paris saponin I associated with the induction of apoptosis through the mitochondrial pathway. *Mol Cancer Ther,* 8, 1179-1188, 2009. DOI: 10.1158/1535-7163.MCT-08-0939

7. Sidhu GS, Oakenfull DG: A mechanism for the hypocholesterolaemic activity of saponins. *Br J Nutr*, 55, 643-649, 1986. DOI: 10.1079/BJN19860070

8. Coleman JJ, Okoli I, Tegos GP, Holson EB, Wagner FF, Hamblin MR, Mylonakis E: Characterization of plant-derived saponin natural products against *Candida albicans. ACS Chem Biol,* 5, 321-332, 2010. DOI: 10.1021/cb900243b

9. Arslan I, Celik A: Saponin rich fractions (SRPs) from soapwort show antioxidant and hemolytic activity. *APCBEE Procedia*, 7, 103-108, 2013. DOI: 10.1016/j.apcbee.2013.08.019

10. Sur P, Chaudhuri T, Vedasiromoni JR, Gomes A, Ganguly DK: Antiinflammatory and antioxidant property of saponins of tea (*Camellia sinensis* (L) O. Kuntze) root extract. *Phytother Res*, 15, 174-176, 2001. DOI: 10.1002/ptr.696

11. Rowland LO, Pleyler JE, Bradley JW: *Yucca schidigera* extract effect on egg production and house ammonia levels. *Poult Sci,* 55, 2086-2093, 1976.

12. Baytop T: Türkiye'de bitkiler ile tedavi, geçmişte ve bugün. İstanbul Üniversitesi Yayınları, No: 3255, Eczacılık Fakültesi Yayınları, No: 40, 520s. İstanbul, 1984.

13. Özçelik H, Yıldırım B: Türkiye çövenlerinin (Gypsophila L. ve Ankyropetalum Fenzl spp.) ekonomik önemi, kullanım olanakları ve korunması üzerine düşünceler. *SDU Fac Forestry J,* 12, 57-61, 2011.

14. Cheeke PR: Actual and potential applications of *Yucca schidigera* and *Quillaja saponaria* saponins in human and animal nutrition. *J Anim Sci,* 77, 1-10, 2000. DOI: 10.1007/978-94-015-9339-7_25

15. AOAC: Official Methods of Analysis Association of AOAC International. 17th ed., AOAC International Maryland, 2000.

16. Crampton EW, Maynard LA: The relation of cellulose and lignin content to nutritive value of animal feeds. *J Nutr,* 15, 383-395, 1983.

17. Lane JH, Eynon L: Determination of reducing sugars by means of Fehling's solution with methylene blue as internal indicator. *J Soc Chem Ind Trans,* 42, 32-36, 1923.

18. Carpenter KJ, Clegg KM: The metabolizable energy of poultry feding stuffs in relation to their chemical composition. *J Sci Food Agric,* 7, 45-51, 1956. DOI: 10.1002/jsfa.2740070109

19. Lalitha T, Seshadri R, Venkataraman LV: Isolation and properties of saponins from Madhuca butyracea seeds. *J Agric Food Chem,* 35, 744-748, 1987. DOI: 10.1021/jf00077a024

20. National Research Council: Nutrient Requirements of Poultry, 9th ed., National Academic Press, Washington, DC, 1994.

21. Anonymous: Poultry carcass-rules for carcass dissecting. Institute of Turkish Standards, TS 5890, Ankara, Turkey, ICS 67.120.10; 67.120.20, 2009.

22. Placer AZ, Linda LC, Johnson B: Estimation of product lipid peroxidation (malonyldialdehyde) in biochemical systems. *Anal Biochem,* 16, 359-364, 1966. DOI: 10.1016/0003-2697(66)90167-9

23. Sun Y, Oberley LW, Li Y: A simple method for clinical assay of superoxide dismutase. *Clin Chem*, 34, 497-500, 1988.

24. Lawrence RA, Burk RF: Glutathione peroxidase activity in selenium-deficient rat liver. *Bioch Bioph Res Comm,* 71, 952-958, 1976. DOI: 10.1016/0006-291X(76)90747-6

25. Sedlak J, Lindsay RH: Estimation of total protein bound and non-protein sulfhydryl groups in tissue with Ellman reagent. *Anal Biochem,* 25, 192-205, 1968. DOI: 10.1016/0003-2697(68)90092-4

26. Hara AR, Radin NS: Lipid extraction of tissues with a low-toxicity solvent. *Anal Biochem*, 90, 420-426, 1978. DOI: 10.1016/0003-2697(78)90046-5

27. Christie WW: Gas chromatography and lipids. The Oil Press, Glaskow, 302, 1992.

28. SPSS Inc: SPSS for Windows Release 11.5 (6 Sep. 2002), Standard Version, Copyright SPSS Inc, Chicago, 2002.

29. Hostettmann K, Marston A: Saponins. Cambridge: Cambridge University Press, UK, 1995.

30. Kutlu HR, Gorgulu M, Unsal I: Effects of dietary *Yucca schidigera* powder on performance and egg cholesterol content of laying hens. *J Appl Anim Res*, 20, 49-56, 2001. DOI: 10.1080/09712119.2001.9706736

31. Aslan R, Dundar Y, Eryavuz A, Bulbul A, Kucukkurt I, Fidan AF, Akinci Z: Effects of different dietary levels of *Yucca shidigera* powder (Deodorase) added to diets on performance, some hemotological and biochemical blood parameters and total antioxidant capacity of laying hens. *Rev Med Vet*, 156, 350-355, 2005.

32. Heywang BW, Bird HR: The effect of alfalfa saponin on the growth, diet consumption, and efficiency of diet utilization of chicks. *Poult Sci*, 33, 239-241, 1954. DOI: 10.3382/ps.0330239

33. Anderson JO: Effect of alfalfa saponin on the performance of chicks and laying hens. *Poult Sci,* 36, 873-876, 1957. DOI: 10.3382/ps.0360873

34. Heywang BW, Thompson CR, Kemmerer AR: Effect of alfalfa saponin on laying chickens. *Poult Sci*, 38, 968-971, 1959. DOI: 10.3382/ps.0380968

35. Jenkins KJ, Atwal AS: Effects of dietary saponins on fecal bile acids and neutral sterols, and availability of vitamins A and E in the chick. *J Nutr Biochem,* 5, 134-135, 1994. DOI: 10.1016/0955-2863(94)90084-1

36. Whitehead CC, Mcnab JM, Griffin HD: The effects of low dietary concentrations of saponin on liver lipid accumulation and performance in laying hens. *Br Poult Sci,* 22, 281-288, 1981. DOI: 10.1080/00071688108447887

37. Cheeke PR, Powley JS, Nakaue HS, Arscott GH: Feed preference responses of several avian species fed alfalfa meal, high-and low-saponin alfalfa, and quinine sulfate. *Can J Anim Sci,* 63, 707-710, 1983. DOI: 10.4141/cjas83-080

38. Southon S, Johnson IT, Gee JM, Price KR: The effect of Gypsophila saponins in the diet on minerals status and plasma cholesterol concentration in the rat. *Br J Nutr*, 59, 49-55, 1988. DOI: 10.1079/

BJN19880008

39. Southon S, Wright AJA, Price KR, Fairweather-Tait SJ, Fenwick GR: The effect of three types of saponin on iron and zinc absorption from a single meal in the rat. *Br J Nutr*, 59, 389-396, 1988. DOI: 10.1079/BJN19880048

40. Afrose S, Hossain MS, Maki T, Tsujii H: Hypocholesterolemic response to Karaya saponin and Rhodobacter capsulatus in broiler chickens. *Asian-Aust J Anim Sci,* 23, 733-741, 2010. DOI: 10.5713/ajas.2010.90481

41. Yang CJ, Yang IY, Oh DH, Bae IH, Cho SG, Kong IG, Uuganbayar D, Nou IS, Choi KS: Effect of green tea by-product on performance and body composition in broiler chicks. *Asian-Aust J Anim Sci*, 16, 867-872, 2003. DOI: 10.5713/ajas.2003.867

42. Kucukkurt I, Dundar Y: Effects of dietary *Yucca schidigera* supplementation on plasma leptin, insulin, iodated thyroid hormones and some biochemical parameters in rats. *Rev Med Vet*, 7, 362-367, 2013.

43. Johnson JS: Heat stress alters animal physiology and post-absorptive metabolism during pre- and postnatal development. *Graduate Theses and Dissertations,* Paper: 13982, 2014.

44. Virden WS, Kidd MT: Physiological stress in broilers: Ramifications on nutrient digestibility and responses. *J Appl Poult Res*, 18, 338-347, 2009. DOI: 10.3382/japr.2007-00093

45. Dabbert CB, Lochmiller RL, Teeter RG: Thermal stress influences clinical chemistry values of northern bobwhite *(Colinus virginianus*). *Comp Haematol Int*, 6, 120-122, 1996. DOI: 10.1007/BF00426054

46. Kucukkurt I, Ince S, Enginar H, Eryavuz A, Fidan AF, Kargioglu M: Protective effects of *Agrostemma githago* L. and *Saponaria officinalis* L. extracts against ionizing radiation-induced oxidative damage in rats. *Rev Med Vet*, 162, 289-296, 2011.

47. Rohaida AR, Alimon AR, Sazili AQ: Fatty acid composition of breast and thigh muscles of broilers fed diets supplemented with candle nut kernel meal subjected to different heat treatments. *Mal J Anim Sci*, 17, 47-60, 2014.

48. Brogna DM, Nasri S, Salem HB, Mele M, Serra A, Bella M, Priolo A, Makkar HP, Vasta V: Effect of dietary saponins from *Quillaja saponaria* L. on fatty acid composition and cholesterol content in muscle Longissimus dorsi of lambs. *Animal*, 5, 1124-1130, 2011. DOI: 10.1017/S1751731111000048

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Research Article

Analysis of *Cytidine Monophospho-N-Acetylneuraminic Acid Hydroxylase (CMAH)* Gene Related to Neonatal Isoerythrolysis in Stray Cats of Izmir, Turkey [1]

Hüseyin CAN [1] Esra ATALAY ŞAHAR [1] Mert DÖŞKAYA [2] Hüseyin Gökhan ÖZDEMİR [3] Ayşe CANER [2] Aysu DEĞİRMENCİ DÖŞKAYA [2] Yüksel GÜRÜZ [2] Cemal ÜN [1]

[1] *Preliminary results of this study have been presented in the 22nd National Biology Congress held in Eskişehir, Turkey between June 23-27, 2014*

[1] Ege University Faculty of Science, Department of Biology, TR-35040 Bornova, İzmir - TURKEY
[2] Ege University Faculty of Medicine, Department of Parasitology, TR-35100 Bornova, İzmir - TURKEY
[3] Municipality of İzmir, Department of Veterinary Affairs, TR-35250 Konak, İzmir - TURKEY

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Abstract

Neonatal isoerythrolysis is a life threatening disease in new born cats. It occurs when type A or type AB kittens are born from a type B queen (female cat). A homozygous 18 bp insertion located in *cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMAH)* gene has been detected in type B cats, causing production of inactive *CMAH* enzyme. Currently, molecular methods are being used to determine type B blood in female cats, which can help prevent neonatal isoerythrolysis in kittens. These molecular assays target the presence of 18 bp insertion in *CMAH* gene. In this study, we aimed to analyze the potential of neonatal isoerythrolysis among stray cats of İzmir, Turkey using PCR detecting the 18 bp insertion in *CMAH* gene. During the study, we analyzed 793 cats' blood sample for the presence of 18 bp insertion in *CMAH* gene. Three cats known to have blood types A, B, and AB were used as control in PCR. According to the PCR results, blood type A control cat displayed a 175 bp product indicating a homozygous type A cat while blood type control B cat showed a 193 bp product in *CMAH* gene (with 18 bp insertion) indicating a homozygous type B cat. Interestingly, blood type AB control cat showed a heterozygous pattern for *CMAH* gene, in which three different bands (175 bp like that of type A, 193 bp product for type B, and the third unique band with approximately 240 bp size) were detected. Among 793 stray cats of İzmir, 791 were homozygous for *CMAH* gene with 175 bp band size (99.7%). The remaining two stray cats showed heterozygous band pattern like blood type AB cat (0.12%). Overall, 175 bp band displaying type A cats are prevalent contrary to the two cats that have type AB pattern and non-existence of homozygous type B cats. These results show that the potential of neonatal isoerythrolysis in stray cats of İzmir is minimal. Future studies are required to scrutinize the reason(s) for non-existence of type B cats in İzmir and presence of unique band in blood type AB.

Keywords: *CMAH gene, Neonatal isoerythrolysis, Insertion, Cat, PCR*

Türkiye, İzmir Sokak Kedilerinde Neonatal İzoeritrolizisle İlişkili *Sitidin Monofosfat-N-Asetilnöraminik Asit Hidroksilaz (CMAH)* Geninin Analizi

Özet

Neonatal izoeritrolizis yeni doğan kedilerde hayatı tehdit eden bir hastalıktır. Bu hastalık, tip A ya da tip AB kediler tip B dişi kedilerden doğduğunda ortaya çıkmaktadır. Tip B kedilerde inaktif *sitidin monofosfat-N-asetilnöraminik asit hidroksilaz (CMAH)* enzim üretimine neden olan *CMAH* geni üzerinde homozigot 18 bp bir insersiyon saptanmıştır. Son zamanlarda, moleküler metotların tip B dişi kedilerin belirlenmesinde kullanımı yavru kedilerde neonatal izoeritrolizisin önlenmesine yardım etmektedir. Bu moleküler teknikler *CMAH* genindeki 18 baz çiftlik insersiyonun varlığını hedeflemektedir. Bu çalışma İzmir, Türkiye de sokak kedileri arasında neonatal izoeritrolizis potansiyelinin *CMAH* geninde 18 baz çiftlik insersiyonu hedefleyen PZR ile analiz edilmesini amaçlamıştır. Çalışma sırasında, 793 sokak kedisinin kan örneği incelenmiştir. A, B ve AB kan tipine sahip olduğu bilinen üç kedi PZR sırasında kontrol olarak kullanılmıştır. Elde edilen sonuca göre, kontrol grubu tip A kedi, homozigot tip A kediyi işaret eden 175 baz çiftlik bir ürüne sahip iken kontrol grubu tip B kedi, homozigot tip B kediyi işaret eden *CMAH* geninde 18 baz çiftlik insersiyonlu 193 baz çiftlik bir ürüne sahipti. İlginç bir şekilde, kontrol grubu tip AB kedi *CMAH* geni için heterozigot bir patern göstermiş olup burada üç farklı bant (tip A gibi 175 baz çiftlik, tip B gibi 193 baz çiftlik ve yaklaşık olarak 240 baz çiftlik büyüklüğünde üçüncü yeni bir bant) saptanmıştır. İzmir'de 793 sokak kedisi arasında, 791'i 175 bç'li *CMAH* geni için homozigot olduğu (%99.7) ve geriye kalan iki kedinin de tip AB kedi gibi heterozigot bant paterni gösterdiği saptanmıştır (%0.12). Sonuç olarak, tip A kedileri işaret eden 175 baz çiftlik bant, tip AB paterne sahip iki kediye ve homozigot olarak saptanmamış tip B kedilere göre sık görülmektedir. Bu sonuçlar İzmir sokak kedilerinde neonatal izoeritrolizis potansiyelinin düşük olduğunu göstermektedir. Gelecek çalışmalarda İzmir'de tip B kedilerin olmaması sebepleri ve tip AB kedide saptanan yeni bant varlığının incelenmesinin uygun olacağı düşünülmektedir.

Anahtar sözcükler: *CMAH gen, neonatal izoeritrolizis, insersiyon, kedi, PZR*

İletişim (Correspondence)
+90 232 3115186
huseyin.can@ege.edu.tr

INTRODUCTION

Cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMAH) gene encodes an enzyme called cytidine monophosphate-N-acetylneuraminic acid hydroxylase. CMAH enzyme produces N-glycolylneuraminic acid (Neu5Gc) derived from N-acetylneuraminic acid (Neu5Ac) through enzymatic hydroxylation [1]. Neu5Gc and Neu5Ac are two of the most abundant sialic acids and both of them are expressed in echinoderm to mammalians [1-4]. These sialic acids have significant biologic roles in cellular recognition, signaling, neuronal development, and host-pathogen interaction in vertebrates, including humans [5-9].

There are many studies to determine the types of sialic acid in different species. According to data obtained from these studies, Neu5Gc is expressed in various mammalians except humans due to a 92 bp deletion causing a mutation in the coding region of single-copy *CMAH* gene which is located in telomeric region on chromosome 6 [10-12]. In humans, the *CMAH* mutation which occurred ~100.000 years ago is thought to arise against some lethal microbial pathogen during an evolutionary selection process [6]. In addition, the loss of functionality in *CMAH* gene in humans resulted with a biochemical difference which is detected nearly on the surface of every cell between humans and great apes [13]. Also, the *CMAH* mutation in human causes the presence of precursor Neu5Ac at high levels [10]. Unlike human, *CMAH* gene has been conserved and is active in species as primitive as echinoderms. Moreover, *CMAH* gene exhibits highly similarity among mammals [14].

From starfish to human, *CMAH* gene is analyzed by molecular techniques to find out the mutations or polymorphisms. For examples, 18 different haplotypes were detected in humans originated from Africa and non-Africa depending on intronic region of *CMAH* pseudogene [15].

In cats, *CMAH* gene is located on B2 chromosome corresponding to human chromosome 6 and related to cat blood types. The major blood types present in cats are type A and B. Among these blood types, different sialic acid residues are found; while type A cats have Neu5Gc, type B cats have Neu5Ac. Also, type B cats possess strong agglutinins against type A cats. A rare blood type in cats is type AB which has both Ne5Ac and Neu5Gc in similar quantities [14].

Lately, genetic mechanism of cat's blood group system has been investigated. According to result of that study, CMAH enzyme encoded by *CMAH* gene converts Neu5Ac to Neu5Gc and thus, different blood group arise in cats. However, a mutation [18 bp insertion = *(AACGAGCAACC GAAGCTG)*] located in *CMAH* gene has been detected in type B cats and it has been stated that the mutation led to production of inactive CMAH enzyme. Because of this, CMAH enzyme can no longer convert Neu5Ac to Neu5Gc [14].

There is a life threatening disease in new born cats called neonatal isoerythrolysis. It occurs when type A or type AB kittens are born from a type B queen (female cat). Currently, molecular methods are being used to determine type B blood in cats, which can help prevent neonatal isoerythrolysis in kittens [16]. These molecular assays target the presence of 18 bp insertion in *CMAH* gene. There is limited data about the prevalence of 18 bp insertion in *CMAH* gene in cats worldwide. In this study, we aimed to detect the prevalence of 18 bp insertion in *CMAH* gene among stray cats of İzmir, Turkey using polymerase chain reaction (PCR). Therefore, risk of neonatal isoerythrolysis for stray cats would be determined depending on prevalence of 18 bp insertion. For this purpose, we analyzed 793 stray cats' blood sample for the presence of 18 bp insertion in *CMAH* gene.

MATERIAL and METHODS

Cats and Sample Collection

Stray cat samples (n:793) were provided from the Veterinary Clinic, Municipality of İzmir, Turkey. The stray cats were brought to Veterinary Clinic from 13 different counties of İzmir [Balçova (n:97), Bayraklı (n:31), Konak (n:452), Buca (n:37), Bornova (n:27), *Çiğli* (n:5), Gaziemir (n:2), Güzelbahçe (n:20), Torbalı (n:1), Narlıdere (n:4), Seferihisar (n:2), Karabağlar (n:44) and Karşıyaka (n:71)]. Blood samples obtained from stray cats were collected in EDTA-coated tubes. All experiments were performed under the instructions and approval of the Institutional Animal Care and Use Committee (IACUC) of Ege University for animal ethical norms (Approval number: 2015-056). The RapidVet-H IC Feline Immuno-Chromatographic Test (Rapidvet, DMS laboratories) for identifying feline A, B and AB blood type have been used according to the manufacturer's protocol. Blood samples of three cats with blood type A, B and AB as determined by the kit were used as positive control.

DNA Extraction and PCR Analysis

DNA isolation from blood samples of stray cats was performed with the QIAamp DNA mini kit according to the manufacturer's protocol (Qiagen).

Conventional PCR targeting the *CMAH* gene (GenBank no. EF127686.1) of stray cats was performed as described with some modification [14]. To detect *CMAH* gene containing 18 bp insertion or without insertion, the following primers were used: Cat *CMAH* F (5'-*ACACAG CAGAGGAAGTGGTG*-3') and Cat *CMAH* R (5'-*CATTGGG TCTGGAGGAACCC*-3'). The PCR amplified a 193 bp product in *CMAH* gene with 18 bp insertion while 175 bp product in *CMAH* gene without 18 bp insertion.

The 30 µl amplification reactions included 2 µl template DNA, the primers (0.66 µM each), 0.375 U Thermo Scientific

Taq DNA Polymerase (Thermo Scientific), 0.016 µM dNTPs, 0.175 mM $MgCl_2$ and 1× Taq reaction buffer. The PCR amplification reaction was performed using the following calculated-control protocol: 2 min initial denaturation step at 95°C, followed by 35 cycles of 1 min at 95°C, 45 sec at 58°C, and 45 sec at 72°C, and a final extension of 10 min at 72°C. Three cats' DNA samples, known to have blood types A, B and AB, were used as PCR control. All PCR products were separated by 3% agarose gel electrophoresis, stained by ethidium bromide and visualized under DNR Bio-imaging systems.

RESULTS

Among the control groups, blood type A control cat displayed a 175 bp product indicating a homozygous type A cat while blood type control B cat showed a 193 bp product in *CMAH* gene (with 18 bp insertion) indicating a homozygous type B cat. Interestingly, blood type AB control cat showed a heterozygous pattern for *CMAH* gene, in which three different bands (175 bp like that of type A, 193 bp product for type B, and the third *unique* band with approximately 240 bp size) were detected. Among 793 stray cats of İzmir, 791 were homozygous for *CMAH* gene with 175 bp band size (99.7%). The remaining two stray cats showed heterozygous band pattern like blood type AB cat (0.12%) *(Fig. 1)*.

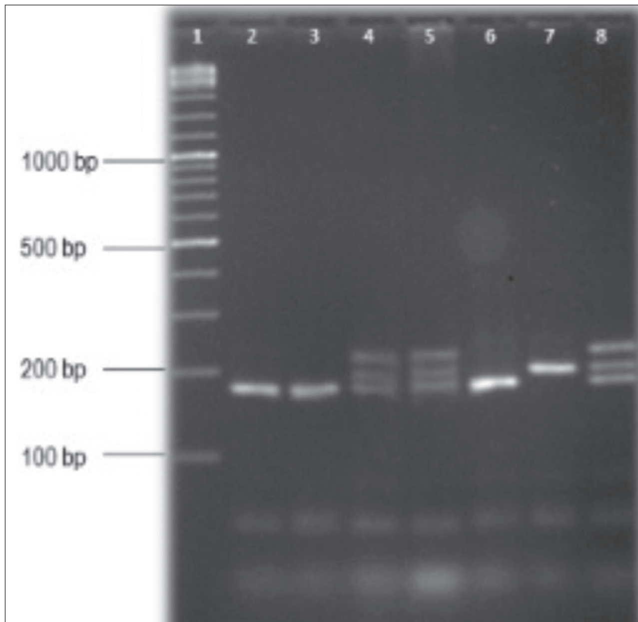

Fig 1. The PCR products obtained from control groups and analyzed samples. **Line 1:** DNA ladder; **Lines 2-5:** Analyzed samples for *CMAH* gene, line 2 and 3 show homozygote pattern while line 4 and 5 show heterozygous pattern; **Lines 6-8:** Control groups containing three cats' blood samples, known to have blood types A, B and AB, respectively

Şekil 1. Kontrol gruplarından ve analiz edilen örneklerden elde edilen PZR sonuçları. **Sıra 1:** DNA merdiveni; **Sıra 2-5:** *CMAH* geni için analiz edilen örnekler, sıra 2 ve 3 homozigot paterni gösterirken sıra 4 ve 5 heterozigot paterni göstermektedir; **Sıra 6-8:** Kan grubunun sırasıyla, A, B ve AB olduğu bilinen üç kedi kanından oluşan kontrol grubları

Table 2. *Evaluation of PCR results*

Tablo 2. *PZR sonuçlarının değerlendirilmesi*

PCR Product Size	Test Result	Evaluation
193 bp	b/b	Type B
175 bp like that of type A, 193 bp product for type B, and unique band with approximately 240 bp	b/N	Type A or AB with b allele
175 bp	N/N	Type A or AB

b/b: *a cat with two copies of b allele;* ***b/N:*** *one b allele carrier cat;* ***N/N:*** *a cat without any b allele*

In addition, blood type A control cat displaying a 175 bp product was detected in all counties of İzmir. Two cats with three different bands were found in only Konak and Bornova counties of İzmir *(Table 1)*.

DISCUSSION

Cat blood group is encoded by unique gene called as *CMAH* gene which is related to production of sialic acid present in cat red cells. CMAH enzyme encoded by *CMAH* gene converts Neu5Ac to Neu5Gc in various mammals, including cats. However, CMAH enzyme is not active in type B cats because this group has homozygous 18 bp insertion on *CMAH* gen which causes production of non-functional CMAH enzyme. In addition to 18 bp insertion, 5 more polymorphisms (A-217G, C-371T, G139A, T265A, G1600A) were detected in homozygous form in all type B cats [14]. Although these polymorphisms and 18 bp insertion were determined in heterozygous form in some type A cats, any homozygous polymorphism or 18 bp insertion was not found in type A cats [14].

18 bp insertion on *CMAH* gene is not very good marker to identify blood type in cats since this marker cannot differentiate between type A and type AB cat. However, it can be used to predict blood groups and to detect the type B or b allele carrier cat without blood sample collections [14] *(Table 2)*.

In a previous study, G139A and C136T SNPs in *CMAH* gene were used to predict blood type in cats and results of SNPs were compared to those of standard blood type microplate agglutination method. Coherence rate of these two assays was found to be 96%. Among these SNPs, homozygous G139A polymorphisms were detected in 10 of 14 type B cats. Also, any SNP was not determined in 59 type A cats [16].

Except of molecular approach, several tests are used for blood typing in cats. Among them, serological techniques such as card agglutination (CARD), immunochromatographic cartridge (CHROM), gel-based (GEL), and conventional slide (SLIDE) and tube (TUBE) agglutination assays are generally used in blood typing in cats. These assays are applied to determine cat blood phenotype [16].

Table 1. *Geographic distribution of CMAH alleles (with insertion, without insertion and unique allele) and CMAH gene status according to PCR results*
Tablo 1. *CMAH allellerinin (insersiyonlu, insersiyonsuz ve yeni allel) coğrafik dağılımı ve PZR sonuçlarına göre CMAH gen durumu*

Counties of İzmir (Number of Analyzed Cats)	*CMAH* Gene without insertion	*CMAH* Gene with insertion	Unique *CMAH* Allele	*CMAH* Gene Status
Konak (n:451)	+	-	-	Homozygous
Konak (n:1)	+	+	+	Heterozygous
Balçova (n:97)	+	-	-	Homozygous
Karşıyaka *(n:71)*	+	-	-	Homozygous
Karabağlar (n:44)	+	-	-	Homozygous
Buca (n:37)	+	-	-	Homozygous
Bayraklı (n:31)	+	-	-	Homozygous
Bornova (n:26)	+	-	-	Homozygous
Bornova (n:1)	+	+	+	Heterozygous
Güzelbahçe (n:20)	+	-	-	Homozygous
Çiğli (n:5)	+	-	-	Homozygous
Narlıdere (n:4)	+	-	-	Homozygous
Gaziemir (n:2)	+	-	-	Homozygous
Seferihisar (n:2)	+	-	-	Homozygous
Torbalı (n:1)	+	-	-	Homozygous

Determination of blood types in cats is important since neonatal isoerythrolysis can be prevented in kittens. Blood types of cats vary depending on the breed of the cat or geographic location. Blood type A among cats has the highest prevalence (85-100%) worldwide. In some regions, the prevalence of type B cats increases such as Greece, England, Australia, and Turkey [17].

In eastern part of Turkey, of the 85 Van cats analyzed, 40% had type A, and 60% had type B blood. In addition, cats from central Anatolia, called Angora were analyzed and among them, 53.6% had type A and 46.4% had type B blood. Type AB cats were not found in both breeds [18]. In another study, of 301 cats typed from four distinct regions of Turkey, 220 had type A blood, 74 had type B and seven had type AB [19].

Currently, determination of blood types by molecular methods has become popular which can help prevent neonatal isoerythrolysis in kittens before breeding program [16]. Our results showed that among control groups, blood type A control cat displayed a 175 bp product indicating a homozygous type A cat while blood type control B cat showed a 193 bp product in *CMAH* gene (with 18 bp insertion) indicating a homozygous type B cat. Interestingly, blood type AB control cat showed a heterozygous pattern for *CMAH* gene, in which three different bands were detected *(Fig. 1)*. Among 793 stray cats of İzmir, 791 were homozygous for *CMAH* gene with 175 bp band size (99.7%). The remaining two stray cats showed heterozygous band pattern like blood type AB cat (0.12%). Type B cats were not detected.

Overall, 175 bp band displaying type A cats are prevalent contrary to the two cats that have type AB pattern and non-existence of homozygous Type B cats. Interestingly, a new allele was found for first time in this study and information about new allele is not available in literature yet. It is thought to be related with *CMAH* gene in type AB cats because primer set used in this study was specific to only *CMAH* gene in cats.

These results show that the potential of neonatal isoerythrolysis in stray cats of İzmir is minimal. Future studies are required to scrutinize the reason(s) for non-existence of type B cats in İzmir and presence of *unique* band in blood type AB.

REFERENCES

1. Nysted J, Anderson H, Hirvonen T, Impola U, Jaatinen T, Heiskanen A, Blomqvist M, Satomaa T, Natunen J, Saarinen J, Lehenkari P, Valmu L, Laine J: Human CMP-N-acetylneuraminic acid hydroxylase is a novel stem cell marker linked to stem cell-specific mechanisms. *Stem Cells*, 28, 258-267, 2010. DOI: 10.1002/stem.250

2. İzzetoğlu S, Şahar U, Şener E, Deveci R: Determination of sialic acids in immune system cells (coelomocytes) of sea urchin, Paracentrotus lividus, using capillary LC-ESI-MS/MS. *Fish Shellfish Immunol*, 36 (1): 181-186, 2014. DOI: 10.1016/j.fsi.2013.10.029

3. Irie A, Suzuki A: CMP-N-Acetylneuraminic acid hydroxylase is exclusively inactive in humans. *Biochem Biophys Res Commun*, 248 (2): 330-333, 1998. DOI: 10.1006/bbrc.1998.8946

4. Ghaderi D, Springer SA, Ma F, Cohen M, Secrest P, Taylor RE, Varki A, Gagneux P: Sexual selection by female immunity against paternal antigens can fix loss of function alleles. *Proc Natl Acad Sci U S A*, 108, 17743-17748, 2011. DOI: 10.1073/pnas.1102302108

5. Irie A, Koyam, S, Kozutsumi Y, Kawasaki T, Suzuki A: The molecular basis for the absence of N-glycolylneuraminic acid in humans. *J Biol Chem*, 273, 15866-15871, 1998. DOI: 10.1074/jbc.273.25.15866

6. Wickramasinghe S, Medrano JF: Primer on genes encoding enzymes in sialic acid metabolism in mammals. *Biochimie*, 93, 1641-1646, 2011.

DOI: 10.1016/j.biochi.2011.06.002

7. Martensen I, Schauer R, Shaw L: Cloning and expression of a membrane-bound CMP-N-acetylneuraminic acid hydroxylase from the starfish *Asterias rubens. Eur J Biochem,* 268, 5157-5166, 2001. DOI: 10.1046/j.0014-2956.2001.02446.x

8. İzzetoğlu S, Karaçalı S: The determination of N-acetylneuraminic acid (Neu5Ac) and N-glycolyl-neuraminic acid (Neu5Gc) types of sialic acids in hematopoietic organ of the silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae). *Kafkas Univ Vet Fak Derg,* 18, 147-150, 2012. DOI: 10.9775/kvfd.2011.5257

9. Yılmaz M, Karapehlivan M, Kaya İ: Effects of zinc sulphate on transcaucasian Barb, *(Capoeta capoeta* [Guldenstaedt, 1773]) plasma nitric oxide, malondialdehyde and total sialic acid levels. *Kafkas Univ Vet Fak Derg,* 18, 61-64, 2012. DOI: 10.9775/kvfd.2011.4792

10. Varki A: Loss of N-glycolylneuraminic acid in humans: Mechanisms, consequences, and implications for hominid evolution. *Am J Phys Anthropol,* 33, 54-69, 2001. DOI: 10.1002/ajpa.10018

11. Chou HH, Takematsu H, Diaz S, Iber J, Nickerson E, Wright KL, Muchmore EA, Nelson DL, Warren ST, Varki A: A mutation in human CMP-sialic acid hydroxylase occurred after the Homo-Pan divergence. *Proc Natl Acad Sci U S A,* 95, 11751-11756, 1998. DOI: 10.1073/pnas.95.20.11751

12. Chou HH, Hayakawa T, Diaz S, Krings M, Indriati E, Leakey M, Paabo S, Satta Y, Takahata N, Varki A: Inactivation of CMP-N-acetylneuraminic acid hydroxylase occurred prior to brain expansion during human evolution. *Proc Natl Acad Sci U S A,* 99, 11736-11741, 2002. DOI: 10.1073/pnas.182257399

13. Gagneux P: Great apes and humans: Genetic differences. *Encyclopedia of Life Sciences*, 2006. DOI: 10.1002/9780470015902.a0006115

14. Bighignoli B, Niini T, Grahn RA, Pedersen NC, Millon LV, Polli M, Longeri M, Lyons LA: Cytidine monophospho-N-acetylneuraminic acid hydroxylase (CMAH) mutations associated with the domestic cat AB blood group. *BMC Genetics,* 8, 27, 2007. DOI: 10.1186/1471-2156-8-27

15. Hayakawa T, Aki I, Varki A, Satta Y, Takahata N: Fixation of the human-specific CMP-N-acetylneuraminic acid hydroxylase pseudogene and implications of haplotype diversity for human evolution. *Genetics,* 172 (2): 1139-1146, 2005.

16. Tasker S, Barker EN, Day MJ, Helps C: Feline blood genotyping versus phenotyping, and detection of non-AB blood type incompatibilities in UK cats. *Small Anim Pract,* 55 (4): 185-189, 2014.

17. Fosset FT, Blais MC: Prevalence of feline blood groups in the Montreal area of Quebec, Canada. *Can Vet J,* 55 (1): 1225-1228, 2014.

18. Arikan S, Duru SY, Gurkan M, Agaoglu ZT, Giger U: Blood type A and B frequencies in Turkish Van and Angora cats in Turkey. *J Vet Med A Physiol Pathol Clin Med,* 50 (6): 303-306, 2003.

19. Arikan S, Gurkan M, Ozaytekin E, Dodurka T, Giger U: Frequencies of blood type A, B and AB in non-pedigree domestic cats in Turkey. *J Small Anim Pract,* 47 (1): 10-13, 2006.

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Research Article

Non-Genetic Factors Affecting Milk Yield, Composition and Somatic Cell Count in Hungarian Holstein Cows

Edit MİKÓNÉ JÓNÁS [1] Savaş ATASEVER [2] Myrtill GRÁFF [1] Hüseyin ERDEM [2]

[1] Department of Animal Husbandry, Faculty of Agriculture, University of Szeged, 6800, Szeged, HUNGARY
[2] Department of Animal Science, Faculty of Agriculture, University of Ondokuz Mayis, TR-55139 Samsun - TURKEY

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Abstract

The purpose of this study was to determine effects of non-genetic factors on milk yield, milk composition and somatic cell count (SCC) of dairy cows. A total of 4891 records of Hungarian Holstein cows raised in a private dairy farm in South Hungary, between 2007 and 2008 were investigated. Fat, protein and lactose were assessed as milk composition parameters. To evaluate milking cows by effective factors; three different stage of lactation (SL) (SL 1= 90d<, SL 2= 91-150d and SL 3=151≤d), five parity, four calving season (CS) and three body condition score (BCS) groups (group1=<3 points; group2=3-3.50 points and group3=>3.50 points) were designed. While fat, protein and lactose decreased, daily milk yield (DMY), 305 daily milk yield (305 DMY) and SCC increased with advanced parity. Fat, protein and SCC increased, but lactose and DMY tended to drop with later SL and BCS. These parameters were highest in cows calved in winter-autumn, summer, winter-spring and winter-autumn, respectively. DMY negatively correlated with investigated parameters except for lactose and 305 DMY. The study revealed that non-genetic factors are associated with milk composition, yield and SCC of milk. Therefore, it is suggested that these factors should primarily be considered to obtain more quality and quantity milk from dairy cows.

Keywords: *Environmental factor, Cow, Body condition score, Milk quality, Somatic cell count*

Macar Siyah Alaca İneklerinde Süt Verimi, Bileşimi ve Somatik Hücre Sayısını Etkileyen Genetik Olmayan Faktörler

Özet

Bu çalışmada süt sığırlarında süt verimi, süt bileşimi ve somatik hücre sayısı (SHS)'nı etkileyen genetik olmayan faktörlerin belirlenmesi amaçlanmıştır. Güney Macaristan'daki özel bir süt sığırı işletmesindeki Macar Siyah Alacaları'nın 2007-2008 yıllarına ait toplam 4891 verim kaydı incelenmiştir. Yağ, protein ve laktoz; süt bileşimine ait parametreler olarak değerlendirilmiştir. Sağmal inekleri etkili faktörler bakımından değerlendirmek üzere; üç farklı laktasyon dönemi (LD) (LD 1= 90<, LD 2= 91-150 ve LD 3=151≤gün), beş laktasyon sırası (LS), dört buzağılama mevsimi (BM) ve üç vücut kondüsyon puanı (VKP) grubu (grup1=<3 VKP; grup2=3-3.50 VKP ve grup3=>3.50 VKP) oluşturulmuştur. İlerleyen LS'na bağlı olarak yağ, protein ve laktoz azalırken, günlük ortalama süt verimi (GOSV), 305 günlük süt verimi (305 GSV) ve SHS yükselmiştir. İleri LS ve VKP gruplarında yağ, protein ve SHS'nda artış, laktoz ve GOSV'nde ise azalış gözlenmiştir. Bu parametreler sırasıyla kış-sonbahar, yaz, kış-ilkbahar ve kış-sonbahar BM'nde buzağılayan ineklerde en yüksek bulunmuştur. GOSV; laktoz ve 305 GSVdışındaki parametrelerle negatif korelasyona sahiptir. Bu araştırma, genetik olmayan faktörlerin süt bileşimi, süt verimi ve SHS ile ilişkili olduğunu ortaya koymuştur. Bu nedenle, süt ineklerinden daha kaliteli ve yüksek miktarda süt elde etmek için bu faktörlerin öncelikli olarak dikkate alınması önerilmektedir.

Anahtar sözcükler: *Çevre faktörü, Inek, Vücut kondüsyon puanı, Süt kalitesi, Somatik hücre sayısı*

INTRODUCTION

Elevating quality and quantity of milk is crucial to achieve more income by dairy herd owners. In addition to genetic factors, multiple factors such as parity, season, stage of lactation, milking interval or feeding management markedly affect milk yield and composition [1,2]. Generally, variation in milk yield is associated with milk composition [3]. Water, fat, protein, ash, lactose and minerals can be classified as the major components of bovine raw milk [4]. Highly wide ranged genetic correlations between milk fat and persistence of lactation have been estimated [5-7]. Plasma proteins migrate to the inflammation site for dealing with the infection, and thus, percentage of protein may increase during this time. A decrease in lactose percentage of milk leads to reduce in milk yield due to

İletişim (Correspondence)
+90 362 3121919, Fax: +90 362 4576034
satasev@omu.edu.tr

lactose plays an active role for transmission of water to the mammary gland [8]. On the other hand, some milk components can be used as reflectors of reproductive performance. Moderate heritabilities for milk yield, fat and protein have been estimated [7,9]. This cases show the importance of non-genetic factors on milk production markers. In addition to reaching high quantity, determination of somatic cell count (SCC) is regarded as the principal process for monitoring quality of cow milk [10-12]. Somatic cells are responsible for natural defence system and contain lymphocytes, macrophages, epithelium cells and polymorphonuclear cells [13]. Studies revealed that high SCC adversely affects milk composition and processing level [8,14]. It has been indicated that elevation of SCC from $50x10^3$ cells/ml to $800x10^3$ cells/ml caused to reduction in milk yield by 6.3% in primiparous cows and 9.6% in cows in the third or later parities [10]. Today, modern dairy industry encourages producers to obtain milk with low SCC via additional payments [8]. In milk production cycle, energy demands are generally higher than their intake in early lactation especially for high-yielding cows [15]. Due to hardness of controlling this balance, an indirect parameter, body condition score (BCS), is commonly used in dairy operations. It has been revealed that BCS losses post-calving are correlated with milk yield, fertility and animal health [16]. That's why; investigating factors affecting the production parameters would highly be useful to dairy owners to take critical decisions for animal selection, husbandry and feeding management of the herds. Information on this subject in dairy cows may also lead for gaining more quality and quantity raw milk. In spite of many environmental factors can be effective on yield and components of milk, of these, parity, lactation period, calving season and body condition score may be classified as the main non-genetic factors. Eliminating the effects of these factors may be seen a gold step to manage an elite herd for dairy breeders.

The present study aims to determine the influence of stage of lactation, calving season and body condition score those referred to non-genetic factors on composition, milk yield and SCC in Hungarian Holstein cows.

MATERIAL and METHODS

The study was conducted in a private dairy farm in Szegvár, South- Hungary. A total of 4891 records of Hungarian Holstein cows, clinically *healthy and* in the lactation, between 2007 and 2008 were evaluated. The *cows were kept* in similar feeding and management conditions: in loose housing stable with deep litter and by means of feeding mainly forage supplemented with concentrated feeds during the experiment period. All cows were kept indoors all the study period. The daily rations were formulated with a ration-optimizing program. The data of measurements was recorded by dairy farm management software and milk recording data including daily milk yield (DMY), 305 daily milk yield (305 DMY), calving time and parity information was collected from the Association of Milk Recording. Milk composition traits (fat, protein, lactose) and SCC analysis were performed by the Fourier Transform Spectrometer and Infrared *Milk Analyzer* (Bentley Instrument Inc., Chaska, MN, USA). To ensure homogeneity of variance, SCC values were transformed into log scale (log10) for statistical analysis.

The cows were monthly recorded by BCS using a 5-grade scoring system, which describes 1 point is emaciated and 5 points refer to an obese cow, and to achieve more sensitivity, 0.50 points were also used.

To evaluate cows by effective factors; periods of milk production (early, middle and late lactation) of milking cows was considered and thus, three different stage of lactation (SL) (*SL 1 = 90 d<, SL 2 = 91-150 d and SL 3 = 151≤d*) were designed. Cows were evaluated in five parity (*cows with parity ≥5 were assessed into 5th group*) and four calving season groups. Besides, milk components, DMY, 305 DMY and SCC data were assessed in three BCS subgroups (*group 1 = <3 points; group 2 = 3-3.50 points and group 3 = >3.50 points*).

The data were tested by analysis of variance (One-Way ANOVA) and effects of the non-genetic factors on fat, protein, lactose, DMY, 305 DMY and logSCC were analyzed using the following linear model:

$$Y_{ijklm} = \mu + a_i + b_j + c_k + d_l + e_{ijklm}$$

where: Y_{ijklm}: is dependent variable *(parameters)*

μ: population mean,

a_i: effect of parity *(i = 1,2,3,4 and 5th lactation)*

b_j: effect of stage of lactation *(j = 90<, 91-150 and 151≤d in lactation)*

c_k: effect of calving season *(k = winter, spring, summer and autumn)*

d_l: effect of BCS (*l = 1,2,3; 1 = <3 points; 2 = 3-3.50 points; 3 = >3.50 points)*

e_{ijklm}: random residual effect.

Relations among investigated traits were estimated by Pearson's correlation coefficients. The means were compared by Duncan's multiple range test based on the 0.05 level of probability and all statistical analyses were performed using SPSS 17.0 for Windows.

RESULTS

Effects of environmental factors on investigated parameters are given in *Table 1*. As seen that all components were significantly (P<0.01) affected by parity. Fat percentage mean of the 2nd parity was found to be different from that calculated for the 4th also 5th parity. Protein percentage means for the advanced parities (4th and 5th) were lower

Table 1. *Means (±SD) of traits by non-genetic factors*
Tablo 1. *Özelliklerin genetik olmayan faktörlere göre ortalamaları (±S)*

Factors	n	Fat (%)	Protein (%)	Lactose (%)	Daily Milk Yield (kg)	logSCC	n	305 Daily Milk Yield (kg)
Parity								
1	1028	4.04±0.77^{ab}	3.38±0.32^{ab}	4.85±0.23^{a}	21.34±6.93^{a}	5.12±0.57^{a}	1000	7156.88 ±1367.43^{A}
2	1038	4.06 ±0.84^{a}	3.41 ±0.37^{b}	4.73±0.25^{b}	24.60±9.33^{b}	5.10±0.57^{a}	1006	8543.07±1619.29^{B}
3	1370	3.98 ±0.85^{abc}	3.36±0.36^{a}	4.67±0.32^{bc}	25.92±10.58^{b}	5.29±0.67^{b}	1319	9315.23±1646.35^{C}
4	923	3.95±0.81^{b}	3.23±0.36^{c}	4.64±0.31^{c}	28.04±10.37^{c}	5.42±0.76^{c}	840	9139.43±1765.26^{CD}
5	534	3.96±0.78^{bc}	3.30±0.36^{c}	4.58±0.32^{d}	27.10±10.48^{c}	5.71±67^{d}	480	8945.02±1740.71^{D}
Total	4893	4.00±0.92	3.36 ±0.36	4.716±0.30	25.21±9.87	5.28±0.66	4645	8613.29±1809.32
Stage of lactation								
1 *(0-90d)*	1013	3.77±0.85^{a}	3.04±0.27^{a}	4.760±0.25^{a}	32.31±8.65^{a}	5.103±0.71^{a}		
2 *(91-150d)*	828	3.86 ±0.85^{b}	3.24±0.27^{b}	4.770±0.28^{a}	30.37±8.17^{b}	5.166±0.69^{a}		
3 *(≥151d)*	3047	4.11±0.79^{c}	3.49±0.33^{c}	4.671±0.31^{b}	21.46 ±8.66^{c}	5.38±0.63^{b}		
Total	4888	4.00±0.82	3.35±0.36	4.706±0.30	25.22±9.86	5.28±0.67		
Calving season								
1 *(winter)*	1160	4.03±0.85^{a}	3.36±0.35^{ab}	4.73±0.29^{a}	26.05±10.22^{a}	5.27±0.68	1083	8749.18±1873.06^{A}
2 *(spring)*	891	3.95±0.84^{b}	3.35±0.39^{a}	4.72±0.33^{a}	23.27±10.14^{b}	5.27±0.64	853	8259.56±1899.24^{B}
3 *(summer)*	1456	3.96±0.81^{b}	3.38±0.37^{b}	4.69±0.29^{b}	24.48±8.81^{c}	5.31±0.64	1393	8352.02±1639.37^{B}
4 *(autumn)*	1384	4.05±0.77^{a}	3.33±0.32^{a}	4.69±0.31^{b}	26.52±10.18^{a}	5.26±0.70	1314	9008.39±1780.90^{C}
Total	4891	4.00±0.82	3.36±0.36	4.71±0.30	25.21±9.87	5.28±0.67	4643	8613.59±1809.65
Body condition score								
1 *(<3points)*	2425	3.91±0.81^{a}	3.30±0.34^{a}	4.71±0.29^{a}	26.44±9.00^{a}	5.27±0.68^{a}	2278	8706.59±1826.10^{A}
2 *(3-3.50 points)*	1762	4.05±0.79^{b}	3.37±0.35^{b}	4.72±0.31^{a}	25.43±10.15^{b}	5.26±0.67^{a}	1680	8649.14±1807.53^{A}
3 *(>3.50 points)*	702	4.16±0.84^{c}	3.50±0.39^{c}	4.68±0.31^{b}	20.38±10.53^{c}	5.37±0.61^{b}	683	8215.71±1692.35^{B}
Total	4889	4.00±0.82	3.36±0.36	4.71±0.30	25.20±9.86	5.28±0.67	4641	8613.55±1807.66

Different superscript letters in the same column indicate statistically significant differences (a,b: P<0.05; A,B: P<0.01); ***logSCC:*** *logarithmic somatic cell count,* ***305 dMY:*** *305 daily milk yield*

than the means for the other parity groups. Besides, protein means between 2nd and 3rd parities was different from each other. For lactose, a clear dropping with later parities was also observed. In contrast, distinctly increase was obtained with advanced parities for DMY and 305 DMY. The overall DMY and 305 DMY means were calculated to be 25.21±9.87 kg and 8613.29±1809.32 kg, respectively. Similarly, while the lowest logSCC mean was calculated in first and second parity, a linear increase was obtained for logSCC means by advanced parity.

When parameters were evaluated by SL, significant differences (P<0.05) were found among all groups *(Table 1)*. For fat and protein, a distinct increase was observed by advancing parity. The means (%) for these parameters were calculated to be 4.00±0.82 and 3.36±0.36, respectively. In lactose evaluation, relatively lower percentage (4.67±0.31) was obtained in the 3rd SL group. Cows in the first SL had highest DMY and 305 DMY when compared to other groups. In the 3rd SL group, DMY or 305 DMY means were calculated to be fairly lower than those calculated in the other SL groups. Also, a linear increment might be observed in logSCC means by SL groups.

In the study, fat percentages obtained in the winter and autumn CS was statistically different (P<0.05) from those estimated in the other CS groups *(Table 1)*. For protein, mean calculated in spring CS was lower (P<0.05) than the other means. Also, lactose means of winter and spring CS were different (P<0.05) from the means of other CS groups. While cows calved in winter and autumn had the highest DMY, the highest 305 DMY mean was obtained from cows calved in autumn season. In this study, no significant effect of CS on logSCC was determined. The overall untransformed SCC was calculated to be 663x10^{3} cells/ml.

In BCS evaluation, significant (P<0.05) increase was determined according to elevated BCS for fat and protein means. Besides, cows with highest BCS had lowest lactose percentage (4.678±0.314) but highest logSCC (5.37±0.61) in this investigation. Also, a severe dropping in DMY was observed in cows with BCS >3.50.

Associations of investigated markers are given in *Table 2*. DMY had negative correlations with all parameters except for lactose and 305 DMY. While fat positively correlated

Table 2. *Pearson's phenotypic correlation coefficients between traits*
Tablo 2. *Özellikler arasındaki Pearson fenotipik korelasyon katsayıları*

Trait	F	P	Lac	BCS	logSCC	305dMY
dMY	-0.281	-0.485	0.308	-0.190	-0.280	0.466
F		0.452	-0.089	0.109	0.098	-0.136
P			-0.122	0.196	0.217	-0.132
Lac				-0.013	-0.412	-0.140
BCS					0.019	-0.060
logSCC						-0.013

***dMY:** daily milk yield, **F:** fat, **P:** protein, **Lac:** lactose, **BCS:** body condition score, **logSCC:** logarithmic somatic cell count, **305dMY:** 305 daily milk yield*

with protein, BCS and logSCC, negative correlations were calculated between lactose and other components except for DMY. Besides, a positive correlation coefficient *(r= 0.019)* was also estimated between BCS and logSCC. Estimated all correlation coefficients were found to be non-significant, statistically.

DISCUSSION

In the present study, fat percentage was found as relatively lower in cows with later parities. Similarly, protein percentages were decreased in advanced parity groups. In an earlier investigation [17], changeable fat and protein percentages in different parities have also been determined. However, our findings disagree with the report of results obtained in previously investigation [18]. Relatively low lactose content of milk collected from cows with later parities was determined in this study. However, obtained higher milk production (DMY and 305 DMY) in advanced parity groups might also assumed as unsurprised case. Undoubtedly, enhancement in body weight and udder size and new gestations might be referred as the main reasons for this fact. Thus, this result was parallel with the findings of some studies [19,20]. Similar to DMY results, logSCC means increased with advanced parities. Such that, relatively more milk production and eroding the tissues in udder gland with advanced age might be assumed as the normal reason of this case.

The study revealed that fat and protein percentages were lower in milk samples collected from cows in lower than 150th d of lactation. This result can be evaluated as a normal case due to new calving. In contrast, lactose ratio decreased in the latest SL group. This finding was inline with the results of the some researchers [21], who found that the lactose curve showed a progressive decrease as stage of lactation advanced. A general concept that milk production reaches to peak level in lactating cows at the beginning phase of the lactation. In this view, our finding is agreement with the results of some studies [17,22]. However, the linear dropping in DMY with later SL might be seen the reason for elevation in fat and protein percentages by SL. Obtained results for logSCC contrast with some earlier investigation results by an earlier work [23]. At this point, it can be advised to dairy owners that cows in higher than 150 d of lactation should be finically managed to obtain more milk quality.

In this study, cows calved in winter and autumn had more fat in milk (P<0.05). Effect of nutrition program and feeds presented to milking cows in these seasons might be seen the major reasons for this case. In other words, elevated the fat level of milk might be determined due to feeding cows with high energy included feeds in winter and autumn, where the herd kept indoors all year. In protein evaluation, an unsteady trend might be observed. Similarly, a group of researchers [17] reported an altered protein levels by season in their study. In a study [24], it was determined the lowest protein percentage in the summer and the highest percentage in the winter. However, while lactose in milk was higher in winter and spring CS groups, this result was found as harmonic with the indication of some researchers [21], who explained this case by inadequate forage supplementation of diet in these months.

Cows calved in winter and autumn had more DMY when compared to others. This finding is parallel to fat evaluation results. Similarly, cows calved in winter had higher 305 DMY. As mentioned earlier, feeding applications and adjustments in nutrition programs in herds in these seasons might be assumed the marked reason for this case. Actually, it was reported that cooler months positively affected milk production in dairy herds [25]. CS had no significant effect on SCC. In spite of calculated SCC mean of this study was found as similar to level obtained by a group of researchers [26], who conducted a study on this subject in Poland conditions, the mean was higher than SCC limits (400x10^3 cells/ml) by EU directives [27]. In this context, recording and closely observing SCC data may be seen a major stage to ensure high quality raw milk from dairy herds.

In BCS evaluation, similar results were found for fat and protein means in the study. As seen that cows with BCS<4 (group 1 and 2) had lower fat and protein percentage when compared to cows with BCS>3.50. In other words, low BCS caused to low fat and protein percentage in milk. Feeding regime of the farm might be caused to

this case. However, lactose mean dropped in the highest BCS group. Actually, this finding was inline with obtained results *(Table 1)* on lactose percentages by SL groups. An attractive result was obtained in DMY means by BCS and cows with BCS<3 had highest milk production. Such that, loss in milk production between cows with BCS<3 and cows BCS>3.50 was estimated to be 22.91%. Actually, this result is harmonic with DMY evaluation by SL. Namely, high producing cows might be referred as cows in the first SL group, and exposing to negative energy balance [28], BCS seems as relatively low in this group. Similarly, in highest BCS groups, cows had higher SCC. Concisely, keeping cows under 4 BCS points might be considered to achieve more quantity and quality milk yield from Hungarian Holstein cows.

In correlation assessment, DMY had negative correlations with all parameters except for lactose and 305 dMY. In a normal lactation cycle, this finding might be assumed to be an expected result. As mentioned earlier, cows should not be allowed to gain high BCS to take more milk production from herds. Actually, a negative relationship between SCC and milk yield have been reported by many authors [29-31]. Also, positive correlations could be regarded between fat, protein, BCS and SCC. Besides, both fat and protein had negative correlations with lactose percentage. This finding agrees with the report of a study [32] that indicated negative associations of lactose with fat and protein contents. Also, lactose negatively correlated with BCS and SCC. Similarly, it was estimated a negative relationship between lactose and SCC of milk in an earlier work [21]. It was emphasized in a previous study [32] that elevated SCC of milk is highly associated with relatively low lactose, moreover udder health of milking cows adversely affected by this case.

In other words, findings obtained here are agreement with literature, and thus, combining all milk markers may be seen a more beneficial process in the farms for milk quality assessment. And last, a positive but non-significant correlation (*r=0.019*) was also estimated between BCS and SCC. A general hypothesis that negative energy balance in cows exposed to early lactation may be seen a major reason of udder inflammation [33]. In a study [23] that conducted in Turkey conditions, it was determined a negative but non-significant correlation coefficient (*-0.030*) between two parameters.

Finally, the present research indicated that non-genetic factors are associated with milk composition, production level and SCC in milk. Keeping records on milk parameters and observing cows are important steps to obtain an elite dairy herd. Therefore, it is suggested that environmental factors should primarily be considered to achieve more quality and quantity milk from milking cows.

REFERENCES

1. Koc A: Daily milk yield, non-fat dry matter content and somatic cell count of Holstein-Fresian and Brown-Swiss cows. *Acta Vet (Beograd)*, 57, 523-535, 2007. DOI: 10.2298/AVB0706523K

2. Millogo V, Ouedraogo GA, Agenäs S, Svennersten-Sjaunja K: Day-to-day variation in yield, composition and somatic cell count of saleable milk in hand-milked zebu dairy cattle. *Afr J Agric Res*, 4, 151-155, 2009.

3. Yilma Z, Gojjam Y, Shumye M: Milk production level and calf rearing system affecting Boran, Ethiopian zebu cattle breed, cow-calf performance. *Lives Res Rural Dev,* 18, 71, 2006.

4. Claeys WL, Verraes C, Cardoen S, De Block J, Huyghebaert A, Raes K, Dewettinck K, Herman L: Consumption of raw or heated milk from different species: An evaluation of the nutritional and potential health benefits. *Food Control*, 42, 188-201, 2014. DOI: 10.1016/j.foodcont.2014.01.045

5. Biassus IO, Cobuci JA, Costa CN, Rorato PRN, Neto JB, Cardoso LL: Persistence in milk, fat and protein production of primiparous Holstein cows by random regression models. *R Bras Zootec*, 39, 2617-2624, 2010. DOI: 10.1590/S1516-35982010001200009

6. Van der Linde R, Groen AF, De Jong G: Estimation of genetic parameters for persistency of milk production in dairy cattle. Proceedings of the 2000 Interbull Meeting, Bled, Slovenia, International Bull Evaluation Service, Department of Animal Breeding and Genetics, Uppsala, Sweden, pp.113-116 Bulletin no. 25, 2000.

7. Jamrozik J, Jansen G, Schaeffer LR, Liu Z: Analysis of persistency of lactation calculated from a random regression test-day model. Proceedings Interbull Meeting, Rotorua, N.Z., International Bull Evaluation Service, Department of Animal Breeding and Genetics, Uppsala, Sweden, pp.64-69 Bulletin no. 17, 1998.

8. Guariglia BAD, dos Santos PA, de Souza Araújo L, Giovannini CI, Neves RBS, Nicolau ES, da Silva MAP: Effect of the somatic cell count on physicochemical components of milk from crossbred cows. *Afr J Biotechnol*, 14, 1519-1524, 2015. DOI: 10.5897/AJB2015.14540

9. Toghiani S: Genetic relationship between production traits and reproductive performance in Holstein dairy cows. *Arch Tierz,* 55, 458-468, 2012.

10. Memisi N, Bogdanovic V, Tomic Z, Kasalica A, Zujovic M, Stanisic N, Delic N: Variability and correlation between basic quality parameters of raw cow milk. *Biotechol Anim Husband*, 27, 959-967, 2011. DOI: 10.2298/BAH1103959M

11. Ural DA: The relationships among some udder traits and somatic cell count in Holstein-Friesian Cows. *Kafkas Univ Vet Fak Derg*, 19, 601-606, 2013. DOI: 10.9775/kvfd.2012.8517

12. Emre, B, Cengiz M, Alacam, E: Evaluation of effects of milking hygiene and management factor on clinical mastitis incidence in dairy cows. *Kafkas Univ Vet Fak Derg*, 17, 31-35, 2011. DOI: 10.9775/kvfd.2010.2367

13. Pavel ER, Gavan C: Seasonal and milking-to-milking variations in cow milk fat, protein and somatic cell counts. *Not Sci Biol*, 3, 20-23, 2011.

14. More SJ: Global trends in milk quality: Implications for the Irish dairy industry. *Irish Vet J*, 62, 5-14, 2009.

15. Rehak D, Volek J, Bartoň L, Vodková Z, Kubešová M, Rajmon R: Relationships among milk yield, body weight, and reproduction in Holstein and Czech Fleckvieh cows. *Czech J Anim Sci*, 57, 274-282, 2012.

16. Roche JR, Friggens NC, Kay JK, Fisher MW, Stafford KJ, Berry DP: Invited review: Body condition score and its association with dairy cow productivity, health, and welfare. *J Dairy Sci,* 92, 5769-5801, 2009. DOI: 10.3168/jds.2009-2431

17. Yoon JT, Lee JH, Kim CK, Chung YC, Kim CH: Effects of milk production, season, parity and lactation period on variations of milk urea nitrogen concentration and milk components of Holstein dairy cows. *Asian-Aust J Anim Sci,* 17, 479-484, 2004. DOI: 10.5713/ajas.2004.479

18. Gurmessa J, Achenef M: Effect of lactation stage, pregnancy, parity and age on yield and major components of raw milk in bred cross Holstein Friesian cows. *World J Dairy Food Sci,* 7, 146-149, 2012. DOI: 10.5829/idosi.wjdfs.2012.7.2.64136

19. Lee JY, Kim IH: Advancing parity is associated with high milk production at the cost of body condition and increased periparturient disorders in dairy herds. *J Vet Sci*, 7, 161-166, 2006. DOI: 10.4142/jvs.2006.7.2.161

20. Ayalew W, Mohammed A, Negussie E: Milk production performance of Holstein Friesian dairy cows at Holetta Bull Dam Farm, Ethiopia. *Livest Res Rural Dev,* 27, 173, 2015.

21. Henao-Velásquez AF, Múnera-Bedoya OD, Herrera AC, Agudelo-Trujillo JH, Cerón-Muòoz MF: Lactose and milk urea nitrogen: Fluctations during lactation in Holstein cows. *R Bras Zootec*, 43, 479-484, 2014. DOI: 10.1590/S1516-35982014000900004

22. Mech A, Dhali A, Prakash B, Rajkhowa C: Variation in milk yield and milk composition during the entire lactation period in Mithun cows *(Bos frontalis)*. *Livest Res Rural Dev*, 20, 75, 2008.

23. Atasever S, Erdem H: Association between subclinical mastitis markers and body condition scores of Holstein cows in the Black Sea region, Turkey. *J Anim Vet Adv,* 8, 476-480, 2009.

24. Bernabucci U, Basiricò L, Morera P, Dipasquale D, Vitali A, Piccioli CF, Calamari L: Effect of summer season on milk protein fractions in Holstein cows. *J Dairy Sci*, 98, 1815-1827, 2015. DOI: 10.3168/jds.2014-8788

25. Mellado M, Antonio-Chirino E, Meza-Herrera C, Veliz FG, Arevalo JR, Mellado J, de Santiago A: Effect of lactation number, year, and season of initiation of lactation on milk yield of cows hormonally induced into lactation and treated with recombinant bovine somatotropin. *J Dairy Sci*, 94, 4524-4530, 2011. DOI: 10.3168/jds.2011-4152

26. Kuczynska B, Puppel K, Golebiewski M, Kordyasz M, Grodzki H, Brzozowski P: Comparison of fat and protein fractions of milk constituents in Montbeliarda and Polish Holstein-Fresian cows from one farm in Poland. *Acta Vet Brno*, 81, 139-144, 2012. DOI: 10.2754/avb201281020139

27. Norman HD, Lombard JE, Wright JR, Kopral CA, Rodriguez JM, Miller RH: Consequence of alternative standards for bulk tank somatic cell count of dairy herds in the United States. *J Dairy Sci,* 94, 6243-6256, 2011. DOI: 10.3168/jds.2011-4645

28. Block SS, Butler WR, Ehrhardt RA, Bell AW, van Amburgh ME, Boisclair YR: Decreased concentration of plasma leptin in periparturient dairy cows is caused by negative energy balance. *J Endocrinol,* 171, 339-348, 2001. DOI: 10.1677/joe.0.1710339

29. Singh M, Ludri RS: Influence of stage of lactation, parity and season on somatic cell counts in cows. *Asian-Aust J Anim Sci,* 14, 1775-1780, 2001. DOI: 10.5713/ajas.2001.1775

30. Koivula, M,Mäntysaari EA, Negussie E, Serenius T: Genetic and phenotypic relationships among milk yield and somatic cell count before and after clinical mastitis. *J Dairy Sci*, 88, 827-833, 2005. DOI: 10.3168/jds.S0022-0302(05)72747-8

31. Hagnestam-Nielsen C, Emanuelson U, Berglund B, Strandberg E: Relationship between somatic cell count and milk yield in different stages of lactation. *J Dairy Sci*, 92, 3124-3133, 2009. DOI: 10.3168/jds.2008-1719

32. Rajčević M, Potočnik K, Levstek J: Correlations between somatic cells count and milk composition with regard to the season. *Agric Conspec Sci*, 68, 221-226, 2003.

33. van Straten M, Friger M, Shpigel NY: Events of elevated somatic cell counts in high-producing dairy cows are associated with daily body weight loss in early lactation. *J Dairy Sci,* 92, 4386-4394, 2009. DOI: 10.3168/jds.2009-2204

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Research Article

Evaluation of Serum Haptoglobin, Ceruloplasmin and Pseudocholinesterase Levels in Cows with Botulism

İsmail AYTEKİN [1] Feyyaz KAYA [1] Hasan ATALAY [2]

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[1] Balikesir University, Faculty of Veterinary Medicine, Department of Internal Medicine, TR-10100 Balikesir - TURKEY
[2] Balikesir University, School of Veterinary Medicine, Animal Nutrition and Nutritional Diseases, TR-10100 Balikesir - TURKEY

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Abstract

The aim of this study was to determine serum pseudocholinesterase, rheumatoid factor, troponin I, C-reactive protein, caeruloplasmin, haptoglobin, urea, creatinin, creatinin kinase (CK), phosporus, calcium, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in cows with or without botulism. The study included 15 holstein cows aged two to five years as the botulism group and control group consisting of 10 healthy cows. The group included both pregnant and dry period cattle. Serum concentration of all the parameters were measured using an autoanalyzer and Nefelometre equipment BNII. Mice inoculation test was performed to make diagnosis and Clostridium Botulinum type C and D toxins were determined in silage and blood that cows with botulism. Serum troponin I, C-reactive protein, rheumatoid factor, creatinine, creatinine kinase, phosphorus, calcium and AST did not differ significantly between two groups. Biochemistry analysis of serum showed that in the botulism group haptoglobin, caeruloplasmin and urea were higher and that pseudocholinesterase and alanine aminotransferase were lower than in the control group. Serum haptoglobin, caeruloplasmin, urea, alanine aminotransferase and especially pseudocholinesterase concentrations may prove beneficial to the prognosis of botulism.

Keywords: *Botulism, Cow, Toxin, Pseudocholinesterase, Ceruloplasmin, Haptoglobin*

Botulismuslu İneklerde Serum Haptoglobulin, Seruloplazmin ve Pseudokolinesteraz Seviyelerinin Değerlendirilmesi

Özet

Bu çalışmada botulismuslu ve sağlıklı ineklerde serum pseudokolinesteraz, seruloplazmin, haptoglobin, romatoid faktör, troponin I, C-reactive protein, üre, kreatin, kreatin kinaz (CK), alanine aminotransferase (ALT) ve aspartate aminotransferase (AST), fosfor ve kalsiyum seviyeleri araştırıldı. Çalışma ve kontrol grubunu gebe ve kuru dönemde iki ile beş yaşları arasında değişen 15 adet botulismuslu ve 10 adet sağlıklı holştayn inek oluşturdu. Parametrelerin serum düzeyleri Nefelometre BNII ve otoanalizör kullanılarak ölçüldü. Botilusmuslu hayvanların kan serumlarından ve silajdan fare inokulasyon testi yapılarak clostridium botulinum tip C ve tip D toksini tespit edildi. Serum troponin I, C-reactive protein, romatoid faktör, kreatin, kreatin kinaz, AST, fosfor ve kalsiyum seviyeleri botulismuslu ve sağlıklı ineklerde önemli bir değişiklik göstermedi. Botulismuslu ineklerde haptoglobin, seruloplazmin ve üre yüksek çıkarken, ALT ve pseudokolinesterazın sağlıklı ineklere göre daha düşük çıktığı tespit edildi. Botulismuslu ineklerde serum üre, ALT, haptoglobin, seruloplazmin ve özellikle pseudokolinesteraz düzeylerinin belirlenmesi botulismusun prognozuna faydalı olabileceği sonucuna varıldı.

Anahtar sözcükler: *Botulismus, İnek, Toksin, Pseudokolinesteraz, Seruloplazmin, Haptoglobin*

INTRODUCTION

Botulism is caused by a neurotoxin produced by *Clostridium botulinum* that a gram positive, spore forming anaerobe microorganism. Botulinum NeuroToxin (BoNT) is an exotoxin that produced during growth and autolysis process of the organism under anaerobic conditions [1,2]. Eight different botulinum toxins, A, B, Ca, Cb, D, E, F and G have been identified. BoNT blocks acetylcholine release at neuromusculer junction [3]. Affected cattle shows many symptoms that includes loss of tongue tone, decreased upper eyelid and tail tone, loss of appetite, ataxia and decreased ruminal movements [4]. Many types of silages are used extensively in ruminants feeding [5]. However silage

İletişim (Correspondence)
+90 266 6136692/211
aytekin0331@gmail.com

may support *C. botulinum* growth and toxin production [6]. Primarily botulism caused by types C and D in cattle. Type C and D toxins produced by *Clostridium botulinum* in rotting material, silage that contaminated dead rodents, birds or reptiles [1,2,7].

Haptoglobin is an APP that free haemoglobin binding protein in blood [8]. Haptoglobin has also antioxidant role via iron stabilization and antiinflammatory activity during innate immun response [9]. Iron is important for bacteria to grow and haptoglobin makes the iron unavailable via binds free haemoglobin. In this way it shows bacteriostatic effect on bacteria such as *E. coli* [10]. Caeruloplasmin is an acute phase protein (APP) that contains copper and oxidizes ferrous iron to it's nontoxic ferric form [11]. It protects not only tissues from iron mediated free radical damage but also involved in various antioxidant and cytoprotective mechanisms [12].

Cholinesterase is a mammalian enzyme found in two forms. These are acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BChE; EC 3.1.1.8) [13]. Pseudocholinesterase or butrylcholinesterase is an enzyme synthesized in various tissues that include liver, brain, lungs and heart and the enzyme has many roles in tissues such as lipoprotein metabolism [14], myelin maintenance [15], neurogenesis and neurite growth [16]. Butyrylcholinesterase hydrolyses butyrylcholine at higher rate than acetylcholine and propionylcholine [17]. However until now many studies were performed physiological functions of the enzyme remains unknown [13].

Diagnosis of botulism is not so challenge but few of biochemistry analysis investigated. The aim of this study was to determine the serum pseudocholinesterase, haptoglobin, caeruloplasmin and some biochemical parameters in cows with and without Botulism.

MATERIAL and METHODS

Animals

This case occured via feeding musty silage to cows incidentally in a dairy farm in Edremit that is a province of Balikesir. The study included 15 Holstein dairy cattle aged to two to five years and mix stage of pregnancy as the Botulism group and a control group consisting of 10 healthy dairy cattle. There are two pregnant cows both botulism group and control group. All cattle were clinically examined before collecting blood samples. The study was approved by the Çanakkale 18 Mart University Ethics Committee (No: 2014/ 03-12).

Serum Biochemistry Analysis

Blood samples were collected from the jugular vein and kept for two hours at room temperature for proper clotting. The samples were centrifuged at 2.500 g at 4°C for 15 min and stored at -20°C until analysed. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), calcium, phosporus, urea, creatinine, troponin I and pseudocholinesterase levels were measured using commercially available kits as per manufacturer's recommendations using a biochemistry auto analyzer Cobas 8000 (Roche, Germany). C-reactive protein, rheumatoid factor, caeruloplasmin and haptoglobin levels were measured using commercially available kits as per manufacturer's recommendations using a Nefelometre equipment BNII (Siemens, Germany).

Statistical analysis was performed using SPSS 20 for Windows. All the cattle that with and without botulism included in the statistical analysis. Results were statistically analysed using the independent samples t test for ALT, haptoglobin, creatinin, pseudocholinesterase, urea and using Mann- Whitney U test for AST, creatinin kinase, calcium, caeruloplasmin and troponin I.

Toxin Isolation

Samples that includes blood serum, ruminal content and silage were sent to the Veterinary Control and Reserach Inst.

RESULTS

Clinical Findings

Anorexia, lethargy, loss of tongue tone, lameness, recumbency, decreased tail tone, reduced rumen contractions, some of them head turned back against flank and death were observed in botulism group. The cattle in control group were completely healthy.

Biochemical Findings

Biochemistry analysis of serum showed that in the Botulism group pseudocholinesterase ($P<0.05$), and ALT ($P<0.01$) were lower and that haptoglobin ($P<0.001$), caeruloplasmin ($P<0.001$) and urea ($P<0.008$) were higher than in the control group *(Table 1)*. Serum troponin I, C-reactive protein, rheumatoid factor, creatinine, creatinine kinase, phosphorus, calcium and AST did not differ significantly between two groups *(Table 1)*.

Mice Inoculation Test

Toxin types C and D were identified in corn silage and blood serum. Mice inoculation test was performed and results were positive.

DISCUSSION

Enzyme Linked Immuno-Sorbent Assay (ELISA) and mice inoculation test are used to diagnosis of botulism but it is lower sensitivity than mice inoculation test. Because of this reason mice inoculation test is the most reliable

Table 1. *Pseudocholinesterase, haptoglobin, caeruloplasmin and biochemical parameters in cows with botulism and healthy group*

Tablo 1. *Botulismuslu ve sağlıklı ineklerde pseudokolinesteraz, haptoglobin, seruloplazmin ve biyokimyasal parametreler*

Parameters	Healthy Cows (n=10)	BotulismCows (n=15)	P Values
ALT (U/L)	31.62±1.70	22.47±1.46	*
AST (U/L)	80.36±3.01	77.91±7.44	NS
C-Reactive Protein (mg/L)	<3.48	<3.48	NS
Phosporus (mg/dL)	5.82±0.37	5.50±0.35	NS
Haptoglobin (mg/dL)	6.37±0.08	7.59±0.19	**
Calcium (mg/dL)	9.55±0.21	9.72±0.36	NS
Creatine Kinase (U/L)	197.50±9.67	452.40±118.18	NS
Creatinine (mg/dL)	0.95±0.03	0.91±0.03	NS
Pseudocholinesterase (U/L)	94.80±15.59	57.06±5.95	***
RheumatoidFactor (IU/mL)	<11.5	<11.5	NS
Caeruloplasmin (mg/dL)	4.37±0.23	7.26±0.32	**
Troponin I (ng/mL)	0.10±0.00	0.11±0.01	NS
Urea (mg/dL)	12.03±1.06	15.84±0.80	****

** P<0.01, ** P<0.001, *** P<0.05, **** P<0.008,* ***NS****- Not Significant,* ***SEM****- Standart Error of Mean*

test for botulism [18,19]. However negative mice inoculation test results do not eliminate the disease due to the toxin may be present at below level of threshold of detection. Additionaly BoNT is rapidly biodegraded in rumen by rumen microflora [20,21]. Our test results are agreement with previous reports.

The common clinical findings in animals with botulism include loss of tongue tone, decreased upper eyelid and tail tone, decreased rumen motility, pupillar and anal reflexes, loss of appetite and ataxia [4,22] which were all observed in the present study.

Braun et al.[23] reported normal or increased levels of biochemical parameters in plasma that include alanine aminotransferase and aspartate aminotransferase in cattle with botulism. In parallel Cobb et al.[24] determined any abnormalities other from hyperglycaemia and neutrophilia in dairy cows with botulism. However Senturk et al.[4] and Senturk et al.[22] found aspartate aminotransferase levels in normal reference ranges, Senturk and Cihan [4] found slightly increased serum aspartate aminotransferase levels in cattle with botulism. In the present study alanine aminotransferase enzyme did differ significantly between the two groups, it's level is in normal reference ranges [25]. Our results agreement with the previous reports [22-24]. The findings highly suggest that hepatocyte integrity and function of the liver was not impaired severely in the animals with botulism.

Although Senturk and Cihan [4] found slightly increased serum creatinine kinase levels, they found increased creatinine levels in cows with botulism. However Senturk et al.[22] found creatinine levels in normal reference ranges, they found increased creatinine kinase levels in cows with botulism. We think that the differences of results are caused by time of sampling. In the present study our results agreement with previous reports [4,22].

Senturk and Cihan [4] found calcium and phosphorus levels in reference ranges. In the present study although calcium levels slightly decreased and phosphorus levels were in reference ranges in cows with botulism [26]. Our results are similar with previous report [4].

Acute phase proteins do not have sufficient specificity, however they are good indicators of inflammation [27]. In healthy ruminants its blood level is negligible but it increases over 100-fold on immune stimulation [28,29]. Kirbas et al.[30] found increased haptoglobin levels in cows with traumatic reticuloperitonitis and their results are in agreement with the previous reports. Sixfold increases in haptoglobin concentrations were determined in dairy cows that suffer from infectious and metabolic disease compared to animals with minor lesions [31]. Gerlach et al.[32] observed the increased levels of serum haptoglobin in chronic botulism with *C. botulinum* proliferation. Similarly in the present study haptoglobin levels were higher in botulism group as previously reported [27-32].

Caeruloplasmin remains less common compared the other acute phase proteins to make diagnosis. However there have been certain studies determined increased caeruloplasmin levels and ferroxidase activity is an indicator of infection in cattle [28,29,33-35]. Similarly Nisbet and Cenesiz [36], and Nazifi et al.[37], found increased caeruloplasmin levels in cattle infected with cystic echinococcosis and *Theileria annulata* respectively. In the present study high levels of caeruloplasmin were observed in botulismus group, which suggests that inflammation due to botulism.

Although C-reactive protein indicates health status of herd it does not consider a primer acute phase protein in cattle [38]. Similarly in the present study we found any statistical importance between the control and botulism groups.

Urea reference ranges is 6-27 mg/dl in cattles [26]. Additionally Saraiva [39], found serum urea reference ranges is 20-30 mg/dl in healthy Nelore cattles. Senturk and Cihan [4], and Senturk et al.[22] found elevated serum urea concentrations cattle with botulism type C and D. In the present study urea did differ significantly between the two groups, but it's level is in normal reference ranges. We think that our results are in reference ranges because blood samples taken in the first stages of infection.

Pseudocholinesterase's primary pharmacological and toxicolocigal importance is hydrolyzing ester- containing drugs and scavenging cholinesterase inhibitors including potent organophosphorus nerve agents before they reach their synaptic targets [40]. Both in veterinary medicine

and human medicine cholinesterases take attraction as a bioscavenger drug, carbamate and organophosphate insecticides [40-43]. Some qualifications of the enzym that includes hydrolyzing carboxylic or phosporic acid ester containing compounds and attachment to certain aminoacids such as proline [44,45]. In non-toxic inflammatory diseases such as metabolic syndrome in humans, diabetes mellitus and obesity in both dogs and humans, elevated serum pseudocholinesterase concentrations were observed [46-49]. In case of systemic inflammation increased oxidative stress and decreased antioxidant status in blood levels were common in end-stage inflammatory disease and some kind of toxicity such as Cd in rats [50,51]. Similarly Aytekin et al.[52] found decreased antioxidant status caused by the elevated oxidant status in sheeps with Bluetongue. All the previous reports as stated above such as, qualifications of the enzyme, increased serum level of the enzyme in case of non toxic inflammatory diseases, decreased antioxidan status in inflammation and our results that include decreased serum pseudocholinesterase level in cows with botulism highly suggest that BoNT may be detoxified by the enzyme like potent organophosphorus nerve agents.

In conclusion, the literature includes many studies on the toxin's structural investigation, therapeutically uses in humans and blocking mechanism of the acetylcholinesterase in synaptic membrane; however few have investigated biochemical parameters such as pseudocholinesterase, haptoglobin, caeruloplasmin were investigated in the present study. Serum haptoglobin, caeruloplasmin, urea, alanine aminotransferase and especially pseudocholinesterase concentrations may prove beneficial to the prognosis of botulism.

REFERENCES

1. Radostits OM, Gay CC, Blood DC, Hinchliff KW: Diseases associated with bacteria II. **In,** Radostits OM, Gay CC, Blood DC, Hinchliff KW (Eds): Veterinary Medicine. A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. 10th ed., 824-825. Saunders, China, 2006.

2. Robert HW: Botulism. **In,** Smith BP (Ed): Large Animal Internal Medicine. 4th ed., 1096, Mosby, USA, 1990.

3. Burgen ASV, Dickens F, Zatman LJ: Action of botulinum toxin on the neuromusculer junction. *J Physiol,*109, 10-24, 1949. DOI: 10.1113/jphysiol.1949.sp004364

4. Senturk S, Cihan H: Outbreak of botulism in a dairy herd in Turkey. *Ir Vet J*, 481-484, 2007. DOI: 10.1186/2046-0481-60-8-481

5. Givens DI, Rulquin H: Utilisation by ruminants of nitrogen compounds in silage-based diets. *Anim Feed Sci Technol,* 114, 1-18, 2004. DOI: 10.1016/j.anifeedsci.2003.09.005

6. Notermans S, Dufrenne J, Oosterom J: Persistence of *Clostridium botulinum* type B on a cattle farm after an outbreak of botulism. *J Appl Environ Microbiol,* 41, 179-183,1981.

7. Böhnel H, Schwagerick B, Gessler F: Visceral botulism - A new form of bovine *Clostridium botulinum* toxication. *J Vet Med A Physiol Pathol Clin Med,* 48, 373-383, 2001. DOI: 10.1046/j.1439-0442.2001.00372.x

8. Polonovski M, Jayle MF: Preparation of a new fraction of the plasma proteins, haptoglobin. *C R Seances Soc Biol Fil*, 129, 457-460, 1938.

9. El Ghmati SM, Vanhoeyveld EM, Vanstrijp JAG, Ceuppens JL, Stevens EAM: Identification of haptoglobin as an alternative ligand for CD11b/CD18. *J Immunol,* 156, 2542-2552, 1996.

10. Eaton JW, Brandt P, Mahoney JR, Lee JT: Haptoglobin a natural bacteriostat. *Science*, 215, 691-693, 1982. DOI: 10.1126/science.7036344

11. Patel BN, Dunn RJ, Jeong SY, Zhu Q, Julien JP, David S: Ceruloplasmin regulates iron levels in the CNS and prevents free radical injury. *J Neurosci*, 22, 6578-6586, 2002.

12. Inoue K, Akaike T, Miyamoto Y, Okamoto T, Sawa T, Otagiri M, Suzuki S, Yoshimura T, Maeda H: Nitrosothiol formation catalyzed by ceruloplasmin. Implication for cytoprotective mechanism *in vivo*. *J Biol Chem*, 274, 27069-27075, 1999. DOI: 10.1074/jbc.274.38.27069

13. Iwasaki T, Yoneda M, Nakajima A, Terauchi Y: Serum butyrylcholinesterase is strongly associated with adiposity, the serum lipid profile and insulin resistance. *J Intern Med*, 46, 1633-1639, 2007. DOI: 10.2169/internalmedicine.46.0049

14. Kutty KM, Payne RH: Serum pseudocholinesterase and very low density lipoprotein metabolism. *J Clin Lab Anal*, 8, 247-250, 1994. DOI: 10.1002/jcla.1860080411

15. Earl CJ, Thompson RH: Cholinesterase levels in the nervous system in tri-ortho- cresyl phosphate poisoning. *Br J Pharamacol*, 7, 685-694, 1952.

16. Layer PG: Novel functions of cholinesterases in development, physiology and disease. *Prog Histochem Cytochem*, 29, 1-94, 1995. DOI: 10.1016/S0079-6336(11)80046-X

17. Dass P, Mejia M, Landes M, Jones R, Stuart B, Thyssen J: Cholinesterase: Review of methods. *Clin Chem*, 10, 135-57, 1994.

18. Thomas RJ: Detection of Clostridium botulinum type C and D toxin by ELISA. *Aust Vet J*, 68, 111–113, 1991. DOI: 10.1111/j.1751-0813.1991.tb00769.x

19. Guiterrez AR, Bodensteiner J, Gutmann L: Electrodiagnosis of infantile botulism. *J Child Neurol*, 9, 362-365, 1994. DOI: 10.1177/088307389400900404

20. Whitlock RH, Williams JM: Botulism toxicosis of cattle. **In,** Smith RA (Ed): *Proceedings of the 32nd Annual Convention of the American Association of Bovine Practitioners.* American Association of Bovine Practitioners. 45-53, Nashville, Tennessee, USA, 1999.

21. Heider LC, McClure JT, Leger ER: Presumptive diagnosis of *Clostridium botulinum* type D intoxication in a herd of feedlot cattle. *Can Vet J*, 42, 210-212, 2001.

22. Senturk S, Catik S, Akgul G, Mecitoglu Z: Botulism in a dairy herd. *Uludag Univ J Fac Vet Med,* 32, 53-56, 2013.

23. Braun U, Feige K, Schweizer G, Pospischil A: Clinical findings and treatment of 30 cattle with botulism. *Vet Rec*, 156, 438-441, 2005. DOI: 10.1136/vr.156.14.438

24. Cobb SP, Hogg RA, Challoner DJ, Brett MM, Livesey CT, Sharpe RT, Jones TO: Suspected botulism in dairy cows and its implications for the safety of human food. *Vet Rec*, 150, 5-8, 2002. DOI: 10.1136/vr.150.1.5

25. Boonprong S, Sribhen C, Choothesa A, Parvizi N, Vajrabukka C: Blood biochemical profiles of thai indigenous and Simmental x Brahman crossbred cattle in the Central Thailand. *J Vet Med A Physiol Pathol Clin Med*, 54, 62-65, 2007. DOI: 10.1111/j.1439-0442.2007.00893.x

26. Radostits OM, Gay CC, Blood DC, Hinchliff KW: Reference laboratory values veterinary medicine. **In,** Radostits OM, Gay CC, Blood DC, Hinchliff KW (Eds): Veterinary Medicine. A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. 7th ed., Saunders, China, 2006.

27. Eckersall PD, Bell R: Acute phase proteins: biomarkers of infection and inflammation in veterinary medicine. *Vet J*, 185, 23-27, 2010. DOI: 10.1016/j.tvjl.2010.04.009

28. Conner JG, Eckersall PD, Wiseman A, Aitchison TC, Douglas TA: Bovine acute phase response following turpentine injection. *Res Vet Sci*, 44, 82-88, 1988.

29. Conner JG, Eckersall PD, Wiseman A, Bain RK, Douglas TA: Acute phase response in calves following infection with *Pasteurella haemolytica, Ostertagia ostertagi* and endotoxin administration. *Res Vet Sci*, 47, 203-207, 1989.

30. Kirbas A, Ozkanlar Y, Aktas MS, Ozkanlar S, Ulas N, Erol HS: Acute

phase biomarkers for inflammatory response in dairy cows with traumatic reticuloperitonitis. *Isr J Vet Med,* 70, 23-29, 2015.

31. Hirvonen J, Hietarkopi S, Saloniemi H: Acute phase response in emergency slaughtered cows. *Meat Sci,* 3, 249-257, 1997. DOI: 10.1016/S0309-1740(97)00020-X

32. Gerlach H, Gerlach A, Schrodl W, Schottdorf B, Haufe S, Helm H, Shehata A, Kruger M: Oral application of charcoal and humic acids to dairy cows influences *Clostridium botulinum* blood serum antibody level and glyphosate excretion in urine. *Clin Toxicol,* 4, 2, 2014. DOI: 10.4172/2161-0495.1000186

33. Conner JG, Eckersall PD, Doherty M, Douglas TA: Acute phase response and mastitis in the cow. *Res Vet Sci,* 41, 126-128, 1986.

34. Chassagne M, Barnouin J, Chacornac JP: Biological predictors for early clinical mastitis occurrence in Holstein cows under field conditions in France. *Prev Vet Med,* 35, 29-38, 1998. DOI: 10.1016/S0167-5877(97)00092-5

35. Sheldon IM, Noakes DE, Rycroft A, Dobson H: Acute phase protein responses to uterine bacterial contamination in cattle after calving. *Vet Rec*, 148, 172-175, 2001. DOI: 10.1136/vr.148.6.172

36. Nisbet C, Cenesiz S, Acici M, Umur S: Determination of the serum malondialdehyde, ceruloplasmin, adenosine deaminase levels in cattle with cystic echinococcosis. *Erciyes Univ Vet Fak Derg*, 5, 1-4, 2008.

37. Nazifi S, Razavi MS, Reiszadeh M, Esmailnezhad Z, Ansari-lari M: Diagnostic values of acute phase proteins in Iranian indigenous cattle infected with *Theileria annulata*. *Vet Arhiv*, 80, 205-214, 2010.

38. Petersen HH, Nielsen JP, Heegard PMH: Application of acute phase protein measurements in veterinary clinical chemistry. *Vet Res,* 35, 163-167, 2004. DOI: 10.1051/vetres:2004002

39. Saraiva LA, Silva TPD, Paraguaio PE, Araújo MS, Sousa SV, Machado LP: Serum urea, creatinine and enzymatic activity of alkaline phosphatase in Nelore cattle raised in the Micro Upper Middle Gurguéia. *Anim Vet Sci,* 2, 105-108, 2014. DOI: 10.11648/j.avs.20140204.14

40. Raveh L, Grauver E, Grunwald J, Cohen E, Ashani Y: The stoichiometry of protection against soman and VX toxicity in monkeys pretreated with human butyrylcholinesterase. *Toxicol Appl Pharmacol*, 145, 43-53, 1997. DOI: 10.1006/taap.1997.8160

41. Munro NB, Shugart LR, Watson AP, Halbrook RS: Cholinesterase activity in domestic animals as a potential biomonitoring for nerve agent and other organophosphate exposure. *J Am Vet Med Assoc*, 199, 103-115, 1991.

42. Atkinson JE, Bolte HF, Rubin LF, Sonawane M: Assessment of ocular toxicity in dogs during six months exposure to a potent organophosphate. *J Appl Toxicol,* 14, 145-152, 1994. DOI: 10.1002/jat.2550140217

43. Sakaguchi K, Nagayama M, Masaoka T, Nishimura A, Kageyama K, Shirai M, Akahori F: Effects of fenthion, isoxathion, dichlorvos and propaphos on the serum cholinesterase isoenzyme patterns of dogs. *Vet Hum Toxicol*, 39, 1-5. 1997.

44. Cokugras AN: Butyrylcholinesterase: Structure and physiological importance. *Turk J Biochem,* 28, 54-61, 2003.

45. Biberoglu K, Schopfer LM, Tacal O, Lockridge O: The proline-rich tetramerization peptides in equine serum butyrylcholinesterase. *FEBS J*, 279, 3844-3858, 2012. DOI: 10.1111/j.1742-4658.2012.08744.x

46. Edward WR, Maria SM, Hongwei Z, Jim SS, Guang S: Relationship between serum butyrylcholinesterase and the metabolic syndrome. *CLB*, 38, 799-805, 2005. DOI: 10.1016/j.clinbiochem.2005.04.008

47. Allam AR, Gumpeny RS, Undurti ND: Elevated butyrylcholinesterase and acetylcholinesterase may predict the development of type 2 diabetes mellitus and Alzheimer's disease. *Med Hypotheses*, 69, 1272-1276, 2007. DOI: 10.1016/j.mehy.2007.03.032

48. Tvarijonaviciute A, Fernando T, José JC: Relationship between serum butyrylcholinesterase and obesity in dogs: A preliminary report. *Vet J*, 186, 197-200, 2010. DOI: 10.1016/j.tvjl.2009.07.030

49. Tvarijonaviciute A, Ceron JJ, Caldin M: Serum butyrylcholinesterase activity in dogs with diabetes mellitus. *Vet J*, 192, 494-497, 2012. DOI: 10.1016/j.tvjl.2011.06.040

50. Stanojkovica I, Stevuljevica JK, Milenkovic B, Spasica S, Vujic T, Stefanovic A, Ilic A, Ivanisevic J: Pulmonary function, oxidative stress and inflammatory markers in severe COPD exacerbation. *Respir Med*, 105, 31-37, 2011. DOI: 10.1016/S0954-6111(11)70008-7

51. Olisekodiak MJ, Igbeneghu CA, Onuegbu AJ, Oduru R, Lawal AO: Lipid, lipoproteins, total antioxidant status and organ changes in rats administered high doses of cadmium chloride. *Med Princ Prac,* 21, 156-159, 2012. DOI: 10.1159/000333385

52. Aytekin I, Aksit H, Sait A, Kaya F, Aksit D, Gokmen M, Baca AU: Evaluation of oxidative stress via total antioxidant status, sialic acid, malondialdehyde and RT-PCR findings in sheep affected with bluetongue. *Vet Rec Open*, 2-54, 2015. DOI: 10.1136/vetreco-2014-000054

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Research Article

Effects of Chromium (III) Picolinate and Chromium (III) Picolinate Nanoparticles Supplementation on Growth Performance, Organs Weight and Immune Function in Cyclic Heat Stressed Broiler Chickens

Omid HAMIDI [1] Mohammad CHAMANI [1] Hasan GHAHRI [2]
Ali Asghar SADEGHI [1] Hassan MALEKINEJAD [3]

[1] Department of Animal Sciences, Faculty of Agriculture and Natural Resources, Tehran Science and Research Branch, Islamic Azad University, Tehran, IRAN
[2] Faculty of Veterinary Medicine, Urmia Branch, Islamic Azad University, Urmia, IRAN
[3] Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Urmia University, Urmia, IRAN

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Abstract

This experiment conducted to investigate the effects of dietary chromium (III) picolinate (CrPic) and chromium (III) picolinate nanoparticles (NanoCrPic) supplementation on growth performance, organs weight and immune function of broilers exposed to heat stress. Heat stress (36°C) was applied for 10 h per day from the 21th to the 42nd days. Among 8 experimental treatments; only group T1 represented the non-heat stressed control group fed with a basal diet in comfort zone whereas group T2 represented the heat stressed control group fed with a basal diet. Heat stressed T3, T4, T5 groups were fed with basal diet supplemented with 500, 1.000, 1.500 ppb of CrPic/kg while T6, T7, T8 groups were fed with bsal diet supplemeted with 500, 1.000, 1.500 ppb of NanoCrPic/kg respectively. Results of the current experiment showed that the non-heat stressed group had a higher final BW, daily weight gain and daily feed intake compared with heat stressed groups during the experiment period (d 21-42), Among heat stressed groups, FCR values improved by supplementation of Cr into the diet. NanoCrPic 1.500 treatment had the lowest (P<0.05) FCR (2.14) of the total experimental period among heat stressed groups. The liver weight values of the day 35 of experiment differed significantly (P<0.05). Serum complement component C3 of experimental broilers was severely affected by the Cr supplementation. The results indicated that the nanoparticle supplementation might be an influential method for reduction of heat stress induced disorders which may attribute to the lowering of FCR and provoking the hepatic related alteration including the liver weigth.

Keywords: *Chromium picolinate nanoparticles, Cyclic heat stress, Immune Function, Organs weight, Performance*

Krom (III) Pikolinat ve Krom (III) Pikolinat Nanopartikül Katkısının Siklik Isı Stresine Maruz Kalan Broiler Civcivlerde Büyüme Performansı, Organ Ağırlığı ve Bağışıklık Fonksiyonları Üzerine Etkileri

Özet

Bu çalışma krom (III) pikolinat (CrPic) ve krom (III) pikolinat nanopartikül (NanoCrPic) katkısının ısı stresine maruz kalan broiler civcivlerde büyüme performansı, organ ağırlığı ve bağışıklık fonksiyonları üzerine etkilerini araştırmak amacıyla yapıldı. Civcivlerde 21. ve 42. günler boyunca toplam 10 gün her gün uygulanmak suretiyle ısı stresi oluşturuldu. Toplam 8 grup oluşturuldu. T1 grubu hayvanlar ısı stresi uygulanmayan ve bazal diyet ile beslenen kontrol grubunu oluştururken T2 grubu hayvanlar bazal diyet alırken ısı stresine maruz bırakıldı. T3, T4 ve T5 gruplarındaki hayvanlar bazal diyet ile birlikte sırasıyla 500, 1000 ve 1500 ppb CrPic/kg; T6, T7 ve T8 gruplarındaki hayvanlar ise bazal diyet ile birlikte sırasıyla 500, 1.000 ve 1.500 ppb NanoCrPic/kg katkısı aldılar. Çalışma sonucunda en yüksek nihai vücut ağırlığı, günlük ağırlık kazanımı ve günlük yem tüketimi ısı stresine maruz bırakılmayan hayvanlarda şekillendi. Isı stresi uygulanan gruplar arasında yem konversiyon oranı diyette Cr katkısı ile iyileşme gösterdi. NanoCrPic 1.500 uygulaması ısı stresi uygulanan gruplar arasında en düşük yem konversiyon oranına (2.14) (P<0.05) neden oldu. Çalışmanın 35. günü sonunda karaciğer ağırlık değerleri anlamlı ölçüde farklılıklar gösterdi (P<0.05). Serum komplement bileşeni C3 deneysel gruplarda şiddetli derecede Cr katkısı ile etkilenmişti. Çalışma sonucunda elde edilen bulgular doğrultusunda nanopartikül katkısı kullanımının ısı stresine bağlı olarak oluşan azaltmış yem konversiyon oranını düzeltme ve karaciğer ağırlık artışına katkıda bulunmada faydalı olacağı kanısına varıldı.

Anahtar sözcükler: *Krom pikolinat nanopartikül, Siklik ısı stresi, Bağışıklık fonksiyonu, Organ ağırlığı, Performans*

İletişim (Correspondence)
+98 912 3221336, Fax: +98 214 4865484
m.chamani@srbiau.ac.ir

INTRODUCTION

Heat stress (HS) is one of the most important commercial challenges that cause poor growth and has a negative influence on feed efficiency of broiler chickens. Broilers exposed to high environmental temperature display various behavioral and physiological disorders include feather pecking, tendency to inactivity, increase of body temperature, panting and respiratory alkalosis [1,2]. Briefly, HS is considered by decreased feed consumption, reduced metabolic rate, high mortality, reduced body weight gain, high feed conversion ratio, peroxidation of lipid, endocrine disorders, immunosuppression and intestinal microbial dysbiosis in poultry [3-6]. Also HS has been shown to decrease the total white blood cell count and antibody production [4], reduction of the peripheral blood lymphocytes number, induction of an electrolyte imbalance [7], decline spleen weight [8] and diminution $CD4^+$ T cells (T-helper lymphocytes) and $CD8^+$ T cells (T-cytotoxic lymphocytes) [9].

Some strategies for resolving this problem have been proposed to manage the negative effects of heat stress, including environmental management, nutritional manipulation as well as the addition of feed additives in the diet. Chromium-supplemented diets has shown to be effective in diminishing the negative effects of stress and improving immunity in broilers [10,11]. Chromium is an ingredient of glucose tolerance factor (GTF) and is essential for carbohydrate, fat, and protein metabolism likely by potentiating the action of insulin [12]. Stress and diseases lead to more urinary excretion of Cr and can cause exacerbating of marginal Cr deficiency [12]. Most poultry feedstuffs are mainly composed of plant source components, which are typically a low in contented of Cr [13]. It has been reported that the inclusion of CrPic in diet enhances daily gain, feed utilization and improve growth performance of broilers consumed low protein diets [14,15]. Chromium has been reported to have immunomodulatory properties [16-18], which is assumed to be an indirect effect of chromium on the secretion of glucocorticoids, because corticosteroids have a depressing effect on the immune system [19,20].

Recently, nanotechnology has rapidly been developing different scientific areas and nanoscale of materials has interested extention because nano-formulation particulates exhibit novel distinguishing quality such as a size, shape, large surface area, high surface activity, high catalytic efficiency and strong adsorbing ability [21]. Limited published data in various experimental conditions implicated higher absorption and bioavailability of chromium nanoparticles [22-24]. Previous researches have shown that chromium nanoparticles had beneficial properties on growth performance, body composition, as well as augmented tissue concentrations of Cr in selected muscles [25] and serum [24]. Also and chromium nanoparticles can improve utilization of Zn, Fe and Ca of broiler chickens [23]. Therefore, the purpose of this study was to investigate the effects of the supplementation of CrPic and NanoCrPic on performance, organs weight and immune function of broiler chickens exposed to cyclic heat stress (10 h/d).

MATERIAL and METHODS

All experiments were carried out under the ethical guidelines of the Islamic Azad University of Tehran Science and Research Branch (93/987, in 2014).

Birds and Grouping

A total of 480 broiler chickens (*Ross 308*), from 21 to 42 days old (equal in both sex), were used in a completely randomized design. Chickens were purchased from a commercial hatchery and were housed in floor pens covered with sterilized and contaminant-free wood shavings with 10 birds/m^2 and with water and food (hanging feeders) provided as *ad libitum*. The broiler chickens were observed for health status and behavior constantly. All chickens consumed a diet based on corn-soybean meal, which provided as mash form was formulated based on NRC 1996 by UFFDA software *(Table 1)*. On day 21, the broiler chickens were weighed and , selected based on weight (630±50 g), re-allocated into 8 different groups: a control- (thermoneutral) group and 7 independent heat-stressed groups and for each group of birds, 4 replications with 15 birds per box were made. Broilers in the control- (TN) and control+ (heat stress) groups were fed with no additive, whereas other groups were fed 500; 1.000; or 1.500 ppb of CrPic, or 500; 1.000 or 1.500 ppb of NanoCrPic, respectively. CrPic purchased from Sigma-Aldrich [($C_{18}H_{12}CrN_3O_6$), Cat. no. C4124, CAS Number = 14639-25-9, USA] and Nanoparticles of chromium picolinate were prepared using the method that described by Lin *et al.*[24], briefly" mixture of dry ingredients contained of 10 g of chromium and 2.5 g of dispersed reagent silica was added to 240 ml of 95% ethanol to make a semi-liquid mixture. The mixture was premixed for 1.5 h and then placed in a grind chamber with 500 g of 0.2 mm zirconium particles. The mixture was then ground for 1.5 h at 960 g. After the grinding, the mixture was passed through a 0.074 mm (200 mesh) sieve to remove large particles. The mixture was then oven-dried at 50°C overnight. The chromium nanoparticle powder was passed through a 0.074 mm sieve again" [24], finally the nanoparticles size was determined by a transmission electronic microscope (Philips Bio Twin 100 The Netherland) according to lin *et al.*[24] and the average diameter of particles was 100 nm *(Fig. 1)*.

The birds of control group (C) were kept comfort zone temperature (23±1°C from 21 to 28 day and 21±1°C from 28-42 day). The birds of heat-stressed groups were kept under 36±1°C ambient temperature from 08:00 to 18:00 h = 10 h/d (from 21-42 day). From 18:00 to 08:00 h, the environmental temperature of the heat-stressed groups was reduced to the equal to that of the control- group. The birds in 42 days old were euthanized by cervical dislocation.

Table 1. *Ingredients and chemical composition of the experimental diets*
Tablo 1. *Deneysel diyetin içeriği ve kimyasal kompozisyonu*

Ingredients (%)	Starter (1–21d)	Finisher (21 – 42 d)							
		Cont - (T1)	Cont + (T2)	Cr500 (T3)	Cr1000 (T4)	Cr1500 (T5)	NCr500 (T6)	NCr1000 (T7)	NCr1500 (T8)
Corn	60.7	66.0	66.0	66.0	66.0	66.0	66.0	66.0	66.0
Soybean meal	30.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
Corn gluten meal	2.5	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Soybean oil	2.3	2.55	2.55	2.55	2.55	2.55	2.55	2.55	2.55
Dicalcium phosphate	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
Limestone	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Salt	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
L-Lysin	0.16	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
DL-Methionine	0.14	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Mineral and vitamin mix[1]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
CrPic (ppb/kg)	-	-	-	500	1000	1500	-	-	-
NanoCrPic (ppb/kg)	-	-	-	-	-	-	500	1000	1500
Calculated nutrient composition	**Starter (1-21d)**	**Finisher (21-42 d)**							
ME (kcal/kg)	3120	3190							
CP (%)	21.1	19.0							
Lysine (%)	1.1	0.95							
Methionine (%)	0.5	0.4							
Calcium (%)	0. 9	0.9							
Total phosphorus (%)	0.4	0.4							

[1] Supplied per kilogram of diet: trans-retinyl acetate, 25 mg; cholecalciferol, 6 mg; menadione, 1.2 mg; thiamine, 2.3 mg; riboflavin, 8 mg; nicotinamide, 42 mg; choline chloride, 400 mg; calcium pantothenate, 10 mg; pyridoxine HCl, 4 mg; biotin, 0.04 mg; folic acid, 1 mg; cobalamin, 0.012 mg; Fe (from ferrous sulfate), 82 mg; Cu (from copper sulfate), 7.5 mg; Mn (from manganese sulfate), 110 mg; Zn (from zinc oxide), 64 mg; I (from calcium iodate), 1.1 mg; Se (from sodium selenite), 0.28 mg

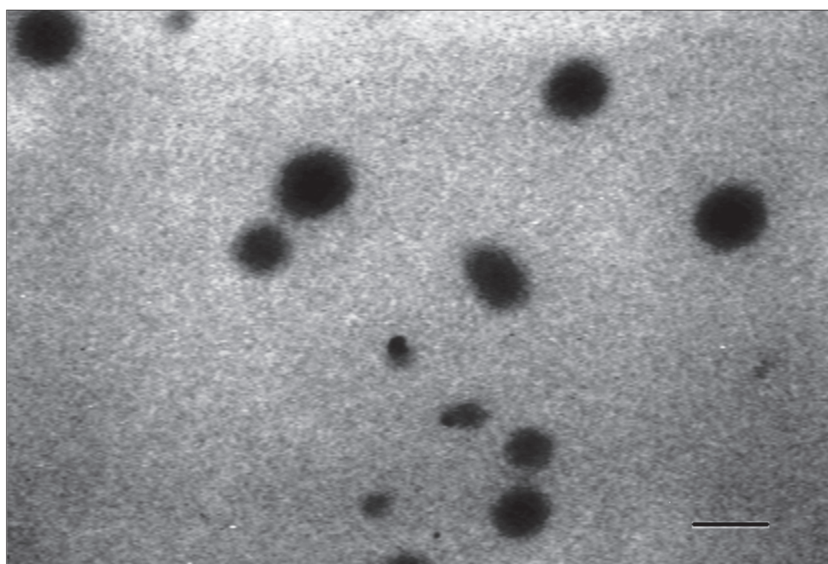

Fig 1. TEM image of nano-chromium (the average diameter was 100 nm)

Şekil 1. Nano-kromun TEM görüntüsü (ortalama çap = 100 nm)

Vaccination

All chickens were immunized intramuscularly with a killed vaccine of Newcastle virus at 8 d of age (Nobilis ND LaSota, Intervet/Schering-Plough Animal Health, Boxmeer, the Netherlands). Live Newcastle disease vaccine was administered orally (drinking water) at 22 d of age.

Performance Parameters

Broilers performance was assessed for mortality rate, BW gain, feed consumption per bird, and feed conversion. The feed conversion ratio was calculated on the basis of feed intake/gain for each replicate. Data were collected during the experimental period (ED21 to ED42).

Organ Weights

On ED28 and ED35 immediately after weighing, 8 birds per group (2 birds per pen) were randomly selected and euthanized by cervical dislocation. At necropsy, heart, liver and lymphoid organs (spleen, and bursa of Fabricius) were then picked up for relative weight determinations, ± 0. 01 g (connective tissue was removed before weighting).

Plasma C3 and C4

At the end of experiment period 8 birds per pen were randomly selected, and blood samples were collected from the wing vein. Blood samples were kept to at 4°C to coagulate, then samples centrifuged at 3.000 × g for 10 min (at 4°C) to separation of serum. Blood serum samples were stored at −20°C until they were analyzed. Serum complement component 3 (C3), and complement component 4 (C4) concentrations were determined by commercial kits (Jiancheng Biological Engineering Research Institute, Nanjing, China, Cat. Nos. E032 and E033). The procedure was carried out according to the advised protocol of the company.

Statistical Analysis

All data were subjected to a one-way ANOVA procedure of SPSS 19.0 for Windows [26], and the differences among means were separated by Duncan's multiple range test. A probability value of less than 0.05 was described to be statistically significant. Because there was no mortality during the experimental period so it has not been analyzed statistically.

RESULTS

Performance Parameters

The effects of different treatments on BW gain, feed intake and feed conversion ratio (FCR) throughout the experiment are presented in Table 2. The control group (TN group) had a higher final BW, daily weight gain and daily feed intake compared with heat stressed groups during

Table 2. *Effects of CrPic and NanoCrPic supplementation on production performance of heat stressed broilers on experimental period (day 21-42)*

Tablo 2. *Deney süresince (21 ile 42. günler arası) ısı stresi uygulanan broiler civcivlerde CrPic ve NanoCrPic katkısı kullanımının üretim performansı üzerine etkileri*

Items	Treatments								SEM
	Control (-)	Control (+)	Cr500	Cr1000	Cr1500	NCr500	NCr1000	NCr1500	
21-28d (1th week)									
Initial weight (day 21) (kg bird^{-1})	0.593	0.661	0.591	0.621	0.619	0.625	0.638	0.615	0.0087
Final weight (day 28) (kg bird^{-1})	0.874^{a}	0.872^{a}	0.837^{ab}	0.855^{ab}	0.832^{ab}	0.805^{b}	0.822^{ab}	0.870^{a}	0.0063*
Daily feed intake (kg bird^{-1})	0.068^{c}	0.083^{a}	0.082^{a}	0.082^{a}	0.077^{ab}	0.072^{bc}	0.081^{a}	0.080^{a}	0.0010**
Daily weight gain (kg bird^{-1})	0.040	0.032	0.031	0.036	0.033	0.035	0.035	0.035	0.0010
FCR	1.72^{b}	2.62^{a}	2.66^{a}	2.28^{ab}	2.36^{ab}	2.07^{ab}	2.32^{ab}	2.29^{ab}	0.0951*
29-35d (2th week)									
Final weight (kg bird^{-1})	1.595^{a}	1.367^{b}	1.437^{b}	1.350^{b}	1.354^{b}	1.325^{b}	1.392^{b}	1.374^{b}	0.0196*
Daily feed intake (kg bird^{-1})	0.154^{a}	0.154^{a}	0.155^{a}	0.149^{ab}	0.148^{ab}	0.140^{b}	0.150^{ab}	0.148^{ab}	0.0011**
Daily weight gain (kg bird^{-1})	0.102^{a}	0.063^{c}	0.092^{ab}	0.069^{bc}	0.075^{bc}	0.066^{bc}	0.081^{bc}	0.068^{bc}	0.0033**
FCR	1.51^{c}	2.44^{a}	1.68^{bc}	2.16^{ab}	1.97^{abc}	2.12^{abc}	1.85^{bc}	2.18^{ab}	0.0756*
36-42d (3th week)									
Daily feed intake (kg bird^{-1})	0.221^{a}	0.167^{bc}	0.179^{b}	0.162^{c}	0.164^{c}	0.165^{bc}	0.165^{bc}	0.170^{bc}	0.0038**
Daily weight gain (kg bird^{-1})	0.082^{ab}	0.064^{ab}	0.071^{ab}	0.070^{ab}	0.070^{ab}	0.067^{ab}	0.059^{b}	0.089^{a}	0.0025**
FCR	2.70^{a}	2.61^{a}	2.52^{ab}	2.31^{ab}	2.34^{ab}	2.46^{ab}	2.80^{a}	1.91^{b}	0.0735*
21-42d (total)									
Final weight (kg bird^{-1})	2.176^{a}	1.763^{c}	1.986^{b}	1.769^{c}	1.840^{bc}	1.730^{c}	1.811^{c}	1.849^{bc}	0.0313*
Daily feed intake (kg bird^{-1})	0.148^{a}	0.135^{bc}	0.139^{b}	0.131^{cd}	0.130^{cd}	0.126^{d}	0.132^{cd}	0.132^{c}	0.0013**
Daily weight gain (kg bird^{-1})	0.075^{a}	0.053^{c}	0.065^{b}	0.058^{bc}	0.059^{bc}	0.056^{bc}	0.058^{bc}	0.064^{b}	0.0015**
FCR	1.97^{b}	2.55^{a}	2.14^{ab}	2.26^{ab}	2.20^{ab}	2.25^{ab}	2.28^{ab}	2.06^{b}	0.0403**

$^{a-d}$ *different superscript letters indicate a significant difference between data presented in the same row, * (P<0.05), ** (P<0.01)*

the experiment period (d 21-42), whereas, all of these parameters improved significantly with the Cr inclusion to diet with different dosage and particle size. Moreover, the FCR value of control group (1.97) was found lower (P<0.01) than that in heat- stressed groups and treatment NanoCrPic 1500 (2.06) had the lowest (P<0.01) FCR value among heat stressed groups. Besides, as there was no mortality during the experimental period it has not been reported.

Organ Weight

The effect of different treatments on organs weight of broilers has been summarized in *Table 3*. Data belongs to two consecutive weeks of experiment (4th to 5th weeks). At the end of the 4th week (one week after CrPic addition), there was a significant difference (P<0.05) between the heart and bursa of Fabricius percentage among experimental treatments. Also, the spleen and liver weights were not affected significantly. As well, in the 5th week of finishing period (d 35), NanoCrPic 500 (T6) had higher liver weight (3.75% of live weight) in comparison with other treatments (P<0.05).

Immune Function

The effects of CrPic and NanoCrPic supplementation on serum complement components in heat stressed broilers are shown in *Table 4*. The results indicated that, serum complement component C3 of experimental broilers was considerably affected by Cr addition. The concentrations of serum complement component C3 in the birds fed 1.500 ppb NanoCrPic diet (0.225 mg/mL) were greater than birds fed chromium free or other levels of Cr and NanoCr diet from d 21 to 42 (P<0.01). No significant differences were observed in serum complement component C4 concentrations among the control and test groups, although C4 values increased in some CrPic and NanoCrPic treatments but not significantly (P>0.05).

DISCUSSION

Heat stress (36°C), applied for 10 h per day from the 21th to the 42nd days of life decreased performance parameters in broilers in the present study. These data are in agreement with those reported elsewhere in similar contexts [5,6,27,28]. Chromium was reported to modulate feed intake of heat stressed chickens [27,29,30], and our findings are corresponded with those documents. Also, the data showed that body weight (P<0.05) and daily weight gain (P<0.01) improved with Cr addition significantly, and these are in agreement with [30]. Concerning to performance observations, it is well established that stress and disease exacerbate urinary excretion of Cr and this occurrence can worth a marginal Cr deficiency [12]. Cr is usually recognized

Table 3. *Effect of CrPic and NanoCrPic supplementation on organs weight (percentage of live body weight) at 28 and 35 days of age*
Tablo 3. *28 ile 35. günler arasında organ ağırlıkları (canlı vücut ağırlık yüzdesi) üzerine CrPic ve NanoCrPic katkısı kullanımının etkileri*

Items	Treatments								SEM
	Contr -	Cont+	Cr500	Cr1000	Cr1500	NCr500	NCr1000	NCr1500	
Day 28(% live weight)									
Heart	0.59^{ab}	0.58^{abc}	0.46^{c}	0.50^{bc}	0.56^{abc}	0.62^{a}	0.57^{abc}	0.52^{abc}	0.0149^{*}
Liver	2.58	2.59	2.40	2.53	2.50	2.77	2.40	2.66	0.0510
Spleen	0.08	0.10	0.10	0.09	0.10	0.07	0.10	0.07	0.0044
Bursa of Fabricius	0.29^{a}	0.22^{ab}	0.23^{ab}	0.23^{ab}	0.22^{ab}	0.23^{ab}	0.29^{a}	0.24^{ab}	0.0110^{*}
Day 35 (% live weight)									
Heart	0.58	0.54	0.46	0.51	0.53	0.54	0.49	0.47	0.0133
Liver	2.66^{b}	2.65^{b}	3.10^{ab}	3.06^{ab}	2.64^{b}	3.75^{a}	2.67^{b}	3.25^{ab}	0.0983^{*}
Spleen	0.07	0.09	0.13	0.08	0.10	0.11	0.11	0.10	0.0067
Bursa of Fabricius	0.21	0.18	0.22	0.19	0.21	0.18	0.17	0.19	0.0093

$^{a-d}$ different superscript letters indicate a significant difference between data presented in the same row (P<0.05)

Table 4. *Effect of CrPic and NanoCrPic supplementation on serum complement components in heat stressed broilers*
Tablo 4. *Isı stresi uygulanan broiler civcivlerde CrPic ve NanoCrPic katkısı kullanımının serum komplement bileşenleri üzerine etkileri*

Items (mg/mL)	Treatments								SEM
	Cont -	Cont +	Cr500	Cr1000	Cr1500	NCr500	NCr1000	NCr1500	
C3	0.197^{ab}	0.173^{b}	0.174^{b}	0.179^{b}	0.181^{b}	0.190^{ab}	0.194^{ab}	0.225^{a}	0.0042^{**}
C4	0.122	0.119	0.117	0.120	0.115	0.117	0.116	0.129	0.0029

$^{a-d}$ different superscript letters indicate a significant difference between data presented in the same row (P<0.01)

as the active component in the Glucose Tolerance Factor (GTF), which increases the sensitivity of tissue receptors to insulin and subsequent increase in glucose uptake by cells and finally increases oxidation of glucose. It was assumed that increased glucose uptake, reduced blood glucose and increased appetite should increase feed intake. Increased feed intake will tend to increase BW gain, because of improving amino acid and other nutrients consumption by tissues and muscle cells and increase protein retention which tends to increased body weight.

In the current study, the NanoCrPic administration resulted in influential effects on feed conversion ratio in the experimental period. It is theorized that when nutrients are digested, large particle size is degraded into small particle size, so they can be absorbed easily through the intestinal mucosa. In addition, the surface area of particles will increase and then enhance the digestion. Therefore, feed at nanoparticle scale may improve intestinal absorption. Some reports have indicated that nanoparticle drugs and minerals could increase absorption [31,32]. Lien *et al*.[33] reported that as compared with regular CrPic, the NanoCrPic significantly increased the CrPic digestibility in rats. According to the description, nanoparticles of chromium absorbed more and easier and made a strong impact.

Significant differences ($P<0.05$) were found in the heart, liver and lymphoid organs (Bursa of Fabricius and spleen) weight of heat stressed chickens treated with general sized Cr and Nano sized Cr. The improvements in lymphoid organs are in agreement with Naghieh *et al*.[30] and Moeini *et al*.[28], although, others reported no significant effects of Cr supplementation on lymphoid organs weight [11,34]. In regards to, Bartlett and Smith [35] suggest that the decrease in lymphoid organ weights might have been as a result of the reduction in feed consumption, so providing fewer nutrients for suitable growth of these organs under heat stress condition. Also, some findings showed that physiological stress is frequently associated with degeneration of lymphoid organs [36] but the effects of Cr on these organs regeneration are not clear yet.

Serum complement component C3 increased significantly ($P<0.01$), with CrPic and NonoCrPic addition to diet of heat stressed broilers and there were lots of reports that indicate improving immune function with Cr inclusion [10,11,28,30,34], and our findings are in agreement with those data. Also Serum complement component C4 increased in some treatments, especially in treatment NanoCrPic 1500 (0.129 mg/mL), but not significantly ($P>0.05$). Bahrami *et al*.[10] and Toghyani *et al*.[34] reported that serum IgG concentration increased in broiler chickens supplemented with Cr under heat-stress conditions. Moreover, Kegley and Spears [37] observed that total IgG increased in feeder calves receiving supplemental chromium nicotinate under stress. In addition, Chang and Mowat [38] and Moonsie-Shageer and Mowat [39] reported that total IgG and IgM increased after transportation stress in calves supplemented with high-Cr yeast. To explain the improvement of immune function, heat stress induce a cascade of neural and hormonal events, beginning with hypothalamic stimulation and the production of corticotrophin-releasing factor, which stimulates the anterior pituitary to release ACTH, and ending with stimulation of adrenal cortical tissue by ACTH to increase the production and release of corticosteroids (e.g. corticosterone and cortisol) primarily corticosterone, in birds [40]. Corticosterone prevents antibody production [41]. Zulkifli *et al*.[42] reported that antibody production in young broiler chicks decreased in heat-stress conditions. This decline might be indirectly owing to an increase in inflammatory cytokines under stress, which stimulates the hypothalamic production of corticotrophin-releasing factor [43]. Chromium supplementation is detected to enhance the immune response, either through a direct effect on the cytokines [17] or through the indirect effect of decreasing the glucocorticosteroid levels [19,20]. Myers *et al*.[44] observed that dietary chromium supplementation has an optimistic effect on the interleukin-6 levels in swine. The exact mechanism by which Cr improves the immune system is not known. However, a reliable result showed that Cr reduces serum cortisol levels. It is possibly not surprising that depletion in serum cortisol content is one of the principal mechanisms by which Cr alleviates heat stress-related depression in immunocompetent of broilers. Additionally, Sahin *et al*.[45] found out that Cr supplementation improved serum insulin concentration while noticeably decreasing corticosterone concentration in laying hens at a low ambient temperature. This is a typical metabolic relation between insulin (anabolic) and corticosterone (catabolic), in which they have opposite effects on metabolism. A Cr deficiency can disrupt the metabolism of carbohydrates and protein and reduce insulin sensitivity in peripheral tissues [29,46]. Dietary Cr supplementation increased the plasma insulin concentration, indicating the physiological part of Cr in empowering the insulin to act as an insulin cofactor [45].

Nanoparticles are of great scientific concern as they are effectively a bridge between bulk materials and atomic or molecular structures. With the knowledge of that the particle, when its dimension is reduced to nanometer size, exhibits new electrical, magnetic, mechanical and biological properties. Previouse reports have shown that nanoparticle drugs and minerals, possibly will increase absorption [31,32]. Lien *et al*.[33] described that as compared with regular CrPic, the NanoCrPic significantly increased the CrPic digestibility in rats. Furthermore, Wang and Xu (25) and Wang *et al*.[47] reported that dietary supplementation with nanoparticle chromium increased the lean ratio, longissimus muscle area and tissue chromium content, while decreasing the fat ratio and back fat thickness in pigs. Zha *et al*.[22] pointed out that Nano size chromium in the rat diet can significantly increase the average body weight gain, feed efficiency and lean mass weight, and

reduce the body fat ratio and serum insulin concentration. Besides, numerous studies have shown that nanoparticles are more inclined to be recognized by the immune system and ingested by immune cells, such as macrophages, monocytes and leukocytes [48]. However, reports concerning nanoparticle chromium supplementation in broiler diets are rare, as only three published reports [23,24,49] were found and there was not a considerable outstand marked study about using nanoparticles chromium in heat stressed broilers. Totally, although all of our findings were reaffirmation of chromium improve impact in heat stressed broilers, there were remarkable differences between normal and Nano sizes of Cr particles in this experiment that can be considered.

We tested the details of Chromium and Nanochromium effects on heat stressed broilers in this study. We concluded that chromium and chromium nanoparticles will be effective in heat stress situations, but the results indicated that nanoparticles may be more effective, although more research is needed to firm further.

REFERENCES

1. Harrison PC, Biellier HV: Physiological response of domestic fowl to abrupt changes of ambient air temperature. *Poult Sci,* 48, 1034-1045, 1969. DOI: 10.3382/ps.0481034

2. Deyhim F, Teeter RG: Sodium and potassium chloride drinking water supplementation effects on acid-base balance and plasma corticosterone in broilers reared in thermoneutral and heat-distressed environments. *Poult Sci,* 70, 2551-2553, 1991. DOI: 10.3382/ps.0702551

3. Lan PT, Sakamoto M, Benno Y: Effects of two probiotic Lactobacillus strains on jejunal and cecal microbiota of broiler chicken under acute heat stress condition as revealed by molecular analysis of 16S rRNA genes. *Microbiol Immunol*, 48, 917-929, 2004. DOI: 10.1111/j.1348-0421.2004.tb03620.x

4. Mashaly MM, Hendricks GL, Kalama MA, Gehad AE, Abbas AO, Patterson PH: Effect of heat stress on production parameters and immune responses of commercial laying hens. *Poult Sci,* 83, 889-894, 2004. DOI: 10.1093/ps/83.6.889

5. Sohail MU, Ijaz A, Yousaf MS, Ashraf K, Zaneb H, Aleem M, Rehman H: Alleviation of cyclic heat stress in broilers by dietary supplementation of mannan-oligosaccharide and Lactobacillus-based probiotic: Dynamics of cortisol, thyroid hormones, cholesterol, C-reactive protein, and humoral immunity. *Poult Sci,* 89, 1934-1938, 2010. DOI: 10.3382/ps.2010-00751

6. Sohail MU, Rahman ZU, Ijaz A, Yousaf MS, Ashraf K, Yaqub T, Zenab H, Anwar H, Rehman H: Single or combined effects of mannan-oligosaccharides and probiotics supplements on the total oxidants, total antioxidants, enzymatic antioxidants, liver enzymes and serum trace minerals in cyclic heat stressed broilers. *Poult Sci,* 90, 2573-2577, 2011. DOI: 10.3382/ps.2011-01502

7. Borges SA, Fischer da Silva AV, Majorka A, Hooge DM, Cummings KR: Physiological responses of broiler chickens to heat stress and dietary electrolyte balance (sodium plus potassium minus chloride, milliequivalents per kilogram). *Poult Sci,* 83, 1551-1558, 2004. DOI: 10.1093/ps/83.9.1551

8. Trout JM, Mashaly MM: The effects of adrenocorticotropic hormone and heat stress on the distribution of lymphocyte populations in immature male chickens. *Poult Sci,* 73, 1694-1698, 1994. DOI: 10.3382/ps.0731694

9. Khajavi M, Rahimi S, Hassan ZM, Kamali MA, Mousavi T: Effect of feed restriction early in life on humoral and cellular immunity of two commercial broiler strains under heat stress conditions. *Br Poult Sci,* 44, 490-497, 2003. DOI: 10.1080/00071660310001598328

10. Bahrami A, Moeini MM, Ghazi SH, Targhibi MR: The effect of different levels of organic and inorganic chromium supplementation on immune function of broiler chicken under heat-stress conditions. *J Appl Poult Res,* 21, 209-215, 2012. DOI: 10.3382/japr.2010-00275

11. Ebrahimzadeh SK, Farhoomand P, Noori K: Immune response of broiler chickens fed diets supplemented with different level of chromium methionine under heat stress conditions. *Asian-Aust J Anim Sci,* 25, 256-260, 2012. DOI: 10.5713/ajas.2011.11217

12. Pechova A, Pavlata L: Chromium as an essential nutrient: A review. *Veterinarni Medicina,* 52 (1): 1-18, 2007.

13. Giri J, Usha KA, Sunita T: Evaluation of the selenium and chromium content of plant foods. *Plant Foods Hum Nutr,* 40, 4959, 1990. DOI: 10.1007/BF02193779

14. Kim S, Han WIK, Choi YJ, Kim YH, Shin IS, Chae BJ: Effect of chromium picolinate on growth performance, carcass composition, and serum traits of broilers fed dietary different levels of crude protein. *Asian Australas J Anim Sci,* 5, 463-470, 1995. DOI: 10.5713/ajas.1995.455

15. Khan RU, Naz S, Dhama K, Saminathan M, Tiwari R, Jeon GJ, Laudadio V, Tufarelli V: Modes of action and beneficial applications of chromium in poultry nutrition, production and health: A review. *Int J Pharmacol,* 10, 357-367, 2014. DOI: 10.3923/ijp.2014.357.367

16. Kegley EB, Spears JW, Brown TT: Effect of shipping and chromium supplementation on performance, immune response, and disease resistance of steers. *J Anim Sci,* 75, 1956-1964, 1997.

17. Borgs P, Mallard BA: Immune-endocrine interactions in agricultural species: Chromium and its effect on health and performance. *Domest Anim Endocrinol,* 15, 431-438, 1998. DOI: 10.1016/S0739-7240(98)00018-6

18. Rajalekshmi M, Sugumar C, Chirakkal H, Ramarao SV: Influence of chromium propionate on the carcass characteristics and immune response of commercial broiler birds under normal rearing conditions. *Poult Sci,* 93, 574-580, 2014. DOI: 10.3382/ps.2013-03373

19. Mirfendereski E, Jahanian R: Effects of dietary organic chromium and vitamin C supplementation on performance, immune responses, blood metabolites, and stress status of laying hens subjected to high stocking density. *Poult Sci,* 94, 281-288, 2015. DOI: 10.3382/ps/peu074

20. Samanta S, Haldar S, Ghosh TK: Production and carcass traits in broiler chickens given diets supplemented with inorganic trivalent chromium and an organic acid blend. *Br Poult Sci,* 49, 155-163, 2008. DOI: 10.1080/00071660801946950

21. Wijnhoven SW, Peijnenburg WJ, Herberts CA, Hagens WI, Oomen AG, Heugens EH, Roszek B, Bisschops J, Gosens I, Van De Meent D, Dekkers S: Nano-silver- A review of available data and knowledge gaps in human and environmental risk assessment. *Nanotoxicology,* 3, 109-138, 2009. DOI: 10.1080/17435390902725914

22. Zha L, Zeng J, Sun S, Deng H, Luo H, Li W: Chromium (III) nanoparticles affect hormone and immune responses in heat-stressed rats. *Biol Trace Elem Res,* 129, 157-169, 2008. DOI: 10.1007/s12011-008-8282-9

23. Sirirat N, Lu JJ, Hung Alex TY, Chen SY, Lien TF: Effects different levels of nanoparticles chromium picolinate supplementation on growth performance, mineral retention, and immune responses in broiler chickens. *J Agri Sci,* 4, 48-58, 2012. DOI: 10.5539/jas.v4n12p48

24. Lin YC, Huang JT, Li MZ, Cheng CY, Lien TF: Effects of supplemental nanoparticle trivalent chromium on the nutrient utilization, growth performance and serum traits of broilers. *J Anim Physiol Anim Nutr,* 99, 59_65, 2015. DOI: 10.1111/jpn.12215

25. Wang MQ, Xu ZR: Effect of chromium nano-particle on growth performance, carcass characteristics, pork quality and tissue chromium in finishing pigs. *Asian-Aust J Anim Sci,* 17, 1118-1122, 2004. DOI: 10.5713/ajas.2004.1118

26. Wilkinson L: "Systat." Wiley Interdisciplinary Reviews: *Comp Stat,* 2, 256-257, 2010. DOI: 10.1002/wics.66

27. Toghyani M, Shivazad M, Gheisari AA, Zarkesh HS: Performance, carcass traits and hematological parameters of heat stressed broiler chicks in response to dietary levels of chromium picolinat. *Int J Poult Sci,* 5, 65-69, 2006. DOI: 10.3923/ijps.2006.65.69

28. Moeini MM, Bahrami A, Ghazi S, Targhibi MR: The effect of different levels of organic and inorganic chromium supplementation on production performance, carcass traits and some blood parameters of broiler chicken under heat stress condition. *Biol Trace Elem Res,* 144, 715-724, 2011. DOI: 10.1007/s12011-011-9116-8

29. Sahin K, Sahin N, Onderci M, Gursu F, Cikim G: Optimal dietary concentration of chromium for alleviating the effect of heat stress on growth, carcass qualities, and some serum metabolites of broiler chickens. *Biol Trace Elem Res*, 89, 53-64, 2002. DOI: 10.1385/BTER:89:1:53

30. Naghieh A, Toghyani M, Gheisari AA, Egbal Saeed S, Mirzazade H: Effect of different sources of chromium on performance and immune responses of broiler chicks. *J Anim Vet Adv,* 9, 354-358, 2010. DOI: 10.3923/javaa.2010.354.358

31. Florence AT, Hillery AM, Hussain N, Jani PU: Nanoparticles as carriers for oral peptide absorption: Studies on particle uptake and fate. *J Control Release,* 36, 39-46, 1995. DOI: 10.1016/0168-3659(95)00059-H

32. Desai MP, Labhasetwar V, Walter E, Levy RJ, Amidon GL: The mechanism of uptake of biodegradable microparticles in caco-2 cell is size dependent. *Pharm Res,* 14, 1568-1573, 1997. DOI: 10.1023/A:1012126301290

33. Lien TF, Yeh HS, Lu FY, Fu CM: Nanoparticles of chromium picolinate enhance chromium digestibility and absorption. *J Sci Food Agric,* 89, 1164-1167, 2009. DOI: 10.1002/jsfa.3569

34. Toghyani M, Zarkesh S, Shivazad M, Gheisari A: Immune responses of broiler chicks fed chromium picolinate in heat stress condition. *J Poult Sci,* 44, 330-334, 2007. DOI: 10.2141/jpsa.44.330

35. Bartlett JR, Smith MO: Effects of different levels of zinc on the performance and immunocompetence of broilers under heat stress. *Poult Sci,* 82, 1580-1588, 2003. DOI: 10.1093/ps/82.10.1580

36. Thaxton P, Siegel HS: Depression of secondary immunity by high environmental temperature. *Poult Sci,* 51, 1519-1526, 1972. DOI: 10.3382/ps.0511519

37. Kegley EB, Spears JW: Immune response, glucose metabolism, and performance of stressed feeder calves fed inorganic or organic chromium. *J Anim Sci,* 73, 2721-2726, 1995.

38. Chang X, Mowat DN: Supplemental chromium for stressed and growing feeder calves. *J Anim Sci,* 70, 559-565, 1992.

39. Moonsie-Shageer S, Mowat DN: Effect of level of supplemental chromium on performance, serum constituents, and immune status of stressed feeder calves. *J Anim Sci,* 71, 232-238, 1993.

40. Siegel HS: Stress, strains and resistance. *Br Poult Sci,* 36, 3-22, 1995. DOI: 10.1080/00071669508417748

41. Gross WB: Effect of short-term exposure of chickens to corticosterone on resistance to challenge exposure with *Escherichia coli* and antibody response to sheep erythrocytes. *Am J Vet Res,* 53 (3): 291-293, 1992.

42. Zulkifli I, Che Norma MT, Israf DA, Omar AR: The effect of early age feed restriction on subsequent response to high environmental temperatures in female broiler chickens. *Poult Sci,* 79, 1401-1407, 2000. DOI: 10.1093/ps/79.10.1401

43. Ogle CK, Valente JF, Guo X, Li BG, Ogle JD, Alexander JW: Thermal injury induces the development of inflammatory macrophages from non-adherent bone marrow cells. *Inflammation,* 21, 569-582, 1997. DOI: 10.1023/A:1027377904641

44. Myers MJ, Farrell DE, Evock-Clover CM, Cpoe CV, Henderson M, Steel NC: Effect of recombinant growth hormone and chromium picolinate on cytokine production and growth performance in swine. *Pathobiology*, 63, 283-287, 1995. DOI: 10.1159/000163962

45. Sahin K, Küçük O, Sahin N: Effects of dietary chromium picolinate supplementation on performance, insulin and corticosterone in laying hens under low ambient temperature. *J Anim Physiol Anim Nutr,* 85, 142-147, 2001. DOI: 10.1046/j.1439-0396.2001.00314.x

46. Sahin K, Sahin N, Küçük O: Effects of chromium, and ascorbic acid supplementation on growth, carcass traits, serum metabolites, and antioxidant status of broiler chickens reared at a high ambient temperature (32°C). *Nutr Res,* 23, 225238, 2003. DOI: 10.1016/S0271-5317(02)00513-4

47. Wang MQ, Xu ZR, Zha LY, Lindemann MD: Effects of chromium nanocomposite supplementation on blood metabolites, endocrine parameters and immune traits in finishing pigs. *Anim Feed Sci Technol,* 139, 69-80, 2007. DOI: 10.1016/j.anifeedsci.2006.12.004

48. Dobrovolskaia MA, Aggarwal P, Hall JB, McNeil SE: Preclinical studies to understand nanoparticle interaction with the immune system and its potential effects on nanoparticle biodistribution. *Mol Pharmacol,* 5, 487-495, 2008. DOI: 10.1021/mp800032f

49. Sirirat N, Lu JJ, Hung Alex TY, Lien TF: Effect of different levels of nanoparticles chromium picolinate supplementation on performance, egg quality, mineral retention, and tissues minerals accumulation in layer chickens. *J Agri Sci*, 5, 150-159, 2013. DOI: 10.5539/jas.v5n2p150

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Research Article

Increased Expressions of eNOS and iNOS Correlate with Apoptosis of Diabetic Nephropathy in Streptozotocin-induced Type 1 Diabetic Rats [1] [2]

Güngör Çağdaş DİNÇEL [1] Serkan YILDIRIM [2]

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1 Laboratory and Veterinary Health Program, Siran Mustafa Beyaz Vocational School, University of Gumushane, TR-29700 Gümüşhane - TURKEY

2 Department of Pathology, Faculty of Veterinary Medicine, University of Ataturk, TR-25000 Erzurum - TURKEY

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Abstract

The present study was designed to evaluate the effects of high level of nitric oxide (NO) production and oxidative stress (OS) on nephropathology and to identify whether NO and OS have any correlation with apoptosis seen in diabetic kidney, elucidating the underlying mechanism(s) involved in the development of nephropathology in streptozotocin (STZ)-induced diabetic rats. Expression levels of caspase 3, caspase 9, endothelial nitric oxide synthase (eNOS), inducible nitric oxide synthase (iNOS), 8-hydroxy-2'-deoxyguanosine (8-OHdG), for the detection of oxidative damage to DNA, were examined in diabetic kidney tissues. Results of the study revealed that the levels of 8-OHdG ($P<0.005$), eNOS ($P<0.005$), iNOS ($P<0.005$), caspase 3 ($P<0.005$) and caspase 9 ($P<0.005$) were remarkably higher in diabetic kidney tissues than in controls. In addition, STZ-treated animals showed significant loss of body weight and renal enlargement. It was suggested that apoptosis, OS and increased NO levels are involved in the pathogenesis of diabetic nephropathy. The results also strongly suggested that STZ-induced apoptosis through activation of the intrinsic pathway and that might be most likely related to increased NO levels. Moreover, high NO production was not only mediated by eNOS but also by iNOS. Increased NO production may contribute to hyperfiltration and microalbuminuria in early diabetic nephropathy. Furthermore, expression of 8-OHdG might give an idea of the progress and may be essential as it has a diagnostic significance for this disease. In conclusion, we believe that eNOS and iNOS overexpressions induce diabetic nephropathy by mediating apoptosis in STZ-induced rats.

Keywords: *Diabetic nephropathy, Type 1 diabetes mellitus, Nitric oxide, Apoptosis, 8-OHdG*

Streptozotosin Kaynaklı Tip 1 Diyabetik Sıçanlarda Meydana Gelen Nefropatilerde Artan eNOS ve iNOS Sunumlarının Apoptozisle İlişkilendirilmesi

Özet

Diyabetik nefropatilerin patogenezi uzun yıllardır çalışılan fakat hala tam açıklığa kavuşmuş bir konu değildir. Bu çalışma streptozotocin (STZ) ile tetiklenmiş diyabetik sıçanlarda nitrik oksit (NO) üretiminin ve oksidatif stresin (OS) nefropatoloji/nefrodejenerasyonların üzerine olan etkilerinin ve bu faktörlerin apoptozisle bir ilişkisinin olup olmadığının araştırılması için tasarlanmıştır. Bu çalışmada kaspaz 3, kaspaz 9, endotelyal nitrik oksit sentaz (eNOS), indüklenebilir nitrik oksit sentaz (iNOS) ve 8-hydroxy-2\'-deoxyguanosine (8-OHdG) sunumları diyabetik böbrek dokularında araştırıldı. Çalışma sonuçlarında, 8-OHdG ($P<0.005$), eNOS ($P<0.005$), iNOS ($P<0.005$), kaspaz 3 ($P<0.005$) and kaspaz 9 ($P<0.005$) sunumlarının diyabetik böbrek dokularında önemli düzeyde arttığı görüldü. Bununla beraber diyabetik hayvanlarda kilo kaybı ve böbrek boyutlarında artış tespit edildi. Bu çalışmada elde edilen bulgulara göre OS ve yükselmiş NO seviyelerinin diyabetik nefropatilerin patogenezinde önemli bir rolünün olduğu düşünülmektedir. Ayrıca, STZ ile tetiklenen apoptozisin iç yolakla gerçekleştiği ve yüksek düzeyde üretilen NO'nun apoptozis düzeyini arttırdığı düşünülmektedir. Bununla beraber NO düzeylerinin artışına sadece eNOS değil iNOS'un da önemli katkı sağladığı görülmektedir. Erken diyabetik nefropatilerde NO'nun hiperfiltrasyona ve mikroalbuminüriye katkı sağladığı düşünülmektedir. 8-OHdG'nin tanısal bir öneminin olduğu ve hastalığın takibinde fikir vereceği düşünülmektedir. Sonuç olarak, diyabetik sıçan modellerinde eNOS ve iNOS'un aşırı salınımlarının apoptozisi tetikleyerek diyabetik nefropatilere neden olduğu düşünülmektedir.

Anahtar sözcükler: *Diyabetik nefropati, Tip 1 diyabetes mellitus, Nitric oksit, Apoptozis, 8-OHdG*

İletişim (Correspondence)

+90 532 2897255

gcdincel@yahoo.com.tr

INTRODUCTION

Diabetes is a metabolic disorder characterized by specific complications such as diabetic nephropathy that causes long-term dysfunction and failure in various organs and tissues [1,2]. Microvascular complications that may occur in diabetic nephropathy may result in end-stage renal disease, further requiring dialysis or transplantation [1,2]. Pathogenesis of nephropathies related to hyperglycemia is an issue that has been studied in the recent years but has still not been fully clarified.

Apoptosis occurs by activation of intrinsic (mitochondrial) and extrinsic (death receptor-mediated) pathways [3]. For the formation of apoptosome complex, initiator procaspase 9 is needed [3]. In intrinsic apoptosis apoptosome complex cleaves initiator procaspase 9 and activates it. Apoptosis is induced when cleaved caspase 9 activates effector caspase 3, caspase 6 and caspase 7 [4-6]. Oxidative stress (OS) is known to induce damage to the mtDNA. If the antioxidative and the DNA damage repair systems are insufficient, cellular dysfunction and ultimately, apoptosis occur [7]. High glucose concentrations were previously defined to increase OS, causing apoptosis in HK2 cells [8].

NO synthesized from L-arginine by NOS isozyme is known to trigger apoptosis [9-11]. NO mediated cytotoxicity was first defined in macrophages and later on high concentration of NO was shown to cause apoptosis [9]. It is explained that NO causes consecutive loss in mitochondrial membrane potential and thus induces cytochrome c release to the cytosol [10,12,13].

Although there are studies regarding hyperglycemia related OS and apoptosis, contribution of NO production and OS related apoptosis in the pathogenesis of diabetic nephropathy is not clear yet. Moreover, the role of nephrodegeneration in the pathogenesis of diabetic nephropathy is not fully defined yet. The purpose of this study was to investigate the relationship between apoptosis and the severity of nephropathological changes that occur in the kidney tissues of rats in which we established type 1 diabetes mellitus. Moreover, the relationship between NO production and hyperglycemia related apoptosis in kidney was studied.

MATERIAL and METHODS

Ethics Statement

This study was performed in strict accordance with the recommendations of the National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs). The experimental protocol was approved by the Committee on the Ethics of Animal Experiments at Ataturk University (Permit Number: 46-02.03/2014).

Experimental Animals

Twenty male Wistar albino rats weighing 250-300 g were randomly allotted to two experimental groups (n = 10 per group). Animals were housed in a well-ventilated and air-conditioned area provided with independently adjustable light-dark cycle (12 h light/12 h dark cycle) and temperature regulation systems. The rooms and animal cages were cleaned daily, and the animals were provided with fresh food and water ad libitum on a daily basis.

Induction of STZ Model of Diabetes

Type 1 diabetes was induced in the rats (diabetic group) by a single intraperitoneal injection of streptozotocin (STZ) (65 mg/kg body weight) dissolved in 0.1 mM sodium citrate, pH 4.5, while the normal control rats (nondiabetic group) were injected with the buffer only. The development of hyperglycemia in rats was confirmed by blood glucose evaluation. Blood glucose was determined by using an automatic glucometer (ACCU-CHEK Active, Roche Diagnostics Ltd, Germany). Plasma glucose level of the animals higher than 250 mg/dL were considered hyperglycemic. This animals were selected for studies and this study covers acute period.

Necropsy and Histopathologic Examination

At the end of 20 days of experiment period, animals were sacrificed by decapitation and kidneys were quickly removed and processed for histopathology and immunohistochemistry analyses. Kidney tissue samples were fixed in 10% neutral buffered formaldehyde for 48 h and washed under tap water overnight. Following routine tissue preparation procedures, tissue samples were dehydrated through graded series of alcohol and xylene and embedded in paraffin blocks. Paraffin serial sections were cut at a thickness of 4-5 µm. Kidneys were sectioned to 4-5 µm thickness, stained with H&E, and examined under a light microscope (Olympus BX51 and DP25 digital camera, Japan).

Immunoperoxidase Examinations

Immunohistochemistry was performed to investigate eNOS, iNOS, caspase 3, caspase 9 and 8-OHdG expressions. Commercial antibodies were visualized on 4- to 5-µm-thick paraffin sections using an indirect streptavidin/biotin immunoperoxidase kit (HRP; Thermo Scientific, USA). All steps were carried out following the procedure described by Dincel and Kul, 2015 [14]. Tissue sections were incubated with the primary antibody (eNOS, iNOS, caspase 3, caspase 9 and 8-OHdG) for 60 min. Finally, sections were incubated in aminoethyl carbazole chromogen (Thermo Scientific, USA) for 5-10 min to induce the color reaction. Mayer's hematoxylin was applied as a counterstain for 30 sec. As a control for non-specific endogenous peroxidase and biotin activities in each test, the primary antibody step was omitted.

Quantitative Histomorphometric Analysis and Statistics

The density of positive staining was measured using a computerized image system composed of a Leica CCD camera DFC420 (Leica Microsystems Imaging Solutions, Ltd., Cambridge, UK), connected to a Lecia DM4000 B microscope (Leica Microsystems Imaging Solutions, Ltd.) was used according to the procedure described by Dincel and Atmaca [15]. The pictures of five random fields selected and consecutive 20x objective microscopic fields were captured by the Leica QWin Plus v3 software (Leica Microsystems Imaging Solutions) at a setting identical to the image system. For the quantification of mean was quantified as the eNOS-, iNOS-, caspase 3-, caspase 9- and 8-OHdG-positive area/total area were measured and calculated by Leica Qwin Plus on the pictures. Data were statistically described in terms of mean and standard deviation (mean±SD) for area %. For evaluating the non-parametric data, Mann-Whitney U-test was performed to compare eNOS, iNOS, caspase 3, caspase 9 and 8-OHdG immunoreactive cells and immunopositively stained areas in the diabetic animals versus the healthy controls. A P value of <0.05 was considered significant. The data were presented as means ± SD. All statistical analyses and graphs were prepared using GraphPad Prism version 6.0 (GraphPad Software, La Jolla California, USA).

RESULTS

Histopathologic Findings

Hematoxylin and eosin (H&E)-stained kidney sections from control group animals exhibited normal architecture *(Fig. 1A)*. The focal endocapillary hypercellularity in glomeruli and calcification on tubules were observed in diabetic nephropathy group *(Fig. 1A)*. Moderate to severe lymphocyte infiltration with tubular degeneration/necrosis were detected in kidney from diabetic nephropathy group *(Fig. 1A)*. In addition the focal mesangial hypercellularity and focal microcystic dilations of tubules were shown in kidney. Morover, the number of atrophic tubules and acute renal hyperemia were also observed in kidney from diabetic nephropathy group *(Fig. 1B)*.

Immunoperoxidase Findings

In this study, eNOS and iNOS, caspase 3 and caspase 9 and 8-OHdG (cytoplasmic) expressions in the kidney were higher in diabetic nephropathy group than in healthy control animals (P<0.005). Statistical analysis of the data on eNOS, iNOS, caspase 3, caspase 9 and 8-OHdG expressions in the kidney, measured by immunostaining in all the groups, are listed in *Table 1*.

8-hydroxy-2′-deoxyguanosine (8-OHdG) Expression

Fairly weak immunoreactivity for 8-hydroxy-2′-deoxyguanosine (8-OHdG) was observed in some cortical tubules *(Fig. 2A)* and medulla cells in healthy control animals *(Fig. 2B)*. Increased 8-OHdG expression was observed only cytoplasmic compartment of cortical tubules, medulla, tubular capillaries and interstitial vessels *(Fig. 2C,D)*. Cytoplasmic immunoreaction was localized in some degenerative/necrotic cortical tubules cells *(Fig. 2C)*. 8-OHdG expressions in the kidney were

Table 1. *Immunoperoxidase test results and statistical data*
Tablo 1. *İmmünopereksidaz test sonuçları ve istatistiksel verileri*

Animals	N	eNOS		P <	iNOS		P <	Caspase 3		P <	Caspase 9		P <	8-OHdG		P <
		Mean	Sd		Mean	Sd		Mean	Sd		Mean	Sd		Mean	Sd	
Control animals	10	1.970	0.323	0.001	1.975	0.284	0.001	1.146	0.481	0.001	1.520	0.345	0.001	2.534	0.335	0.001
STZ-treated animals	10	2.939	0.527		2.784	0.390		4.207	0.955		4.182	0.983		4.722	0.318	

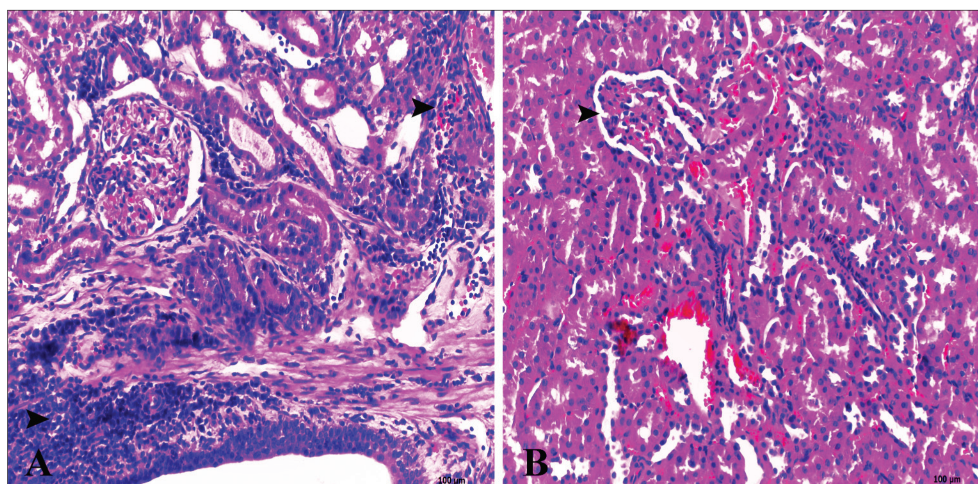


Fig 1. The focal mesangial hypercellularity in glomeruli, moderate to severe lymphocyte infiltration *(arrowheads)* (A) and atrophic/degenerative glomeruli *(arrowhead)*, H&E (B)

Şekil 1. Glomeruluslarda fokal mezanşial hücresellik artışı, orta düzeyden şiddetliye dönen lenfosit infiltrasyonları *(okbaşları)* (A) ve atrofik/dejeneratif glomerulus *(okbaşı)*, H&E (B)

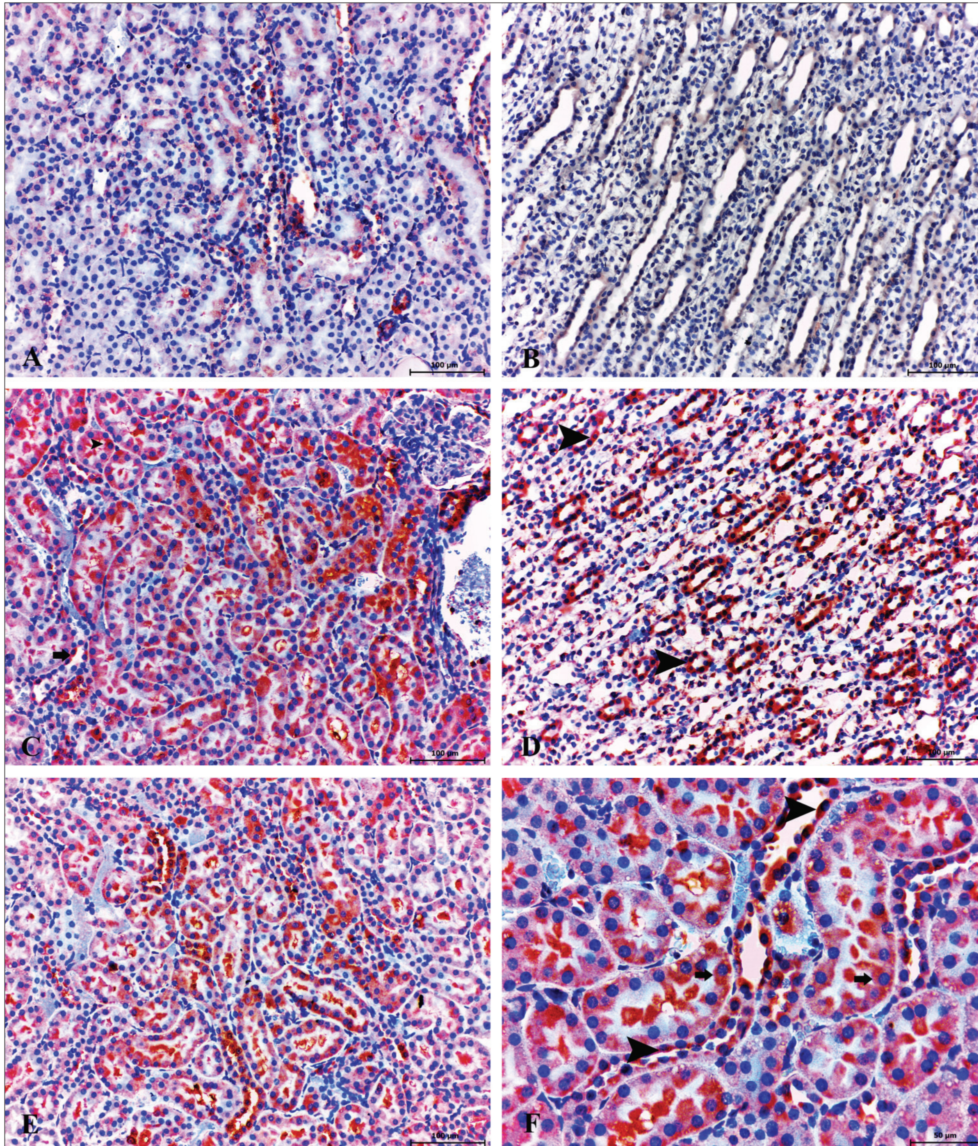


Fig 2. Healthy control group; low expression of 8-OHdG in macula densa other tubular segments and collecting ducts. (A,B) Increased cytoplasmic expression of 8-OHdG in endothelial cells, distal *(arrow)* and proximal *(arrowhead)* convoluted tubules (C), collecting ducts *(arrowheads)* (D), necrotic/degenerative distal *(arrowheads)* and proximal *(arrows)* convoluted tubules. (E,F), ABC technique (anti-8-OHdG)

Şekil 2. Sağlıklı kontrol grup, makula densa, diğer tubular segmentlerdeki ve toplayıcı kanallardaki zayıf 8-OHdG sunumları. (A,B) Endotel hücrelerde, distal *(ok)* ve proksimal *(okbaşı)* konvolüt tubullerde (C), toplayıcı kanallarda *(okbaşları)* (D), Nekrotik/dejeneratif distal *(okbaşları)* ve proksimal *(oklar)* konvolüt tubüllerde artmış sitoplazmik 8-OHdG sunumları. (E,F), ABC teknik (anti-8-OHdG)

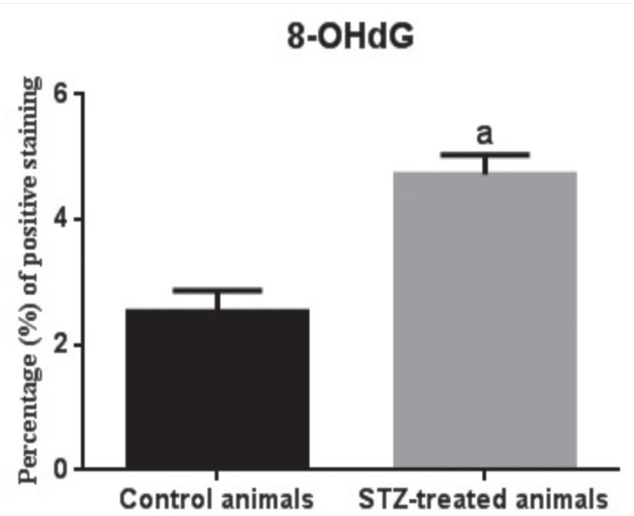


Fig 3. Comparison of 8-OHdG immunopositivity. Statistical difference is indicated as letters. "a" represent values statistically higher than control group

Şekil 3. 8-OHdG immünopozitifliklerin karşılaştırılması. İstatistiksel farklar 'a' ile gösterilmiştir. 'a' istatistiksel olarak kontrol grubundan yüksek olduğunu vurgulamaktadır

statistically higher in in diabetic nephropathy group than in healthy control animals ($P<0.005$) *(Fig. 3)*.

The most conspicuous finding of the present study was that 8-OHdG expression was markedly increased in

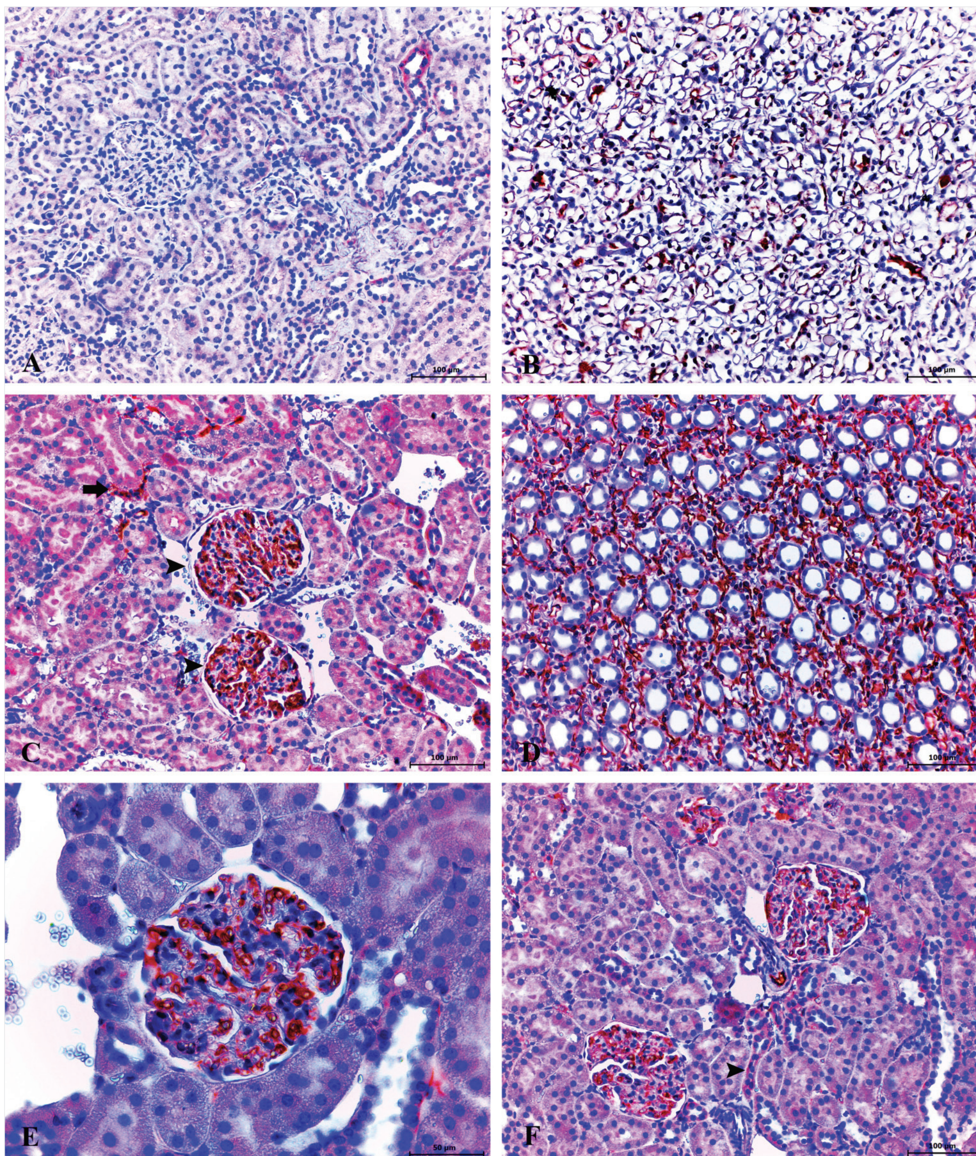


Fig 4. Healthy control group; very slight expression of eNOS in distal/proximal convoluted tubules and glomerulus (A,B) Strong expression of eNOS in glomerulus *(arrowheads)*, distal convoluted tubules *(arrow)* (C), medullary endothelial cells (D), normal/atrophic glomerulus, distal convoluted tubules *(arrowhead)* (E,F). ABC technique (anti-eNOS)

Şekil 4. Sağlıklı kontrol grup; distal/proksimal konvolüt tubuller ve glomeruluslarda zayıf eNOS sunumları (A,B) Glomerus *(okbaşları)* ve distal konvolüt tubullerde *(ok)* (C), medullar endotelyal hücrelerde (D), normal/atrofik glomerulus and distal konvolüt tubullerde *(okbaşı)* güçlü eNOS sunumları (E,F). ABC teknik (anti-eNOS)

proximal and distal convoluted tubules and medullary cells *(Fig. 2E,F)*.

This study showed that 8-OHdG, pivotal marker for measuring the effect of endogenous oxidative damage to DNA, was a good biomarker for risk assessment of this disease. In addition to this biomarker might used to estimate the DNA damage in humans/animals after exposure to hyperglycemia.

Endothelial Nitric Oxide Synthases (eNOS) and Inducible Nitric Oxide Synthases (iNOS) Expressions

Weak immunoreactivity for eNOS and iNOS was observed in some cortical tubules and medulla cells in healthy control animals *(Fig. 4A,B) (Fig. 5A)*. eNOS expressions increased significantly in the vascular endothelial and capillaries of the glomerulus and some necrotic/degenerative glomerulus *(Fig. 4C,D,E,F)*, which was also significantly higher in the diabetic group than the levels in the healthy control group ($P<0.005$) *(Fig. 6)*. Importantly, strong eNOS expression markedly increased in distal convoluted tubules in diabetic kidney tissues. iNOS expressions especially increased significantly in the capillaries of the glomerulus, cortical, medullar tubules and some necrotic/degenerative glomerulus *(Fig. 5B,C,D)*, which was also significantly higher than the levels in the healthy control group ($P<0.005$) *(Fig. 6)*. Another conspicuous finding of the present study was that strong iNOS expression markedly increased in the infiltrating mononuclear cells and proximal convoluted tubules in diabetic kidney tissues.

Caspase 3 and Caspase 9 Expressions

Fairly weak immunoreactivity for caspase 3 and caspase 9 was observed in some cortical tubules and medulla cells in healthy control animals *(Fig. 7A,B) (Fig. 8A,B)*. Strong caspase 3 and caspase 9 immunoreactivity is observed in glomerular capillaries *(Fig. 7F) (Fig. 8C,F)* tubular capillaries

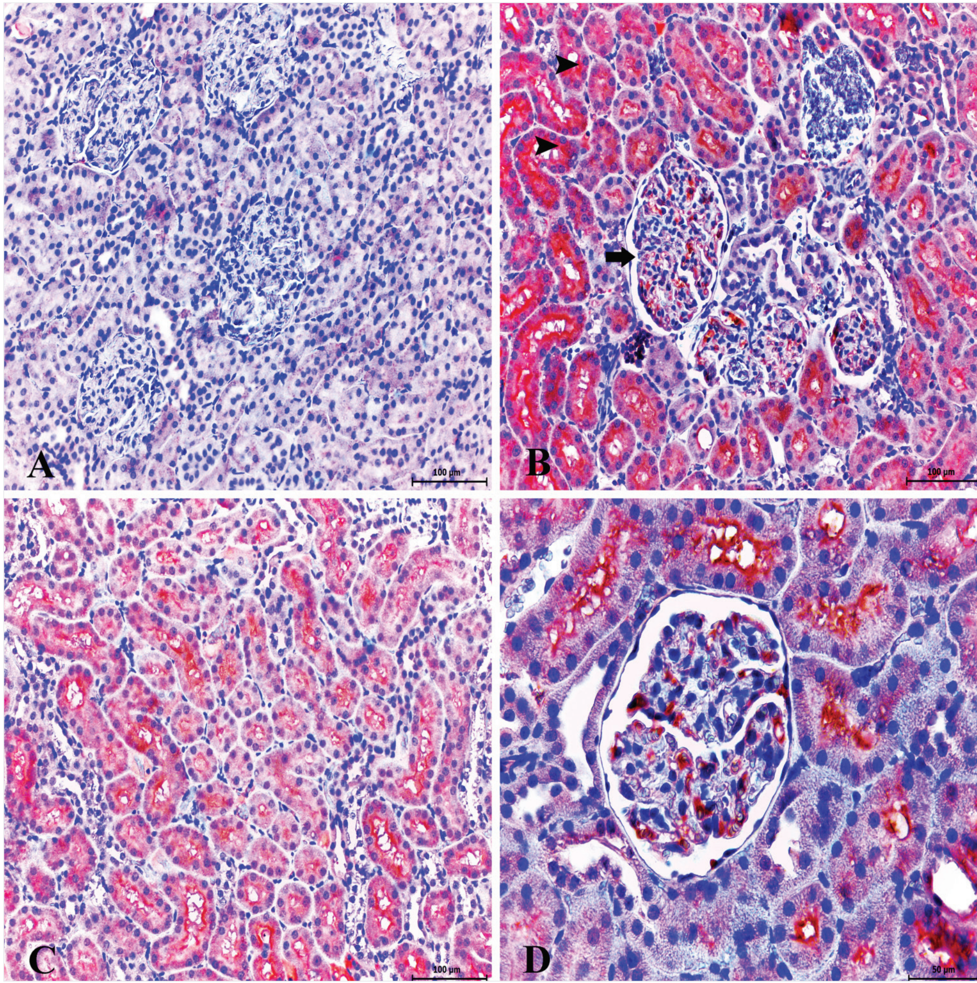
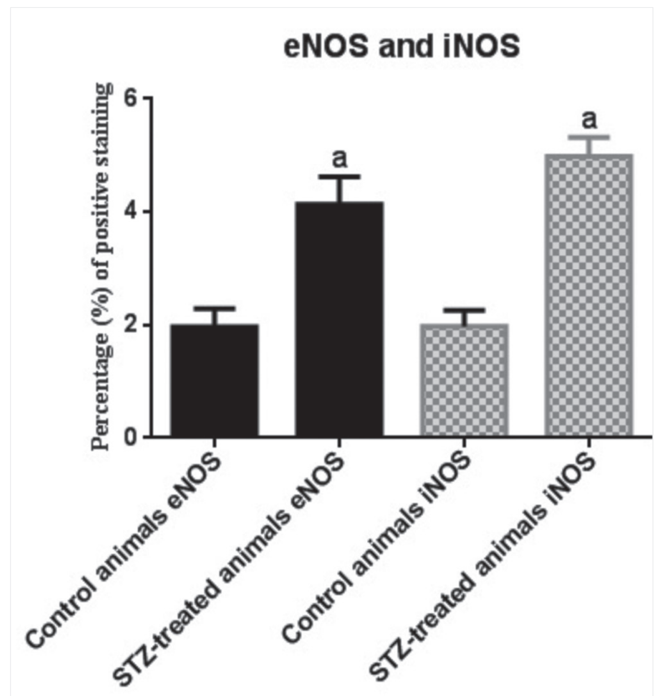


Fig 5. Healthy control group; very slight expression of iNOS in distal/proximal convoluted tubules (A) Strong expression of iNOS in glomerulus *(arrow)* and proximal convoluted tubules *(arrowheads)* (B,C,D). ABC technique (anti-iNOS)

Şekil 5. Sağlıklı kontrol grup; distal/proksimal konvolüt tubüllerde hafif iNOS sunumları (A) Glomerulus *(ok)* ve proksimal konvolüt tubüllerde *(okbaşları)* güçlü iNOS sunumları (B,C,D). ABC teknik (anti-iNOS)



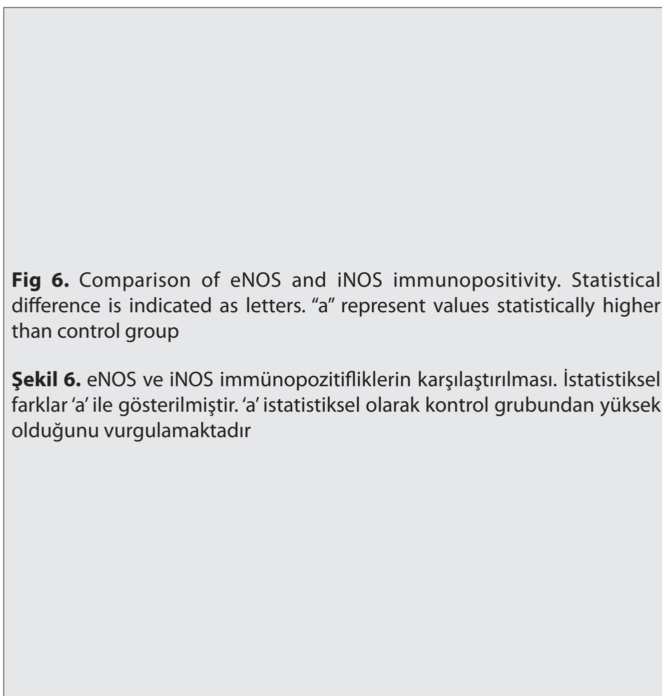


Fig 6. Comparison of eNOS and iNOS immunopositivity. Statistical difference is indicated as letters. "a" represent values statistically higher than control group

Şekil 6. eNOS ve iNOS immünopozitifliklerin karşılaştırılması. İstatistiksel farklar 'a' ile gösterilmiştir. 'a' istatistiksel olarak kontrol grubundan yüksek olduğunu vurgulamaktadır

(Fig. 7D,E) (Fig. 8D,E) medulla and cortical tubules *(Fig. 7C) (Fig. 8D,F)*, which was also significantly higher than the levels in the healthy control group (*P*<0.005) *(Fig. 9)*. Importantly, strong caspase 3 and caspase 9 expressions

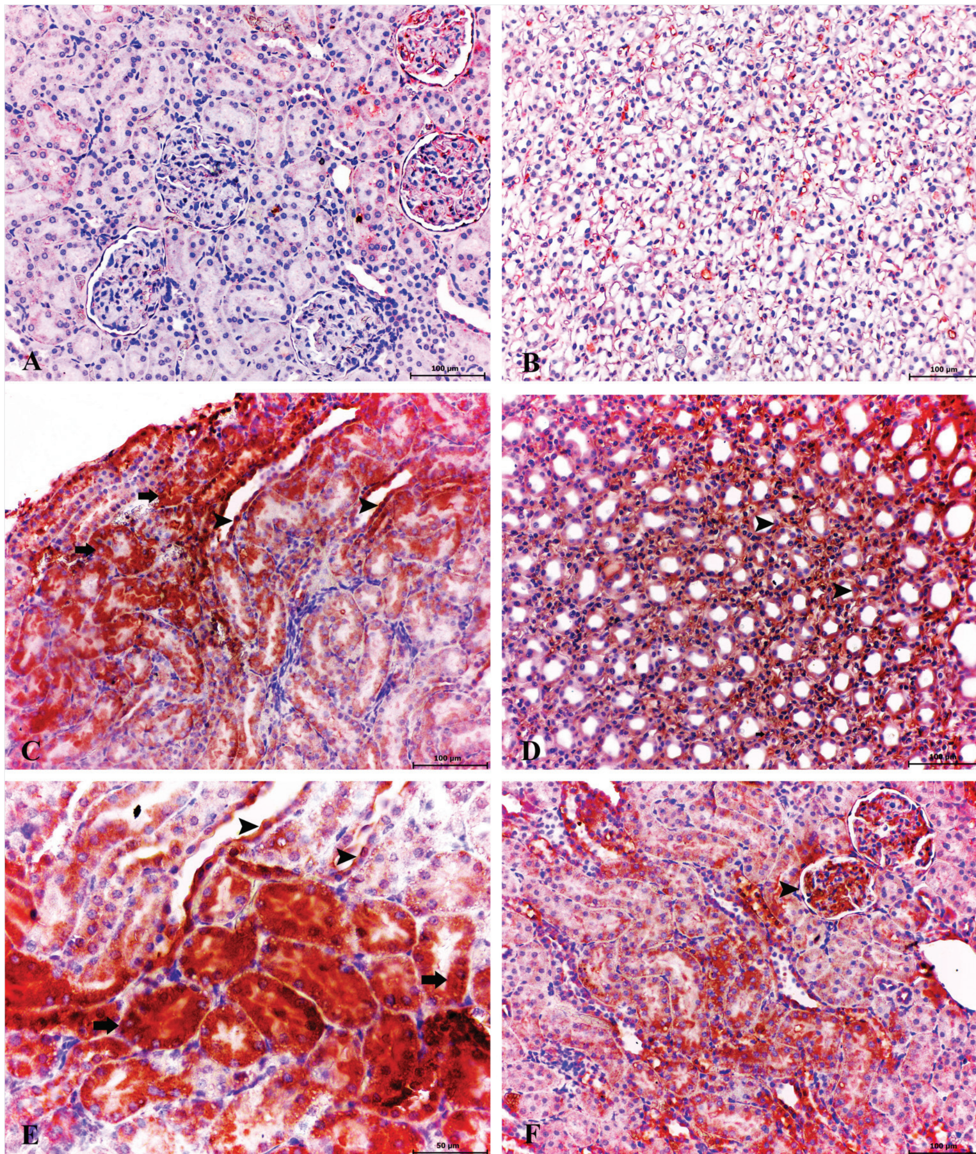


Fig 7. Healthy control group; very slight expression of caspase 3 in proximal convoluted tubules, glomerulus (A) collecting ducts (B) Strong expression of caspase 3 in distal *(arrowheads)* and proximal *(arrows)* convoluted tubules, collecting ducts *(arrow-heads)*, endothelial cells *(arrow)* (C,D,E) and glomerulus *(arrowhead)* (F). ABC technique (anti-caspase 3)

Şekil 7. Sağlıklı kontrol grup; glomerulus, proksimal konvolüt tubüllerde (A) ve toplayıcı kanallarda (B) zayıf kaspaz 3 sunumları. Distal *(okbaşları)* ve proksimal *(oklar)* konvolüt tubullerde, toplayıcı kanallar *(okbaşları)*, endotel hücrelerde *(ok)* (C,D,E) ve glomeruluslarda *(okbaşı)* güçlü kaspaz 3 sunumları. ABC teknik (anti-Kaspaz 3)

markedly increased in proximal and distal convoluted tubules in diabetic kidney tissues.

DISCUSSION

There are many unanswered questions regarding the pathogenesis of hyperglycemia related nephropathies. In this study, we show that NO has severe immunopathological roles and it is not only synthesized by eNOS but also by iNOS. Moreover, it was shown that hyperglycemia induces apoptosis in diabetic kidneys and the greatest contribution to this is provided by high level of NO production and OS.

NO at physiological concentrations inhibits formation of apoptosome complex by blocking cytochrome c release in double membrane of mitochondria [16]. Thus, intrinsic apoptotic pathway activation is prevented. On the other hand, above physiological limits NO was shown to trigger apoptosis [11,17,18]. Moreover, NO plays a role in the modulation of the systemic and the renal circulation [19-21]. Therefore, it is very important that NO is kept at physiological limits for the continuity of cellular life. In this study, NO at very high concentrations was shown to be a very important factor in the induction of intrinsic apoptotic pathways. eNOS and iNOS from endothelial, tubular, mesenchymal cells and infiltrating mononuclear cells are responsible for producing pathological levels of NO. According to this situation, apoptosis that is induced by NO and OS was determined as an important factor in the pathogenesis of diabetic nephropathy related degeneration, necrosis and atrophy.

When intrinsic apoptosis pathways are activated, formation of apoptosome complex triggers activation of initiator caspase 9 [3]. In some of our diabetic animals we observed severe caspase 9 activation in kidney while caspase 3 activation was found at control levels. This situation suggests that in these animals apoptosis is triggered through intrinsic pathways. Interestingly,

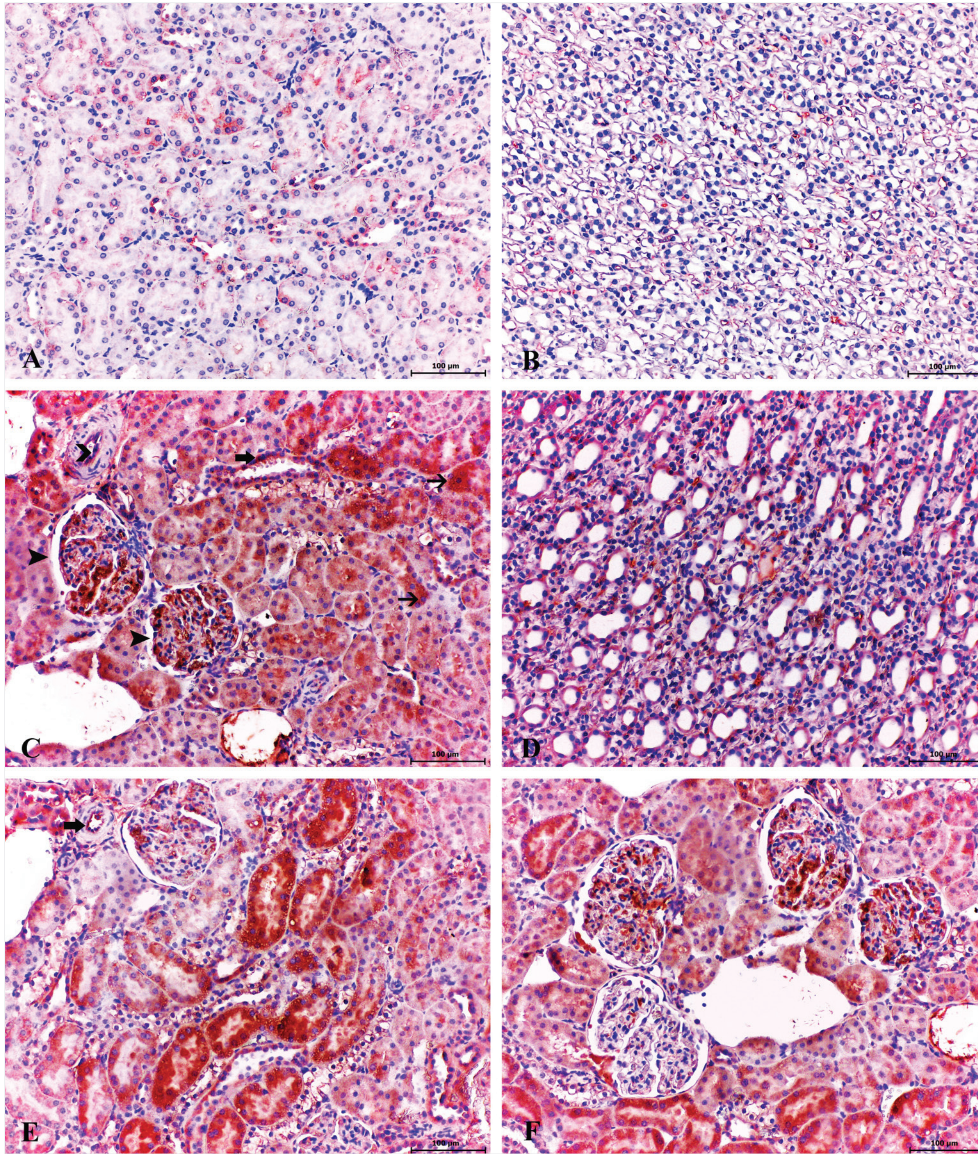


Fig 8. Healthy control group; very slight expression of caspase 9 in distal, proximal convoluted tubules and collecting ducts (A,B) Strong expression of caspase 9 in glomerulus *(arrowheads),* distal *(arrow)*/proximal *(thin arrow)* convoluted tubules, endothelial cells *(thin arrowhead)* (C), collecting ducts (D), endothelial cells *(arrow)* and convoluted tubules (E,F). ABC technique (anti-caspase 9)

Şekil 8. Sağlıklı kontrol grup; distal, proksimal konvolüt tubullerde ve toplayıcı kanallarda zayıf kaspaz 9 sunumları (A,B) Glomerulus *(okbaşları),* distal *(ok)*/proksimal *(ince ok)* konvolüt tubuller ve endotel hücrelerde *(ince okbaşı)* (C), toplayıcı kanallarda (D), endotelyal hücreler *(ok)* ve konvolüt tubullerde güçlü kaspaz 9 sunumları (E,F). ABC teknik (anti-Kaspaz 9)

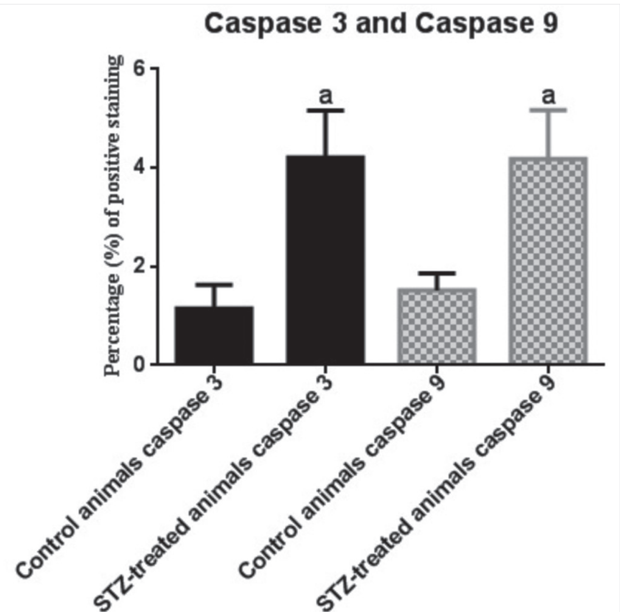


Fig 9. Comparison of caspase 3 and caspase 9 immunopositivity. Statistical difference is indicated as letters. "a" represent values statistically higher than control group

Şekil 9. Kaspaz 3 ve kaspaz 9 immünopozitifliklerin karşılaştırılması. İstatistiksel farklar 'a' ile gösterilmiştir. 'a' istatistiksel olarak kontrol grubundan yüksek olduğunu vurgulamaktadır

in some cases, we detected severe levels of caspase 3 activation while caspase 9 activation was at control levels, suggesting that apoptosis observed in these animals are at late stage. This difference in the activity of caspases may be explained by physiological differences of the animals. In this kind of investigations eventhough animals used are identical (species, age, weight and gender), this negative correlation between caspase 9 and caspase 3 has to be taken into consideration when the results are evaluated.

NO increases fluid shear stress and the greatest contributor to this is eNOS [22,23]. There are also studies showing that OS also increases fluid shear stress [24,25]. We think that one of the most important finding of this study is that severe eNOS and iNOS expressions and oxidative DNA damage significantly contribute to occurrence of fluid shear stress in diabetic nephropathy. Therefore, it is very probable that in order to prevent fluid shear stress, therapies with anti-oxidants and inhibition of NO may be applied together and this may play a key role in the prevention of nephropathology that may occur.

Studies made on diabetic nephropathy show that apoptosis and nephropathology that occur in kidney are highly complex processes. In this study, we observed that in diabetic nephropathy eNOS and iNOS expression pathologically increases, resulting in high levels of NO production that eventually damages mitochondria and triggers intrinsic apoptotic pathway. Hyperglycemia ethiologically does induce degeneration in kidneys of animals and humans but here we think that severe NO production also presents a major contribution to this pathological process. We believe that ROS/reactive nitrogen species that are produced from activated infiltrating mononuclear and other renal cells and induction of eNOS and iNOS expression in endothelial, mesenchymal and tubular cells cause generation of large amounts of NO that causes OS and finally results in apoptosis. In summary, nephropathology seen in diabetes are not only caused by hyperglycemia, severely expressed proinflammatory cytokines or high levels of NO, but also as we show here are caused by apoptosis and OS.

Conflict of Interests

The authors report no conflict of interests.

REFERENCES

1. Kanwar YS, Wada J, Sun L, Xie P, Wallner EI, Chen S, Chugh S, Danesh FR: Diabetic nephropathy: Mechanisms of renal disease progression. *Exp Biol Med,* 233, 4-11, 2008. DOI: 10.3181/0705-MR-134

2. Dronavalli S, Duka I, Bakris GL: The pathogenesis of diabetic nephropathy. *Nat Clin Pract Endocrinol Metab*, 4, 444-452, 2008. DOI: 10.1038/ncpendmet0894

3. Elmore S: Apoptosis: A review of programmed cell death. *Toxicol Pathol*, 35, 495-516, 2007. DOI: 10.1080/01926230701320337

4. Rowinsky EK: Targeted induction of apoptosis in cancer management: the emerging role of tumor necrosis factor-related apoptosis-inducing ligand receptor activating agents. *J Clin Oncol*, 23, 9394-9407, 2005. DOI: 10.1200/JCO.2005.02.2889

5. Loreto C, Almeida LE, Trevilatto P, Leonardi R: Apoptosis in displaced temporomandibular joint disc with and without reduction: An immunohistochemical study. *J Oral Pathol Med*, 40, 103-110, 2011. DOI: 10.1111/j.1600-0714.2010.00920.x

6. Caltabiano R, Leonardi R, Musumeci G Bartolonid G, Rusue MC, Almeidaf LE, Loretoc C: Apoptosis in temporomandibular joint disc with internal derangement involves mitochondrialdependent pathways: An *in vivo* study. *Acta Odontol Scand*, 71, 577-583, 2013. DOI: 10.3109/00016357.2012.700060

7. Roos WP, Kaina B: DNA damage-induced cell death by apoptosis. *Trends Mol Med*, 12, 440-450, 2006. DOI: 10.1016/j.molmed.2006.07.007

8. Verzola D, Bertolotto MB, Villaggio B, Ottonello L, Dallegri F, Salvatore F, Berruti V, Gandolfo MT, Garibotto G, Deferrari G: Oxidative stress mediates apoptotic changes induced by hyperglycemia in human tubular kidney cells. *J Am Soc Nephrol*, 15, 85-87, 2004. DOI: 10.1097/01.ASN.0000093370.20008.BC

9. Hibbs JBJ, Taintor RR, Vavrin Z: Macrophage cytotoxicity: role for L-arginine deiminase and imino nitrogen oxidation to nitrite. *Science*, 235, 473-476, 1987. DOI: 10.1126/science.2432665

10. Bonfoco E, Krainc D, Ankarcrona M, Nicotera P, Lipton SA: Apoptosis and necrosis: Two distinct events induced, respectively, by mild and intense insults with N-methyl-D-aspartate or nitric oxide/superoxide in cortical cell cultures. *Proc Natl Acad Sci USA*, 92, 7162-7166, 1995.

11. Dincel GC, Kul O: eNOS and iNOS trigger apoptosis in the brains of sheep and goats naturally infected with the border disease virus. *Histol Histopathol*, 10, 1233-1242, 2015. DOI: 10.14670/HH-11-621

12. Brookes PS, Salinas EP, Darley-Usmar, Anderson PG: Concentration dependent effects of nitric oxide on mitochondrial permeability transition and cytochrome c release. *J Biol Chem*, 275, 20474-20479, 2000. DOI: 10.1074/jbc.M001077200

13. Moriya R, Uehara T, Nomura Y: Mechanism of nitric oxideinduced apoptosis in human neuroblastoma SH-SY5Y cells. *FEBS Lett*, 484, 253-260, 2000. DOI: 10.1016/S0014-5793(00)02167-0

14. Dincel GC, Kul O: Increased expressions of ADAMTS-13, neuronal nitric oxide synthase, and neurofilament correlate with severity of neuropathology in border disease virus-infected small ruminants. *PLoS One*, 10, e0120005, 2015. DOI: 10.1371/journal.pone.0120005

15. Dincel GC, Atmaca HT: Nitric oxide production increases during *Toxoplasma gondii* encephalitis in mice. *Exp Parasitol*, 156, 104-112, 2015. DOI: 10.1016/j.exppara.2015.06.009

16. Natalie JT, Hajime H, Bronk S, Gores GJ: Nitric oxide inhibits apoptosis downstream of cytochrome c release by nitrosylating caspase 9. *Cancer Res*, 62, 1648-1653, 2002.

17. Pender MP, Rist JM: Apoptosis of inflammatory cells in immune control of the nervous system: Role of glia. *Glia*, 36, 137-144, 2001. DOI: 10.1002/glia.1103

18. Brown GC. Nitric oxide and neuronal death. *Nitric Oxide*, 23, 153-165, 2010. DOI: 10.1016/j.niox.2010.06.001

19. Baylis C, Harton P, Engels K: Endothelial derived relaxing factor controls renal hemodynamics in the normal rat kidney. *J Am Soc Nephrol*, 1, 875-881, 1990.

20. Haynes WG, Noon JP, Walker BR, Webb DJ: Inhibition of nitric oxide synthesis increases blood pressure in healthy humans. *J Hypertens*, 11, 1375-1380, 1993. DOI: 10.1113/jphysiol.2009.177204

21. Bech JN, Nielsen CB, Pedersen EB: Effects of systemic NO synthesis inhibition on RPF, GFR, Una and vasoactive hormones in healthy humans. *Am J Physiol Lung Cell Mol Physiol*, 270, 845-851, 1996. DOI: 10.14814/phy2.12144

22. Corson MA, James NL, Latta SE, Nerem RM, Berk BC, Harrison

DG: Phosphorylation of endothelial nitric oxide synthasein response to fluid shear stress. *Circ Res*, 79, 984-991, 1996. DOI: 10.1161/01.RES.79.5.984

23. Kemeny SF, Figueroa DS, Clyne AM: Hypo- and hyperglycemia impairendothelial cell actin alignment and nitricoxide synthase activation in response shear stress. *PLoS One*, 8, e66176, 2013. DOI: 10.1371/journal.pone.0066176

24. Rouhanizadeh M, Takabe W, Ai L, Yu H, Hsiai T: Monitoring oxidative stress in vascular endothelial cells in response to fluid shear stress: From biochemical analyses to micro- and nanotechnologies. *Methods Enzymol*, 441, 111-150, 2008. DOI: 10.1016/S0076-6879(08)01207-X

25. Edirisinghe I, Rahman I: Cigarette smoke-mediated oxidative stress, shear stress, and endothelial dysfunction: Role of VEGFR2. *Ann N Y Acad Sci*, 1203, 66-72, 2010. DOI: 10.1111/j.1749-6632.2010.05601.x

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Research Article

More than an Uterotonic Agent: Oxytocin Prevents Peritoneal Adhesion

Hatice IŞIK [1,2] Ahmet ŞAHBAZ [1] Öner AYNIOĞLU [1]
Ülkü BAYAR ÖZMEN [1] Osman CENGİL [3] Banu DOĞAN GÜN [4]

[1] Bülent Ecevit University, Faculty of Medicine, Department of Gynecology and Obstetrics, TR-67600 Zonguldak - TURKEY
[2] Mevlana University, Faculty of Medicine, Department of Gynecology and Obstetrics, TR- 42040 Konya - TURKEY
[3] Bülent Ecevit University, Faculty of Medicine, Department of Animal Research, TR-67600 Zonguldak - TURKEY
[4] Bülent Ecevit University, Faculty of Medicine, Department of Pathology, TR-67600 Zonguldak - TURKEY

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Abstract

Prevention of postoperative adhesions (PPA) has become an important issue. The aim is to investigate the effect of Oxytocin (OT) on PPAs. A total of thirty female Wistar-albino rats were randomly divided into three groups (10 rats/group). The cecal peritone of Group I rats (controls) were scraped, to trigger adhesion formation, and no treatment were given. After cecal scrubbing, 1 mL saline solution was applied to each rat in Group II (i.p. saline treated group) and 80 IU/kg of OT (Pituisan®, Ege Vet, Turkey) to Group III (i.p. OT treated group) intraperitoneally. All animals were sacrificed 10 days after surgery and adhesions graded in terms of severity and histopathologic characteristics. The median scores for the extent, severity, and degree of adhesions in Group I and Group II were statistically significant and considerably higher than those scores for Group III (P<0.001). The inflammation, neovascularization, and fibrosis scores for Group III were statistically significant and considerably lower than those scores for Groups I and II (P<0.001, P<0.001 and P=0.002 respectively). OT, significantly prevented adhesion formation improving wound healing possibly by suppressing adhesion formation with anti-inflammatory and antioxidant properties. OT may be useful in the prevention of PPA in humans.

Keywords: *Oxytocin, Postoperative, Peritoneal adhesion*

Uterotonik Bir Ajandan Fazlası: Oksitosin Postoperatif Adezyonları Önlüyor

Özet

Postoperatif adezyonların (PPA) önlenmesi önemli bir konu olmuştur. Bu çalışmada amaç Oksitosinin (OT) PPA üzerindeki etkisini araştırmaktır. Toplam 30 Wistar-albino dişi rat üç eşit gruba ayrıldı (10 rat/grup). Grup I ratlarda (kontrol) sekum peritonu adezyon oluşturmak amacıyla sıyrıldı ve hiçbir tedavi verilmedi. Grup II'ye (i.p. salin tedavi grubu) sekum sıyrılmasından sonra intraperitoneal 1 ml saline ve Grup III (i.p. OT tedavi grubu)'e 80I U/kg (Pituisan®, Ege Vet, Turkey) OT uygulandı. Bütün hayvanlar cerrahiden 10 gün sonra öldürüldü ve adezyonlar şiddeti ve histolojik özellikleri açısından derecelendirildi. Grup I ve II'deki adezyonların yaygınlık, şiddet ve derecelerinin medyan skorlarının Grup III'dekilere göre istatistiksel olarak arttığı görüldü (P<0.001). Grup III'deki inflamasyon, neovaskülarizasyon ve fibrozis skorları Grup I ve II'deki skorlara göre istatistiksel olarak anlamlı ve belirgin ölçüde düşük bulundu (P<0.001, P<0.001 and P=0.002 respectively). Oksitosin, anti-enflamatuarve anti- oksidan özellikleri ile adezyon oluşum sürecindeki basamakları baskılayarak, yara iyileşmesini düzenleyerek ratlarda adezyon oluşumunu ciddi ölçüde engellemiştir. OT insanlarda PPA önlenmesinde faydalı olabilir.

Anahtar sözcükler: *Oksitosin, Postoperatif, Peritoneal adezyone*

INTRODUCTION

Peritoneal adhesions occur due to the disruption of normal peritoneal healing generally after abdominal or pelvic surgery [1]. Postoperative peritoneal adhesions (PPA) can cause chronic pelvic pain, small bowel obstruction, dyspareunia, dysmenorrhea, or infertility. Reoperations due to adhesions can cause various complications such as iatrogenic organ damage and can sometimes make laparoscopic surgery impossible [1].

İletişim (Correspondence)
+90 532 2375927
k.hgonbe@gmail.com

Two mechanisms have been reported in peritoneal healing; normal physiological repair and adhesion formation. The inflammatory process begins due to the peritoneal insult, and then fibrin deposits occur. Activation of plasminogen degrades fibrinous exudate to plasmin. When fibrinolysis of the fibrinous exudate is inhibited, fibrin molecules organize, and then a collagen matrix and adhesions form [2].

Since adhesions cause many health problems and financial burdens, the prevention of PPA has become an important issue for health scientists. Numerous agents have been used to prevent PPA, but most of them are experimental.

Oxytocin (OT) is a neurohypophysial nonapeptide that is synthesized in the paraventricular and supraoptic nuclei in the hypothalamus. OT has central and peripheral effects. The main functions of OT are uterine contractions at parturition and myoepithelial contractions in the mammary gland. Also, OT receptors have been found in some peripheral tissues, such as the kidney, heart, thymus, pancreas, and adipocytes [3]. Experimental studies have shown that OT suppresses neutrophil infiltration and controls inflammatory cytokines [4,5]. Subcutaneous injection of OT has been shown to be helpful in wound healing by exerting anti-oxidant and anti-inflammatory properties [6]. In ischemia/reperfusion experimental models, OT has been found to be protective against many tissue injuries such as heart, liver, and skeletal muscle [7-9].

As far as we know, there has been no study of the effects of OT on PPAs. This study investigates whether OT prevents PPA formation.

MATERIAL and METHODS

The Animals and the Experimental Model

A total of 30 female Wistar-albino rats weighing 200±20 g were used for this study. The rats were housed in pairs in steel cages and were fed ad libitum with standard pelleted food approved by the Turkish Standards Institute and had free access to tap water. The room temperature (22±2°C) and humidity were controlled with 12-h light/dark cycles. All experimental procedures were approved by the Bulent Ecevit University School of Medicine Animal Care Committee with the ID number: 2013/09-1.

Rats were anesthetized with intraperitoneal injections of Ketamine hydrochloride (50 mg/kg, Ketalar; Pfizer, Istanbul, Turkey) and Xylazine hydrochloride (10 mg/kg, Rompun, Bayer, Istanbul, Turkey). After the disinfection and shaving of the anterior abdominal skin, a 2-cm midline incision was performed in the lower abdomen for each rat. Uterine horns and cecum were identified. To form adhesions the cecum abrasion model was used [10]. Cecums were scrubbed with a sterile sponge on the antimesenteric surface until punctate bleeding and serosal petechiae occurred. Rats were randomly divided into three groups each containing ten rats.

Group I (control): Only adhesion formation was performed without any treatment.

Group II (i.p. saline treated group): After cecal scrubbing, 1 mL saline solution wasapplied intraperitoneally.

Group III (i.p. OT treated group): After adhesion formation, 80 IU/kg of OT (Pituisan®, Ege Vet, Turkey) was applied intraperitoneally.

The midline incisions in the rats were closed with 3/0 monofilament sutures. All the rats were housed in cages under standard conditions with free access to food and water. Two weeks after the first operation, a second laparotomy wasperformed with a reverse U incision to evaluate PPA. Macroscopic evaluations of the adhesions were done according to Linsky's classification by the second author blinded to the groups [11]. Adhesions were evaluated for involvement, resistance, and severity. According to the involvement of adhesions, the scores were as follows: no adhesions=0, adhesions up to 25% of the scrubbed area=1, adhesions up to 75% of scrubbed area=2, adhesions on the whole scrubbed area=3. The scores for resistance were as follows: no adhesions=0, adhesions that can easily be separated=1, adhesions that can be separated with traction=2, adhesions that can be separated only by sharp dissection=3. For the severity of the adhesions, the scoring was as follows: no adhesions=0, filmy and avascular=1, moderately filmy and vascular=2, dense and significantly vascular=3. The total scores, calculated by summing the three scores, ranged between 0 and 18.

Histopathological Evaluation

Adhesion areas and cecum were excised, fixated in formal solution samples, and sent for histopathological evaluation. After dehydration with ethanol, specimens were embedded in paraffin blocks, and then 5 µm-thick sections were sliced from embedded tissues and stained with hematoxylin and eosin. A light microscope was used to evaluate fibrosis, inflammation, and vascularization. Histopathological evaluations of the samples were performed by a pathologist (B.D.G.) who was blind to groups. A light microscope was used for the evaluation of sections. To assess inflammation, neovascularization, and fibrosis, a semiquantitative scoring system was used [12]. The degree of inflammation was classified as grade 0 (absent or normal in number), grade 1 (slight increase), grade 2 (moderate infiltration), or grade 3 (massive infiltration); fibrosis as grade 0 (none), grade 1 (slight), grade 2 (moderate), or grade 3 (dense); and neo-vascularization as grade 0 (none), grade 1 (one to two vessels), grade 2 (three to nine vessels), or grade 3 (10 or more vessels).

Statistical Analysis

Statistical analyses were performed with SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). Variables were expressed as median (minimum-maximum). Differences among the groups were analyzed by the Kruskal-Wallis test. Dual comparisons among groups with significant values were evaluated with Dunn's test after the Kruskal-Wallis test. A P-value of less than 0.05 was considered statistically significant for all of the tests.

RESULTS

During the study period, one rat from Group I (control group) and one rat from Group II (SF-treated group) were lost due to the operation. The remaining 28 rats all tolerated the study period. No complications were observed during the follow-up period.

The macroscopic and the histopathological adhesion scores of three groups are presented in *Table 1*. In Group I (control group), 22% of the rats had grade 2 adhesions, and 78% of the rats had grade 3 adhesions. In Group II (SF treated group), 33% of adhesions were grade 2, and 67% were grade 3, whereas in Group III (OT-treated group), 30% of the rats had no adhesions, and 70% had grade 1 adhesions with respect to macroscopic evaluation. The median scores for the extent, severity, and degree of adhesions in Group I and Group II were statistically significant and considerably higher than those scores for Group III (P<0.001).

In pathological examinations, the scores for Group III with respect to inflammation, neovascularization, and fibrosis were statistically significant and considerably lower than those scores for Groups I and II (P<0.001, P<0.001 and P=0.002 respectively). No statistically significant difference were observed between Group I and Group II with respect to the macroscopic and pathological adhesion scores. The microscopic evaluation of the inflammation, vascularization, and fibrosis are presented in *Fig. 1* as none, mild-moderate, and severe.

DISCUSSION

The present study reveals that intraperitoneal administration of 80 IU/kg OT reduces adhesion formation. Peritoneal adhesion formation after any kind of visceral surgery is still an important clinical problem although various preventive strategies against adhesion formation have been investigated. The mesothelial cell lining of the peritoneum has an important role in peritoneal healing [13,14]. In the case of peritoneal injury (bleeding, cauterization or local ischemia) peritoneal adhesions can

Table 1. *The macroscopic and the histopathological adhesion scores. Same letters denote similar groups with regards to adhesion scores*
Tablo 1. *Makroskopik ve histopatolojik adezyon skorları. Aynı harfler adezyon skorları açısından benzer grupları göstermektedir*

Adhesion Scores	Control (n = 9) median (min-max)	Saline Solution (n = 9) median (min-max)	Oxytocin (n = 10) median (min-max)	P
Extent	3 (2-3)[a]	3 (2-3)[a]	1 (0-1)[b]	< 0.001
Severity	3 (2-3)[a]	3 (2-3)[a]	1 (0-1)[b]	< 0.001
Degree	3 (1-3)[a]	3 (1-3)[a]	1 (0-1)[b]	0.001
Total Score	9 (7-9)[a]	8 (6-9)[a]	3 (0-3)[b]	< 0.001
Inflammation	3 (2-3)[a]	2 (2-3)[a]	1 (1-1)[b]	< 0.001
Neovascularization	3 (2-3)[a]	2 (2-3)[a]	1 (0-1)[b]	< 0.001
Fibrosis	2 (1-3)[a]	2 (2-3)[a]	1 (0-1)[b]	0.002

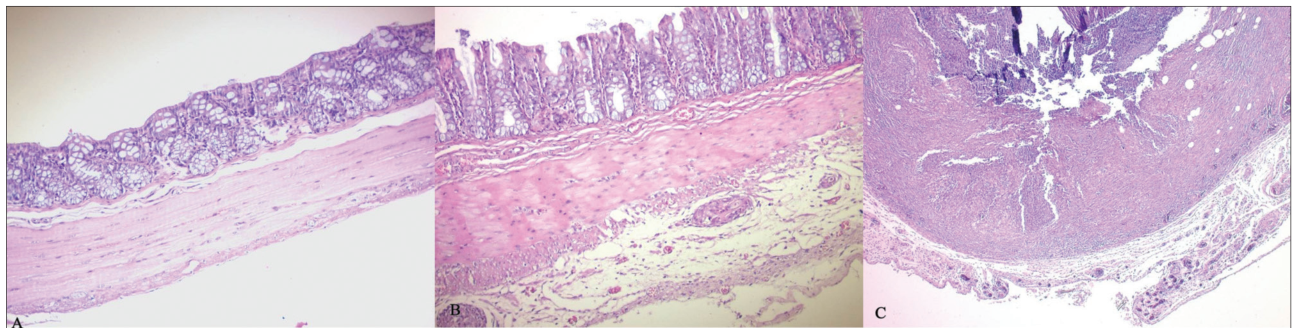

Fig 1. The histopatologic specimens with Hematoxylin-Eosin (HEx40) staining. **A-** no fibrosis, inflammation or vascular proliferation, **B-** mild-moderate inflammation, neo-vascularisation and fibrosis, **C-** severe inflammation, increased vascularisation and dense fibrosis

Şekil 1. Hematoxylin-Eosin (HEx40) ile boyanan histolojik kesitler **A-** fibrozis, inflamasyon ve vasküler proliferasyon yok, **B-** hafif-orta düzeyde inflamasyon, neovaskülazisyan ve fibrozis, **C-** şiddetli inflamasyon, artmış vaskülarizasyon ve yoğun fibrozis

occur since tissue plasminogen activator (tPA) expression is reduced and levels of plasminogen activator inhibitor types1 and 2 (from inflammatory, mesothelial, and endothelial cells) are increased. Normally, fibrinolytic activity provides remesothelization; however, in the case of ischemia, fibrinolytic activity decreases, causing fibrin deposition. In our study we performed peritoneal injury and ischemia via the cecal abrasion method.

Inflammation also plays a vital role in the formation of adhesions. Inflammatory mediators, such as cytokines, interleukins, and transforming growth factor-β, decrease fibrinolytic activity in the peritoneum, so that adhesion formation aggregates.

Reactive oxygen radicals (ROS) formed during hypoxia activate fibroblasts, causing the production of cytokines such as vascular endothelial growth factor (VEGF), tissue growth factor (TGF), and cyclooxygenase-2, which all increase adhesion formation [15,16].

Adhesions are complex structures composed of inflammatory cells, fibrosis, and new vessels. If the soft fibrin that results from adhesion formation is not removed by the fibrinolytic activity, dense fibrins will develop within 2 weeks [17]. In our study we performed the second operation 2 weeks after the first operation. The follow-up time was sufficient for adhesion maturation.

Many experimental methods have been used to introduce peritoneal adhesions such as uterine horn cauterization, large bowel anastomosis, ileal transection, peritoneal damage, scraping, and suturing [18,19]. Since scraping is one of the most effective methods for causing adhesions to form, in this study we used this model to evaluate the effects of OT on PPA.

Various treatment modalities have been used to prevent adhesion formation. The proposed mechanisms with these agents include reducing inflammation, increasing fibrinolysis capacity, preventing fibroplast proliferation, or separating the deperitonealized areas. Also, different antioxidant agents such as Vitamin C, Vitamin E, N-acetyl cysteine, and melatonin have been used to prevent adhesions [20,21]. Since oxytocin is known to have anti-inflammatory and anti-oxidant effects, we tried to evaluate its impact on the prevention of adhesions. Oxytocin had not been studied for the adhesion prevention effect before.

OT has been demonstrated to cause the release of nitric oxide (NO), which inhibits neutrophil infiltration and adhesion formation [22]. In the case of sepsis due to cecal ligation and puncture, massive inflammatory mediators such as NO are produced and it has been reported that via the central NO-cGMP pathway the arginine vasopressin (AVP) and OT gene expressions are increased [23]. Also Ahmed MA et al.[24] suggested that OT decelerated atherosclerosis by inhibiting proinflammatory responses. OT modulates immune and inflammatory response by decreasing interleukin-6 and TNF-α.levels, increasing prostacyclin, and insulin-like growth factor (IGF)-I levels [6]. OT treatment decreased macroscopic and microscopic scores for inflammation and fibrosis in our study.

Vascularization increases in adhesion formation [25]. The present study revealed that the mean neo-vascularization score of the OT-treated group was lower than those of the control and saline-treated groups. The negative effect of OT on neo-vascularization is proposed to be due to decreased levels of inflammatory cytokines including VEGF [26]. However we did not study VEGF levels, which was a limitation in our study.

OT has been known as an anti-oxidant agent that decreases ROS levels. Tas Hekimoglu A et al.[27] reported remote oxidative stress (OS) in liver tissue in renal ischemia reperfusion study. However, they suggested that OT application prevented the rising of OS. The previous studies also reported the protective effect of OT on ischemia reperfusion-related injuries in ovaries, heart and skeletal muscle [7,9,28]. In our study we also detected the protective effect of OT on inflammation, neo-vascularization, fibrosis, and then adhesion formation.

Oxytocin was used intraperitoneally at the dose of 80 IU/kg, which was the same dose used in the previous study [28]. It has been shown that high doses of OT have some side effects in humans such as cardiac arrhythmia, fatal afibrinogenemia, nausea, vomiting, premature ventricular contractions, subarachnoid hemorrhage, and hypertensive episodes. In the present study, we did not observe any OT-related systemic side effects with the dose given intraperitoneally.

Saline has been reported to decrease adhesion formation in some studies [29]. However, others argued that saline did not decrease adhesion formation [30]. Likewise in our study, adhesion formation was not prevented in the saline-treated group.

The present study showed that oxytocin prevented adhesion formation in rats. This effect of oxytocin would be suitable to protect human females against inflammation in the postpartum period especially those who gave birth by Cesarean section since oxytocin is released in large amounts and is also given to women intravenously in the postpartum period.

Conflict of Interest

The authors report no conflict of interest.

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REFERENCES

1. Nair S, Saed GM, Atta HM, Rajaratnam V, Diamond MP, Curiel DT, Al-Hendy A: Towards gene therapy of postoperative adhesions: Fiber and transcriptional modifications enhance adenovirus targeting towards human adhesion cells. *Gynecol Obstet Invest,* 76, 119-24, 2013. DOI: 10.1159/000353426

2. Kamel RM: Prevention of postoperative peritoneal adhesions. *Eur J Obstet Gynecol Reprod Biol,* 150, 111-118, 2010. DOI: 10.1016/j.ejogrb.2010.02.003

3. Gimpl G, Fahrenholz F: The oxytocin receptor system: structure, function and regulation. *Physiol Rev*, 81, 629-683, 2001.

4. Tuğtepe H, Sener G, Biyikli NK, Yüksel M, Cetinel S, Gedik N, Yeğen BC: The protective effect of oxytocin on renal ischemia/reperfusion injury in rats. *Regul Pept*, 140, 101-108, 2007. DOI: 10.1016/j.regpep.2006.11.026

5. Düşünceli F, Işeri SO, Ercan F, Gedik N, Yeğen C, Yeğen BC: Oxytocin alleviates hepatic ischemia-reperfusion injury in rats. *Peptides,* 29, 1216-1222, 2008. DOI: 10.1016/j.peptides.2008.02.010

6. Petersson M, Lundeberg T, Sohlström A, Wiberg U, Uvnäs-Moberg K: Oxytocin increases the survival of musculocutaneous flaps. *Naunyn Schmiedeberg's Arch Pharmacol,* 357, 701-704, 1998. DOI: 10.1007/PL00005227

7. Alizadeh AM, Faghihi M, Khori V, Wiberg U, Uvnäs-Moberg K: Oxytocin protects cardiomyocytes from apoptosis inducedby ischemia-reperfusion in rat heart: Role of mitochondrial ATP-dependentpotassium channel and permeability transition pore. *Peptides,* 36, 71-77, 2012. DOI: 10.1016/j.peptides.2012.03.023

8. Rashed LA, Hashem RM, Soliman HM: Oxytocin inhibits NADPH oxidaseand P38 MAPK in cisplatin-induced nephrotoxicity. *Biomed Pharmacother*, 65, 474-480, 2011. DOI: 10.1016/j.biopha.2011.07.001

9. Erkanli K, Erkanli Senturk G, Aydin U, Arbak S, Ercan F, Tuncdemir M, Isiksacan N, Bakir I: Oxytocin protects rat skeletal muscle against ischemia/reperfusion injury. *AnnVasc Surg,* 27, 662-670, 2013. DOI: 10.1016/j.avsg.2012.10.012

10. Hoffman NE, Siddiqui SA, Agarwal S, McKellar SH, Kurtz HJ, Gettman MT, Ereth MH: Choice of hemostatic agent influences adhesion formation in a rat cecal adhesion model. *J Surg Res,*155, 77-81, 2009. DOI: 10.1016/j.jss.2008.08.008

11. Linsky CB, Diamond MP, Cunningham T, Constantine B, DeCherney AH, diZerega GS: Adhesion reduction in the rabbit uterine horn model using an absorbable barrier, TC-7. *J Reprod Med,* 32, 17-20, 1987.

12. Hooker GD, Taylor BM, Driman DK: Prevention of adhesion formation with use of sodium hyaluronate based bioresorbable membrane in a rat model of ventral hernia repair with polypropylene mesh: A randomized controlled study. *Surgery,* 125, 211-216, 1999. DOI: 10.1016/S0039-6060(99)70267-9

13. Cheong YC, Laird SM, Li TC, Shelton JB, Ledger WL, Cooke ID: Peritoneal healing and adhesion formation/reformation. *Hum Reprod Update,* 7, 556-566, 2001. DOI: 10.1093/humupd/7.6.556

14. Hellebrekers BW, Trimbos-Kemper TC, Emeis JJ, Kooistra T: Use of fibrinolytic agents in the prevention of postoperative adhesion formation. *Fertil Steril*, 74, 203-212, 2000. DOI: 10.1016/S0015-0282(00)00656-7

15. Awonuga AO, Belotte J, Abuanzeh S, Emeis JJ, Kooistra T: Advances in the pathogenesis of adhesion development: The role of oxidative stress. *Reprod Sci,* 21, 823-836, 2014. DOI: 10.1177/1933719114522550

16. Agacayak E, Tunc SY, Icen MS, Alabalik U, Findik FM, Yuksel H, Gul T: Honokiol decreases intra-abdominal adhesion formation in a rat model. *Gynecol Obstet Invest,* 79, 160-167, 2015. DOI: 10.1159/000367661

17. Barbul A: Wound healing. **In,** Brunicardi FC, Andersen DK, Billiar TR, Dunn DL, Hunter JG, Pollock RE (Eds): Schwartz's Principles of Surgery. 8th ed., 223-248, Mc-Graw-Hill, Philadelphia, 2005.

18. Maghsoudi H, Askary B: The effect of piroxicam on the formation of postoperative intraabdominal adhesion in rats. *Saudi J Gastroenterol,* 14, 198-201, 2008. DOI: 10.4103/1319-3767.43276

19. Rajab TK, Wallwiener M, Planck C, Brochhausen C, Kraemer B, Wallwiener CW: A direct comparison of seprafilm, adept, intercoat, and spraygel for adhesion prophylaxis. *J Surg Res*, 161, 246-249, 2010. DOI: 10.1016/j.jss.2008.11.839

20. Attar R, Yildirim G, Kumbak B, Ficicioglu C, Demirbag S, Yesildaglar N: Efficacy of melatonin and hyaluronate/carboxymethyl-cellulose membrane in preventing adhesion reformation following adhesiolysis in a rat uterine model. *J Obstet Gynaecol Res*, 37, 125-131, 2011. DOI: 10.1111/j.1447-0756.2010.01329.x

21. Yetkin G, Uludag M, Citgez B, Karakoc S, Polat N, Kabukcuoglu F: Prevention of peritoneal adhesions by intraperitoneal administration of vitamin E and human amniotic membrane. *Int J Surg*, 7, 561-565, 2009. DOI: 10.1016/j.ijsu.2009.09.007

22. Iseri SO, Sener G, Saglam B, Gedik N, Ercan F, Yegen BC: Oxytocin ameliorates oxidative colonic inflammation by a neutrophil-dependent mechanism. *Peptides*, 26, 483-491, 2005. DOI: 10.1016/j.peptides.2004.10.005

23. Oliveira-Pelegrin GR, Aguila FA, Basso PJ, Rocha MJ: Role of central NO-cGMP pathway in vasopressin and oxytocin gene expression during sepsis. *Peptides*, 31, 1847-1852, 2010. DOI: 10.1016/j.peptides.2010.06.031

24. Ahmed MA, Elosaily GM: Role of oxytocin in deceleration of early atherosclerotic inflammatory processes in adult male rats. *Int J Clin Exp Med*, 4, 169-178, 2011.

25. Herrick SE, Mutsaers SE, Ozua P, Sulaiman H, Omer A, Boulos P, Foster ML, Laurent GJ: Human peritoneal adhesions are highly cellular, innervated, and vascularized. *J Pathol*, 192, 67-72, 2000. DOI: 10.1002/1096-9896(2000)9999:9999<::AID-PATH678>3.0.CO;2-E

26. Erbaş O, Ergenoglu AM, Akdemir A, Yeniel AÖ, Taskiran D: Comparison of melatonin and oxytocin in the prevention of critical illness polyneuropathy in rats with experimentally induced sepsis. *J Surg Res*, 183, 313-320, 2013. DOI: 10.1016/j.jss.2012.11.043

27. Tas Hekimoglu A, Toprak G, Akkoc H, Evliyaoglu O, Ozekinci S, Kelle I: Oxytocin ameliorates remote liver injury induced by renal ischemia-reperfusion in rats. *Korean J Physiol Pharmacol,* 17, 169-173, 2013. DOI: 10.4196/kjpp.2013.17.2.169

28. Akdemir A, Erbas O, Gode F, Ergenoglu M, Yeniel O, Oltulu F, Yavasoglu A, Taskiran D: Protective effect of oxytocin on ovarian ischemia-reperfusion injury in rats. *Peptides*, 55, 126-130, 2014. DOI: 10.1016/j.peptides.2014.02.015

29. Tarhan OR, Barut I, Sezik M: An evaluation of normal saline and taurolidine on intra-abdominal adhesion formation and peritoneal fibrinolysis. *J Surg Res*, 144, 151-157, 2008. DOI: 10.1016/j.jss.2007.09.006

30. Panahi F, Sadraie SH, Khoshmohabat H, Shahram E, Kaka G, Hosseinalipour M: Macroscopic and pathological assessment of methylene blue and normal saline on postoperative adhesion formation in a rat cecum model. *Int J Surg*, 10, 537-541, 2012. DOI: 10.1016/j.ijsu.2012.08.009

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Research Article

Investigation of *Salmonella* spp. and *Listeria monocytogenes* in Seafood by Cultural Methods and PCR [1]

Serkan İKİZ [1] Emek DÜMEN [2] Beren BAŞARAN KAHRAMAN [1]
Gülay Merve BAYRAKAL [2] Tolga KAHRAMAN [2] Sevgi ERGİN [3]

[1] *This work was supported by the Research Fund of the University of Istanbul. Project number: 48022*

[1] Department of Microbiology, Faculty of Veterinary Medicine, Istanbul University, TR-34320 Avcılar, Istanbul - TURKEY
[2] Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Istanbul University, TR-34320 Avcılar, Istanbul - TURKEY
[3] Department of Clinical Microbiology, Cerrahpaşa School of Medicine, Istanbul University, TR-34098 Fatih, Istanbul - TURKEY

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Abstract

The present study was conducted to investigate the presence of *Salmonella* spp. and *Listeria monocytogenes* in 700 seafood (400 raw fish, 100 raw shrimps and 200 raw molluscs) collected from retailers. Isolations were performed by conventional culture methods. The isolates were also confirmed by PCR assays. *Salmonella* spp. and *L. monocytogenes* were detected in 9.9% and 3.86% fish and shellfish samples, respectively. The highest rates of *Salmonella* spp. (12.5%) were found in fish samples and *L. monocytogenes* (7.0%) were in shrimp samples. Therefore, it is essential to ensure improving the quality of production technology from fishing to retail outlet and developing the sanitation conditions of food contact surfaces and handling areas.

Keywords: *Seafood, Fish, Shrimp, Mollusc, Pathogen*

Deniz Ürünlerinde *Salmonella* spp. ve *Listeria monocytogenes* Varlığının Kültürel Metotlar ve PCR İle Araştırılması

Özet

Bu çalışma perakendecilerden toplanan 700 deniz ürününde (400 çiğ balık, 100 çiğ karides ve 200 çiğ molüsk (yumuşakça)) *Salmonella* spp. and *Listeria monocytogenes* varlığının araştırılması amacıyla yapıldı. İzolasyonda konvansiyonel kültür metotları kullanıldı ve tüm izolatların PCR ile konfirmasyonları gerçekleştirildi. Balık ve kabuklu deniz hayvanlarından *Salmonella* spp. %9.9 ve *L. monocytogenes* %3.86 oranında belirlendi. *Salmonella* spp. en yüksek oranda balık örneklerinde (%12.5) saptanırken *L. monocytogene*s ise karides örneklerinde (%7.0) saptandı. Bu çalışmanın sonuçları, avlanması/yetiştirilmesinden perakende satış aşamasına kadar üretim teknolojisinin kalitesinin iyileştirilmesinin ve gıda temas yüzeyleri ile ambalajlama alanlarında sanitasyon koşullarının geliştirilmesinin gerekliliğinin önemini göstermektedir.

Anahtar sözcükler: *Deniz ürünleri, Balık, Karides, Molüsk, Patojen*

INTRODUCTION

Seafood refers not only fish but also of shellfish which include crustacea (shrimp) and molluscs (mussel and calamari) [1]. Seafood is a rich source for a great number of nutritive and important components that have high amount of the vitamins such as A, D, E and B_{12}, the well balanced content of essential amino acids, the presence of antioxidants such as tocopherols, the exceptional concentrations of essential elements such as selenium and iodine, and the good digestibility of protein due to low amounts of connective tissue [2].

Seafood are highly perishable products. They may harbour pathogens which cause serious food safety problems for consumers. Some pathogens including *Salmonella* spp. and *L. monocytogenes* have been implicated in seafood-borne diseases. These pathogens are naturally

İletişim (Correspondence)

+90 212 4737070/17047

ser@istanbul.edu.tr

present in sea water or can contaminate seafood during processing. The handling problems and poor hygiene conditions are the main reasons [1].

There have been some reports on the presence and prevalence of *Salmonella* spp. in Morocco [3], in Spain [4], in Vietnam [5], in India [6]; *L. monocytogenes* in Spain [7], in USA [8], in India [9], in Denmark [10], in Iran [11]. In Turkey the prevalence of these pathogens has not been extensively investigated.

Turkey has a favourable potential in terms of production and consumption of marine products due to surrounded by sea on three sides by the Black Sea in the north, the Mediterranean Sea in the south and the Aegean Sea in the west. In the north-west is also an important sea, the Sea of Marmara. The total shoreline is 8300 km long and half of Turkey's population lives in coastal cities. Considering the consumption, Turkey (8.2 kg per capita per year) ranks in the 7th place among European countries [12].

The present study was carried out to determine the presence of *Salmonella* spp. and *L. monocytogenes* in seafood obtained from retail markets in Istanbul which has approximately an area of 5.343 km^2 and a population of 14.377.018 million people (18.66% of the country). Furthermore Istanbul gets seafood from all the surrounding seas of Turkey, and has a geographical importance due to its location and that represents a transit corridor between Europe and Asia.

MATERIAL and METHODS

Sample Collection

A total of 700 seafood (400 raw fish, 100 raw shrimps and 200 raw molluscs) were collected from retailers in Istanbul. All samples were kept in sterile jars and immediately transferred to the laboratory in cold boxes.

Bacteriological Analysis

Conventional culture-based study of samples was performed as recommended by FDA Bacteriological Analytical Manual for the isolation of *Salmonella* spp. Pre-enrichment was done by suspending 25 g of sample in 225 ml lactose broth (LB - Oxoid, USA) followed by incubation at 37°C for 24 h. One ml mixture was transferred to Rappaport-Vassiliadis (RV - Oxoid, USA) and Muller-Kauffmann Tetrathionate Broth Base (MKTTn - Oxoid, USA). MKTTn and RV broth was incubated for 24 h at 42°C. After incubation samples were streaked on Bismuth Sulfite agar (BS - Oxoid, USA), Brilliant Green agar (BG - Oxoid, USA) and Xylose-Lysine Deoxycholate Agar (XLD - Oxoid, USA), incubated for 24 h at 37°C. The typical colonies were identified by biochemicals tests and confirmed with Salmonella antiserum (O and H-Vi polyvalent antisera) [13].

The isolation of *L. monocytogenes* were performed according to International Standardization Organization (ISO) procedures. 25 gr/ml sample was inoculated into 225 ml Buffered Listeria Enrichment Broth Base (BLEB, Oxoid, USA) and the samples were incubated at 30°C for 4 h. At the end of the 4th h 25 mg/L natamycine was added to each sample and the incubation period was continued up to 48 h at 30°C. At the 24th h of the incubation samples were inoculated onto Oxford Agar and Palcam Agar Plates (Oxoid, USA) and were again inoculated for 48 h at 35°C. After 48 h, all the samples (both from Oxford and Palcam Agars and from BLEB) were inoculated onto Chromogenic Listeria Agar (Oxoid, USA). Then, suspected colonies were passaged onto Triptic Soy Agar with Yeast Extract (TSA, Oxoid, USA) for purification. Suspected isolates which matched to all identification parameters according to reference method (Gram staining, catalase activity, motility test, fermentation of maltose, rhamnose, mannitol, and xylose, hydrolization of esculin, reduction of nitrate) were evaluated as positive. CAMP test with *Staphylococcus aureus* and Henry illumination tests were also applied to all suspected samples [14,15].

PCR

All the culture positive samples were confirmed by PCR assays. The DNAs of all the isolates was extracted by Roche High Pure PCR Template Preparation Kit (Roche, France), according to the manufacturer›s instructions. The extracts were kept at –20°C to be used as target DNA for PCR assays.

Salmonella-specific *invA* primers (5'-GTGAAATTATCGCC ACGTTCGGGCAA-3' and 5'-TCATCGCACCGTCAAAGGAACC-3') were used for the detection of Salmonella in this study [6,16]. The PCR program consisted of an initial denaturation step at 95°C for 2 min, followed by 35 cycles of DNA denaturation at 95°C for 30 s, primer annealing at 64°C for 30 s, and primer extension at 72°C for 30 s. After the last cycle, a final extension step at 72°C for 5 min was added. PCR product were analysed by gel electrophoresis with 2% agarose (Sigma-Aldrich, USA) and visualised. Observed bands at 284 bp were evaluated as positive.

L. monocytogenes specific *actA* gene was reproduced by using specific designed primers (5'-GCTGATTTAAGAGA TAGAGGAACA-3'and 5'-TTTATGTGGTTATTTGCTGTC -3') [17]. The PCR program consisted of an initial denaturation step at 94°C for 2 min, followed by 35 cycles of DNA denaturation at 94°C for 60 s, primer annealing at 50°C for 60s, and primer extension at 72°C for 60 s. PCR products were analysed by gel electrophoresis with 1.5% agarose (Sigma-Aldrich, USA) and visualised. Observed bands at 827 bp were evaluated as positive.

Each of PCR test, positive controls *(Salmonella enterica* serovar Typhimurium, ATCC 23564, field isolate of *L. monocytogenes)*, and negative control (sterile dH$_2$O) were used separately along with seafood samples.

RESULTS

Salmonella spp. and *L. monocytogenes* were detected in 9.9% and 3.86% fish and shellfish samples, respectively *(Table 1)*. All the *Salmonella* spp. and *L. monocytogenes* were confirmed by PCR assay *(Fig. 1)*.

Table 1. *Prevalence of Salmonella spp. and L. monocytogenes in various seafood*

Tablo 1. *Çeşitli deniz ürünlerinde Salmonella spp. ve L. monocytogenes prevalansı*

Products	Number of Samples	Number of *Salmonella* spp. Positive Samples	Number of *L. monocytogenes* Positive Samples
Fish	400	50 (12.5%)	10 (2.5%)
Shrimp	100	2 (2.0%)	7 (7.0%)
Mollusc	200	17 (8.5%)	10 (5.0%)
Total	700	69 (9.9%)	27 (3.86%)

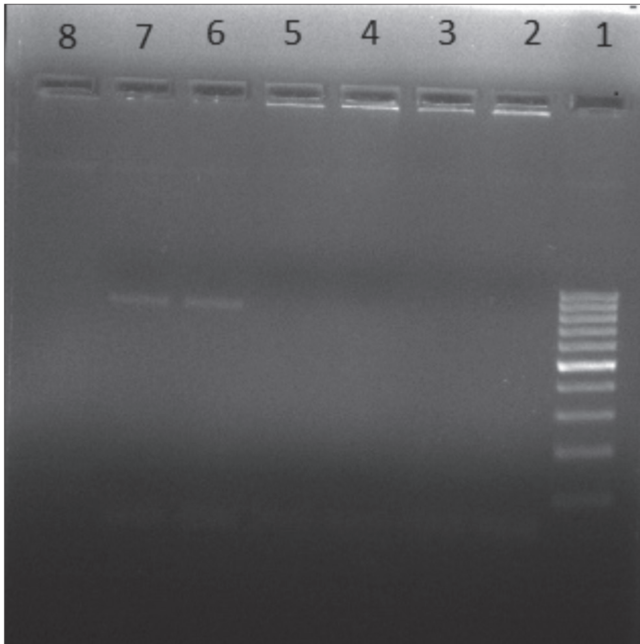

Fig 1. PCR result of a *L. monocytogenes* positive sample

Line 1 DNA marker (100 bp); Line 2-5 Negative samples; Line 6 Positive sample (827 bp); Line 7 Positive control; Line 8 Negative control

Şekil 1. *L. monocytogenes* pozitif örneğe ait PCR sonucu

Hat 1 DNA merdiveni (100 bp); Hat 2-5 Negatif örnekler; Hat 6 Pozitif örnek (827 bp); Hat 7 Pozitif kontrol; Hat 8 Negatif kontrol

DISCUSSION

According to Turkish Food Codex [18], the presence of *Salmonella* spp. and *L. monocytogenes* in 25 g of fish and shellfish is not acceptable.

The present study demonstrated that *Salmonella* spp. was isolated from 12.5% of raw fish samples. Regarding the contamination rate, our results were slightly similar to the study obtained by Kusumaningrum et al.[19] and Hatha & Lakshmanaperumalsamy [20]. In another study, no *Salmonella* spp. was isolated [21]. Contrary to this, the studies which had higher results (90.0%, 43.8% and 30.5%) than ours were reported by Jegadeeshkumar et al.[22], Budiati et al.[23] and Kumar et al.[6] respectively. The reason for high contamination rate should be due to the use of contaminated raw materials, detection methods and the geographical conditions.

The prevalence of *Salmonella* spp. in shrimp samples tested in this study was lower in comparison to those detected by Kumar et al.[6] (29.0%), Kumar et al.[24] (26.7%) and Hatha & Lakshmanaperumalsamy [20] (%15.2). On the other hand, Koonse et al.[25] reported the prevalence rate of *Salmonella* spp. was 1.6% in shrimp samples. Our findings (2.0%) showed similarity with the mentioned results. These differences may be originated from sampling procedures and the sanitation applications. Ahmed [26] stated that the presence of *Salmonella* spp. is an indicator of aldulteration in shrimp industry which is one of the most important commodities seen in global fishery trade.

In this study, *Salmonella* spp. was detected in 8.5% of molluscs samples. This result was in acceptance to the findings reported by Setti et al.[3] and Simental & Martinez-Urtaza [27]. According to the classification of the seafood, the highest risk category includes raw fish and molluscs especially mussels [28]. In Spain, Martinez-Urtaza et al.[4] demonstrated that 3.0% of samples were positive for *Salmonella* spp. The prevalence of *Salmonella* spp. was related with different hygiene applications and poor manufacturing processes.

In our study, *L. monocytogenes* was detected in 2.0% fish samples. Higher results were found by Jeyasekaran et al.[22], Farber [29] and Ellner et al.[30] at rates of 17.2%, 13.3% and 50.0% in fish samples, respectively. Similar results were detected by Gesche & Ferrer [31], Dhanashree et al.[32] and Davies et al.[33]. In contrast, Fuchs & Surendran [34] and Cenet [35] could not detected. Parihar et al.[36] reported that *L. monocytogenes* is not usually found on fish captured from open waters and contamination may take place long before the fish raw material reaches retail trade or processing factories.

According to the results from this study, the prevalence of *L. monocytogenes* was determined as 7.0% in shrimp samples. Likewise, Hofer and Ribeiro [37], Berry et al.[38] and Jeyasekaran et al.[39] demonstrated that *L. monocytogenes* was isolated from 8.8%, 6.7% and 10.7% of shrimp, respectively. On the contrary, lower results were reported by some authors [21,40]. Differences between the findings obtained from several studies can be related to the contaminations after process, preservation conditions and inadequately personal hygiene.

In the present study, of the analysed 200 molluscs

samples, 5% were positive for *L. monocytogenes*. In Spain 7.5% of mussels [41] and in Argentina 4.5% of mussels [42] were investigated *L. monocytogenes* contamination. In another study in Brazil, no *L. monocytogenes* was isolated [43].

In conclusion, the result of this study confirmed that fish and fish products may be contaminated with pathogens which can cause serious public health problems. In Turkey, seafood consumption has been increasing [44]. Therefore, it is essential to ensure improving the quality of production technology from fishing to retail outlet and developing the sanitation conditions of food contact surfaces and handling areas. Also, food safety training should be provided for all staff to increase the level of awareness and the sense of responsibility regarding food hygiene.

REFERENCES

1. Venugopal V: Seafood Processing: Adding Value Through Quick Freezing, Retortable Packaging and Cook-chilling. CRC Press, Taylor & Francis Group, Florida, USA, 2006.

2. Nollet LML, Toldra F: Seafood and Seafood Product Analysis. CRC press, Taylor & Francis Group, Florida, USA, 2010.

3. Setti I, Rodriguez-Castro A, Pata MP, Cadarso-Suarez C, Yacoubi B, Bensmael L, Moukrim A, Martinez-Urtaza J: Characteristics and dynamics of Salmonella contamination along the coast of Agadir, Morocco. *Appl Environ Microbiol*, 75, 7700-7709, 2009. DOI: 10.1128/AEM.01852-09

4. Martinez-Urtaza J, Saco M, de Novoa J, Perez-Pieiro P, Peiteado J, Lozano-Leon A, Garcia-Martin O: Influence of environmental factors and human activity on the presence of Salmonella serovars in a marine environment. *J Appl Environ Microbiol*, 70, 2089-2097, 2004. DOI: 10.1128/AEM.70.4.2089-2097.2004

5. Van TT, Moutafis G, Istivan T, Tran LT, Coloe PJ: Detection of *Salmonella* spp. in retail raw food samples from Vietnam and characterization of their antibiotic resistance. *Appl Environ Microbiol*, 73, 6885-6890, 2007. DOI: 10.1128/AEM.00972-07

6. Kumar R, Surendran PK, Thampuran N: Evaluation of culture, ELISA and PCR assays for the detection of Salmonella in seafood. *Lett Appl Microbiol*, 46, 221-226, 2008. DOI: 10.1111/j.1472-765X.2007.02286.x

7. Herrera FC, Santos JA, Otero A, Garcia-Lopez MA: Occurrence of foodborne pathogenic bacteria in retail prepackaged portions of marine fish in Spain. *J Appl Microbiol*, 100, 527-536, 2006. DOI: 10.1111/j.1365-2672.2005.02848.x

8. Draughon FA, Anthony BA, Denton ME: *Listeria* species in fresh rainbow trout purchased from retail markets. *Dairy Food Environ Sanit*, 19, 90-94, 1999.

9. Karunasagar I, Segar K, Karunasagar I, Goebel W: Incidence of *Listeria* spp. in tropical seafoods. **In,** *Listeria 1992*. Abstract No. 155. *Eleventh International Symposium on Problems of Listeriosis (ISOPOL XI)*, May l lth-14th, Copenhagen, Denmark, 1992.

10. Jorgensen LV, Huss HH: Prevalence and growth of *Listeria monocytogenes* in naturally contaminated seafood. *Int J Food Microbiol*, 42, 127-131, 1998. DOI: 10.1016/S0168-1605(98)00071-3

11. Zarei M, Maktabi S, Ghorbanpour M: Prevalence of *Listeria monocytogenes*, *Vibrio parahaemolyticus, Staphylococcus aureus*, and *Salmonella* spp. in seafood products using multiplex polymerase chain reaction. *Foodborne Pathog Dis*, 9, 108-112, 2012. DOI: 10.1089/fpd.2011.0989

12. Can MF, Günlü A, Can HY: Fish consumption preferences and factors influencing it. *Food Sci Technol (Campinas)*, 35, 339-346, 2015.

13. Food and Drug Administration (FDA): Bacteriological Analytical Manual. 6th ed., Association of Analytical Chemists, Arlington, VA, USA, 1992.

14. ISO 11290-1: Microbiology of food and animal feeding stuffs horizontal method for the detection and enumeration of *Listeria monocytogenes*, Part 1: Detection Method. Geneva, Switzerland, 1996.

15. Dumen E, Issa G, Ikiz S, Bagcıgil F, Ozgur Y, Kahraman T, Ergin S, Yesil O: Determining existence and antibiotic susceptibility status of *Listeria monocytogenes* isolated from dairy products, serological and molecular typing of the isolates. *Kafkas Univ Vet Fak Derg*, 17 (Suppl. A): S111-S119, 2011. DOI: 10.9775/kvfd.2010.3632

16. Rahn K, De Grandis SA, Clarke RC, McEwen SA, Galan JE, Ginocchio C, Curtiss RIII, Gyles CL: Amplification of an invA gene sequence of *Salmonella typhimurium* by polymerase chain reaction as a specific method of detection of *Salmonella*. *Mol Cell Probes* 6, 271-279, 1992. DOI: 10.1016/0890-8508(92)90002-F

17. Zhou X, Jiao X: Polymerase chain reaction detection of *Listeria monocytogenes* using oligonucleotide primers targeting act A gene. *Food Control,* 16, 125-130, 2005. DOI: 10.1016/j.foodcont.2004.01.001

18. Turkish Food Codex (TFC): Microbiological criteria notification. Resmi Gazete, Tebliğ No: 2011/28157, 2011 (in Turkish).

19. Kusumaningrum HD, Suliantari LN, Dewanti-Hariyadi R: Multidrug resistance among different serotypes of Salmonella isolates from fresh products in Indonesia. *Int Food Res J*, 19, 57-63, 2012.

20. Hatha AAM, Lakshmanaperumalsamy P: Prevalence of Salmonella in fish and crustaceans from markets in Coimbatore, South India. *Food Microbiol*, 14, 111-116, 1997. DOI: 10.1006/fmic.1996.0070

21. Adesiyun AA: Prevalence of *Listeria* spp., *Campylobacter* spp., *Salmonella* spp., *Yersinia* spp. and toxigenic *Escherichia coli* on meat and seafoods in Trinidad. *Food Microbiol*, 10, 395-403, 1993. DOI: 10.1006/fmic.1993.1046

22. Jegadeeshkumar D, Saritha V, Moorthy K, Sureshkumar BT: Prevalence, antibiotic resistance and RAPD analysis of food isolates of *Salmonella* species. *Int J Biol Technol*, 1, 50-55, 2010.

23. Budiati T, Rusul G, Wan-Abdullah WN, Arip YM, Ahmad R, Thong KL: Prevalence, antibiotic resistance and plasmid profiling of Salmonella in catfish *(Clarias gariepinus)* and tilapia *(Tilapia mossambica)* obtained from wet markets and ponds in Malaysia. *Aquaculture*, 372, 127-132, 2013. DOI: 10.1016/j.aquaculture.2012.11.003

24. Kumar R, Surendran PK, Thampuran N: Distribution and genotypic characterization of Salmonella serovars isolated from tropical seafood of Cochin, India. *J Appl Microbiol*, 106, 515-524, 2009. DOI: 10.1111/j.1365-2672.2008.04020.x

25. Koonse B, Burkhardt W, Chirtel S, Hoskin GP: Salmonella and the sanitary quality of aquacultured shrimp. *J Food Protect*, 68, 2527-2532, 2005.

26. Ahmed FE: Seafood Safety. National Academy Press, Washington DC, 1991.

27. Simental L, Martinez-Urtaza J: Climate patterns governing the presence and permanence of Salmonella in coastal areas of Bahia de Todos Santos, Mexico. *Appl Environ Microbiol*, 74, 5918-5924, 2008. DOI: 10.1128/AEM.01139-08

28. Huss HH, Reilly A, Ben Embarek KP: Prevention and control of hazards in seafood. *Food Control*, 11, 149-156, 2000. DOI: 10.1016/S0956-7135(99)00087-0

29. Farber JM: *Listeria monocytogenes* in fish products. *J Food Protect*, 54 (12): 922-924, 1991.

30. Ellner R, Utzinger D, Garcia V: Aislamiento de *Listeria* sp. de diversos alimentos en Costa Rica. *Rev Costarric Ci Med*, 12, 33-39, 1991.

31. Gesche E, Ferrer J: Deteccion de *Listeria monocytogenes* en agua de mar y pescado provenientes de areas de recoleccion de productos marinos. *Alimentos (Chile)* 20, 87-92, 1995.

32. Dhanashree B, Otta SK, Karunasagar I, Goebel W, Karunasagar I: Incidence of *Listeria* spp. in clinical and food samples in Mangalore, India. *Food Microbiol*, 20, 447-453, 2003. DOI: 10.1016/S0740-0020(02)00140-5

33. Davies AR, Capell C, Jehanno D, Nychas GJE, Kirby RM: Incidence of foodborne pathogens on European fish. *Food Control*, 12, 67-71, 2001. DOI: 10.1016/S0956-7135(00)00022-0

34. Fuchs RS, Surendran PK: Incidence of *Listeria* in tropical fish and

fishery products. *Lett Appl Microbiol*, 9, 49-51, 1989. DOI: 10.1111/j.1472-765X.1989.tb00288.x

35. Cenet O: An investigation for *Listeria monocytogenes* on trout fletos using different methods. *J Vet Fac YYU*, 18 (2): 41-44, 2007.

36. Parihar VS, Barbuddhe SB, Danielsson-Tham ML, Tham W: Isolation and characterization of *Listeria* species from tropical seafoods. *Food Control*, 19, 566-569, 2008. DOI: 10.1016/j.foodcont.2007.06.009

37. Hofer E, Ribeiro R: Ocorrencia de especies de *Listeria* em camara o industrializado. *Rev Microbiol*, 21, 207-208, 1990.

38. Berry TM, Park DL, Lightner DV: Comparison of the microbial quality of raw shrimp from China, Ecuador or Mexico at both wholesale and retail levels. *J Food Prot*, 57, 150-153, 1994.

39. Jeyasekaran G, Karunasagar I, Karunasagar I: Incidence of *Listeria* spp. in tropical fish. *Int J Food Microbiol*, 31, 333-340, 1996. DOI: 10.1016/0168-1605(96)00980-4

40. Gecan JS, Bandler R, Staruszkiewicz WF: Fresh and frozen shrimp: A profile of filth, microbiological contamination and decomposition. *J Food Prot*, 57, 154-158, 1994.

41. Ben Embarek PK: Presence, detection, and growth of *Listeria monocytogenes* in seafoods: A review. *Int J Food Microbiol*, 23, 17-34, 1994. DOI: 10.1016/0168-1605(94)90219-4

42. Laciar AL, Vaca L, Centobi ONP: Aislamento de *Listeria* spp. en productos de pescaderia. **In,** *VIII Congreso Argentino de Microbiologia. Associacio´n Argentina de Microbiologia*. Buenos Aires, Argentina, 6-9 September 1998. Book of Abstracts. P. K-9, p. 324. 1998.

43. Antoniolli MA, Mendes SMC, Bonelli RR, Jordani E: Influencia de diferentes tempos de cozimento sobre a populac¸ao bacteriana do mexilhao *Perna perna* (L) cultivado no litoral de Santa Catarina. **In,** *Congresso Latino-Americano de Microbiologia e Higiene de Alimentos.* Lindoia, SP, 22-26 November 1998, Livro de Resumos. P. Q.28.1, p. 119, 1998.

44. Aydin H, Dilek MK, Aydin K: Trends in fish and fishery products consumption in Turkey. *Turkish J Fish Aquat Sci,* 11, 499-506, 2011.

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Research Article

The Protective Effect of Kefir on Carbon Tetrachloride-induced Histopathological Changes in the Livers of Rats

Şule Yurdagül ÖZSOY [1]

[1] Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Pathology, TR-31001 Alahan, Antakya/Hatay - TURKEY

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Abstract

The aim of the study was to investigate the protective effects of kefir in the liver damage of rats, at experimental carbon tetrachloride (CCl_4) intoxication by histologically and immunohistochemically. During the 45 days trial period, 18, female, Wistar albino rats were used. One of them was control, three experimental group was created. Twice a week 0.5 cc carbon tetrachloride (CCl_4) + olive-oil (1:1) suspension was injected subcutaneously to the second and third group. At third group additionally to this administration 30 ml kefir was given daily by oral gavage. CCl_4-induced hepatocellular damage and apoptosis was observed but these adverse findings reduced with kefir administration. These findings indicate that kefir may have a protective role at liver damage.

Keywords: *Apoptosis, Carbon tetrachloride, Histopathology, Liver, Rat*

Karbon Tetraklorüre Bağlı Ratların Karaciğerinde Oluşan Histopatolojik Değişikliklere Karşı Kefirin Koruyucu Etkisi

Özet

Çalışmada deneysel karbon tetraklorür (CCl_4) toksikasyonu oluşturulan ratlarda kefirin karaciğer hasarına karşı koruyucu etkilerinin histolojik ve immunohistokimyasal olarak araştırılması amaçlandı. Kırkbeş günlük deneme boyunca, 18 adet, dişi, Wistar albino rat kullanıldı. Biri kontrol olmak üzere, üç deneme grubu oluşturuldu. Grup 2 ve 3'e haftada 2 kez 0.5 cc karbon tetraklorür (CCl_4) + zeytinyağı (1:1) süspansiyonu subkutan yolla verildi. Grup 3'e buna ek olarak günlük 30 ml kefir oral gavaj yoluyla verildi. CCl_4'e bağlı hepatoselüler dejenerasyon ve apoptosis gözlendi, ancak kefir eklenmesi ile bu olumsuz değişiklikler azaldı. Bu bulgular kefirin karaciğer hasarında koruyucu rolü olabileceğini gösterdi.

Anahtar sözcükler: *Apoptozis, Karbon tetraklorür, Histopatoloji, Karaciğer, Rat*

INTRODUCTION

Carbon tetrachloride is obtained by the chlorination of carbondisulfide or reacting of the same compound with sulfur monochloride. This material absorbed by respiration, skin and gastrointestinal tract. They are used as anthelminthic, against parasites in veterinary medicine [1]. When carbon tetrachloride used in high doses the accumulation of it causes damage in liver even cirrhosis can be created. It also makes degeneration in many other organs in the body [1-3].

In regard to FAO/WHO; probiotics means 'for life' organisms, are useful for humans and animals [4]. The probiotics include some yeast such as; *Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus helveticus, Lactobacillus lactis, Lactobacillus salivarius, Lactobacillus plantarum, Streptococcus thermophilus, Enterococcus faecium, Enterococcus faecalis, Bifidobacterium spp., Escherichia coli* and *Saccharomyces*. Some of them also content *Bacillus subtilis* [5,6]. It has been reported that probiotic applications might be protective against to the urogenital, gastrointestinal and surgical infections [7,8].

A fermented milk product kefir drink, obtained from kefir grains, is Caucasian origin and ethyl alcohol and lactic acid fermentation are shaped together in it [9,10]. The polysaccharide structure, white-yellowish color kefir grains contains microorganisms such as, lactobacilli, lactococci, leuconostocs, acetobacteri and fungi (*Kluyveromyces marxianus, Torulaspora delbrueckii, Saccharomyces cerevisiae, Candida kefir*) [11-13]. It was reported that kefir has antioxidant,

İletişim (Correspondence)
+90 326 2455845
suleozsoy@yahoo.com

antifungal [14], antibacterial, antitumor, immunological [15,16], cholesterol-lowering [17] and anti- the apoptotic effect [18].

Both physiological and pathological inducible cell death mechanism apoptosis known as programmed cell death or cell suicide. Organism refers to apoptosis during organogenesis of multicellular organisms or completed the development in living, damaged or the removal of cells that potentially tumor predisposed [19,20]. Apoptosis occurs by one of two pathways: (1) a death receptor pathway, and (2) the mitochondrial pathway [21]. It was reported that in cultured rat hepatocytes, the hydrophobic bile acid glycochenodeoxycholate, GCDC, at pathophysiologically relevant concentrations (20-100 μM) induces apoptosis, as documented by cell shrinkage, nuclear condensation and lobulation, caspase activation, DNA fragmentation, and phosphatidylserine externalization [22].

In the study, it was aimed that detection the protective effect of kefir at histologic and apoptotic changes with TUNEL method, induced by carbon tetrachloride in the livers of rats.

MATERIAL and METHODS

A total of 18 female Wistar albino rats were used in the study. Three experimental group (one of them was control) formed and each group consisting 6 animals. All groups were fed with pellet (standart commercial rat chow) and drinking water was given *ad libitum*. The research project and animal housing conditions were approved by the Mustafa Kemal University Ethical Committee for Animal Studies (approval 2014-01/11). Rats were obtained from the Mustafa Kemal University Laboratory Animal Breeding Unit. The rats were assigned randomly to three groups. The first group was the control group, were fed only rat chow and drinking water. Twice a week 0.5 cc carbon tetrachloride (CCl_4) + olive-oil (1:1) suspension was injected subcutaneously to the second and third group. A total of 12 injections applied for 45 days. At third group additionally to this administration 30 ml kefir was given daily by oral gavage. Kefir drink was prepared as; kefir grain to sterile milk, 3% (w/v) and fermenting at 30°C for 24 h. After fermentation kefir was diluted 1:3, before given to rats.

At the end of the 45-day experimental period animals were sacrificed by decapitation under anesthesia [intra-muscular injection of ketamine (50 mg/kg) and xylazine (20 mg/kg)]. Necrospy was performed and liver tissues took out and routine process was done. Initially tissue samples were fixed in 10% neutral buffered formalin, embedded in paraffin then were cut in 5-6 μm for hematoxylin and eosin (H & E) staining [23] and for in situ detection of apoptotic cells.

In-situ Detection of Apoptotic Cells by TUNEL Assay: DNA fragmentation was assessed in situ in liver sections using the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-digoxigenin nick endlabelling (TUNEL) method used as catalog procedure *(In Situ Cell Death Detection Kit, POD, Roche, Mannheim, Germany)*. In summary sections were de-waxed and rehydrated using routine methods. Firstly sections were held in 3% H_2O_2 for 20 min later in proteinase K (20 mg/ml; Roche, *Mannheim, Germany)* for 15 min at room temperature. As following step; sections initially washed with Phosphate buffer solution (PBS, pH 7.4) for 3 times for 5 min, later 50 μl TUNEL reaction mixture (including TdT & dUTP) was dropped and incubated in 37°C humid camera for 1 h. Again washed with PBS. Later sections were incubated with conjugate anti-fluorescein-POD for 40 min at room tempereture and washed three times with PBS. To visiualize reaction products, samples were incubated with AEC *(3-Amino 9-Ethyl Carbasole, Dako, Glostrup, Denmark)* for 5 min, and counterstained with Mayer's hematoxyline stain. As a control, samples were treated with labeling solution instead of TdT.

Finally all sections were examined by light microscopy *(Olympus CX31)* and microphotographed *(Olympus DP12)*.

RESULTS

At control group liver was normal in colour and consistency, any macroscopical change was observed. Liver sections of the control group showed a normal histological appearance of the sinusoids and hepatic central vein, any fatty degeneration was observed *(Fig. 1)*.

At CCl_4 treated group livers yellowish pigmentation and crumbly-fatty consistency noticed at macroscopical examination. Liver histopathology revealed centrolobular lipid accumulation with necrosis in the hepatocytes *(Fig. 2)*. Also sinusoidal congestion, locally yellowish-green gall pigmentation, increase in the number of kupffer cells and inflammatory cell infiltration around the necrotic tissue was noted. With TUNEL staining DNA fragmentation was observed at some liver epithelium cells. TUNEL reaction in cell cytoplasms was demonstrated as granular staining *(Fig. 3)*.

At kefir added group macroscopical appearance of the liver was resembled to control group, normally in colour and pigmentation. The parenchymal structure of the liver was preserved via kefir administration. Kefir significantly reduced fatty degeneration, hepatocytes necrosis, sinusoidal congestion and inflammatory cell infiltration *(Fig. 4)*. Compatible with this histopathological results any staining was observed with TUNEL reaction test.

At positive and negative control sections dropping terminal transferase-free solution instead of TUNEL reaction mixture, test gave negative result in all sections.

DISCUSSION

Due to chemicals or infectious agents the liver

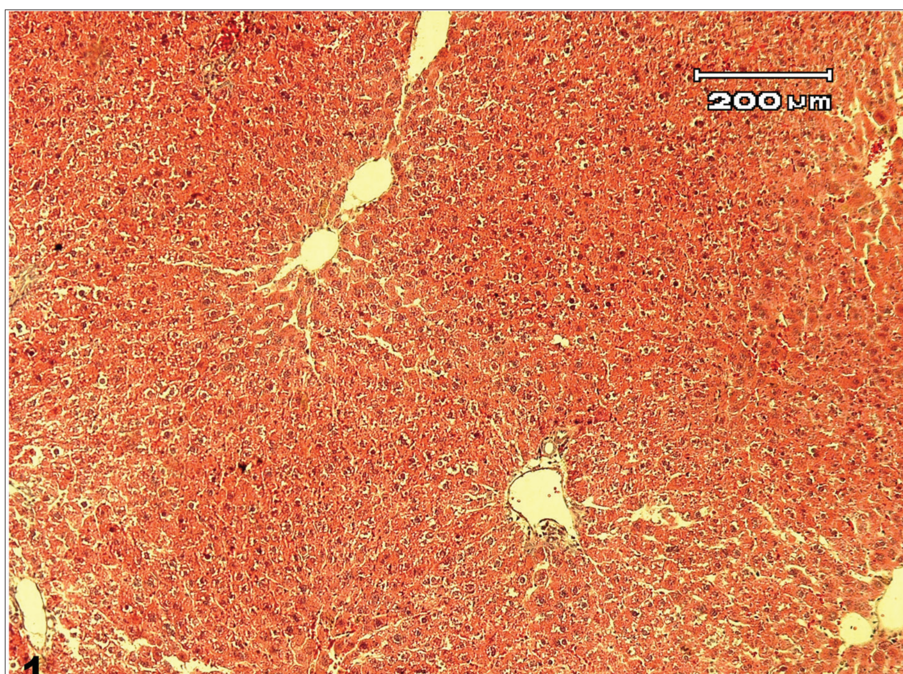


Fig 1. Control group, liver, normal histological appearance of the sinusoids and hepatic central vein, H&E

Şekil 1. Kontrol grup, karaciğer, sinuzoidler ve hepatik sentral damarın normal histolojik görüntüsü, H&E

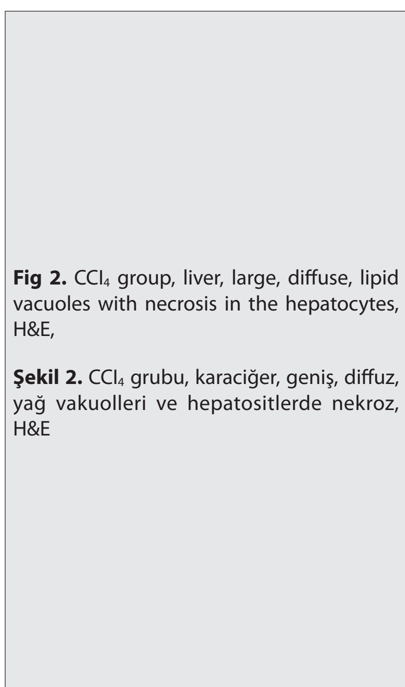
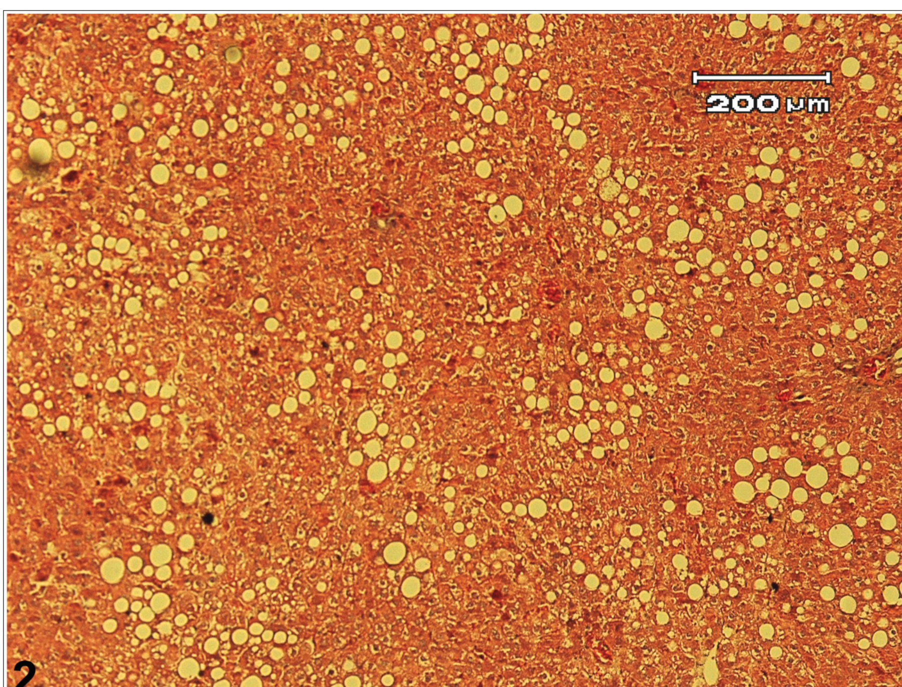


Fig 2. CCl_4 group, liver, large, diffuse, lipid vacuoles with necrosis in the hepatocytes, H&E,

Şekil 2. CCl_4 grubu, karaciğer, geniş, diffuz, yağ vakuolleri ve hepatositlerde nekroz, H&E



disorders are one of the world problems and unfortunately no effective treatment that prevent disease damage, progression and complications has yet been found [24,25]. But each passing day investigators studied with new agents to prevent liver damage. Many hepatotoxin agent was found in the environment [26,27], carbon tetrachloride is one of them [28]. Carbon tetrachloride and other halogenated hydrocarbons are used as liquid cleaner (detergents) and oil-repelling substances immemorial. In veterinary medicine they are used against the parasitic anthelminthic [2]. Low doses of CCl_4 caused fatty degeneration of the liver cells, while high doses caused the necrosis of liver cells has been reported [1,29]. In the our study due to 45 day, low doses CCl_4 administration; hepatocellular degeneration, necrosis and lipid accumulation was observed as described before.

Human and animal beings can encounter with many hepatotoxic agents during their life beacuse of this each passing day both humans, pet owners and of course investigators use a lot of functional foods such as kefir. In the developing world kefir drink increasingly become as popular. Its known that Caucasian origin acidic and midly alcolic fermented milk kefir drink contain benefical microorganisms and can treat some diseases [9] and has

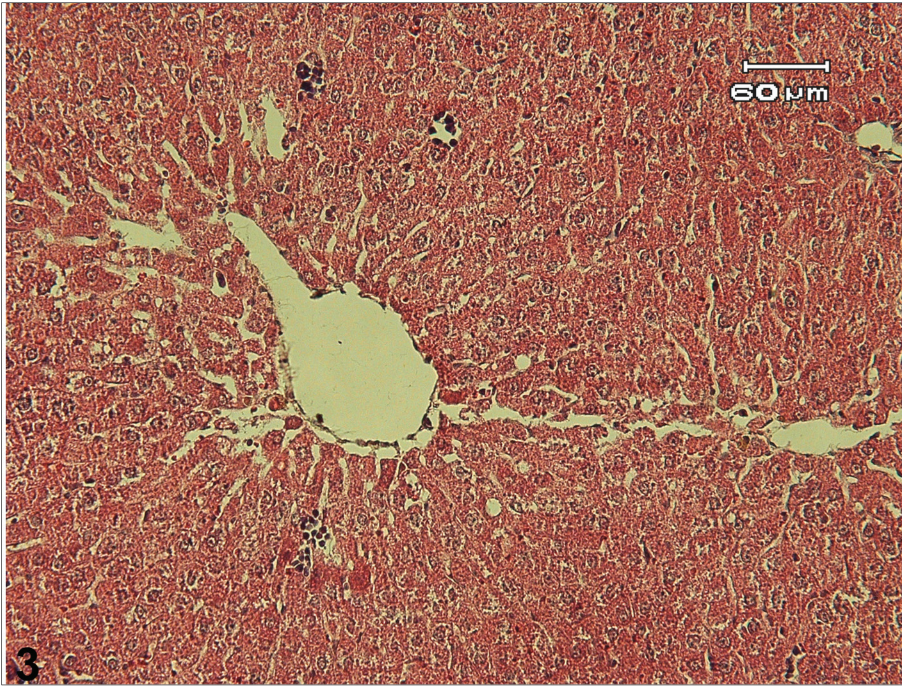


Fig 3. Kefir group, liver, only few lipid vacuoles, H&E

Şekil 3. Kefir grubu, karaciğer, birkaç yağ vakuolü**,** H&E

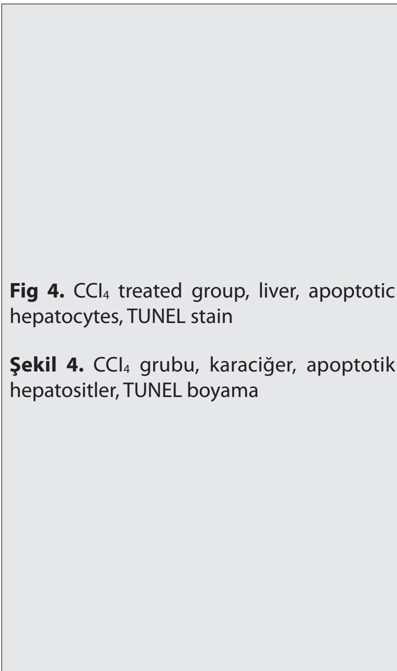
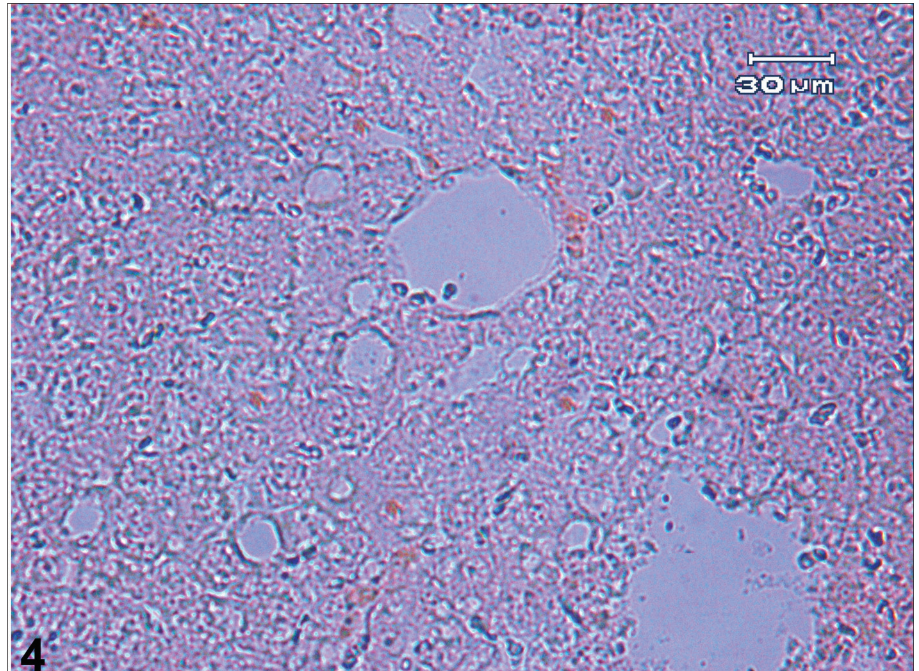


Fig 4. CCl_4 treated group, liver, apoptotic hepatocytes, TUNEL stain

Şekil 4. CCl_4 grubu, karaciğer, apoptotik hepatositler, TUNEL boyama



an effect on natural immune system it modulates the immune system [30] and also previous studies reported that kefir including many benefical general health properties such as antioxidant features [14,31]. In the study its clearly observed that affiliated to CCl_4 administration severe liver damage was formed but kefir drink significantly reduced pathological changes. Initial positive change observed at macroscopical appearance of the liver. While in CCl_4 tretaed group yellowish pigmentation and crumbly-fatty consistency noticed, with kefir administration nearly to normally colour and consitency was observed. Due to CCl_4 toxication histopathologically diffuse, big lipid vacuoles were replaced with few and small lipid vacuoles with kefir addition. Also kefir protect the paranchymal structure of the liver. This situation was explained at previous studies by this; kefir can able to inhibite the adipocyte differentiation due to its ability to eliminate lipid accumulation at mature adiposites. GPDH (gliserol 3 fosfat dehidrogenaz) is an enzyme that converse glycerole to triglyceride and regular kefir consumption also can be significantly reduced GPDH activity [32]. Kefir has other benefical effect on decreasing chlosterol levels [33-35]. By apoptosis dying cells are promptly removed by phagocytosis and replaced by new cells generated by mitosis, also apoptosis is an essential feature

of a wide variety of acute and chronic diseases, including liver diseases [36]. In the study cytoplasmic reactions that were detected with TUNEL test, were associated with apoptotic bodies that including nucleous residuals. We absorved apoptosis in some hepatocytes depending on CCl_4 exposed. But although severe lipid accumulation, hepatocellular degeneration and inflammatory reaction; apoptotic changes were very mild. Based on kefir supplementation microscopically nearly to control group liver paranchymal structure was observed. Otherwise any reaction that was related to apoptosis was determinated.

In conclusion, our results indicate that CCl_4 induce histopathological changes and apoptosis at hepatocytes. Kefiran intake decreased these adverse alterations and did not show any negative effects in the liver of rats. As a results the study shows that kefir is a healthy food that protect liver from CCl_4 toxication and inhibits hepatocellular degeneration, lipid accumulation and apoptosis.

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REFERENCES

1. Sanlı Y: Veteriner Farmakoloji. *Ankara Üniv. Vet. Fak. Yay,* No: 412, Ankara, 1988.

2. Vural N: Toksikoloji. *Ankara Üniv. Eczacılık Fak. Yay*, No: 56, Ankara, 1984.

3. Özsoy N, Okyar A, Arda-Pirinçci P, Can A, Bolkent Ş, Akev N: Evaluation of Smilax excelsa L. Use in Experimentally Induced Nephrotoxicity. *Kafkas Univ Vet Fak Derg,* 19, 807-814, 2013. DOI: 10.9775/kvfd.2013.9253

4. FAO/WHO: Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria; *FAO/WHO*: Amerian Córdoba Park Hotel, Córdoba, Argentina, 1-34, 2001.

5. Fuller R: Probiotics in man and animals. *J Appl Bacteriol,* 66, 365-378, 1989. DOI: 10.1111/j.1365-2672.1989.tb05105.x

6. Robert P, Fedor RN, Madsen KL: Probiotics and nutraceuticals: Non-medicinal treatments of gastrointestinal diseases. *Curr Opinion Pharma,* 5, 596-603, 2005. DOI: 10.1016/j.coph.2005.06.009

7. Falagas ME, Betsi GI, Athanasiou S: Probiotics for prevention of recurrent vulvovaginal candidiasis - A review. *J Antimicrob Chemother,* 58, 266-272, 2006. DOI: 10.1093/jac/dkl246

8. Szymanski H, Pejcz J, Jawien M, Chmielarczyk A, Strus M, Heczko PB: Treatment of acute infectious diarrhoea in infants and children with a mixture of three *Lactobacillus rhamnosus* strains - A randomized, double-blind, placebo-controlled trial. *Aliment Pharmacol Ther,* 23, 247-253, 2006. DOI: 10.1111/j.1365-2036.2006.02740.x

9. Korolev NS: Starters for fermented milks. Sections 4, Kefir and Kumys Starters. *Bulletin of the IDF 227*, Chapter 2. International dairy Federation, Brussels, Belgium, 1988.

10. Zubillaga M, Weill R, Postaire E, Goldman C, Caro R, Boccio J: Effect of probiotics and functional foods and their use in different diseases. *Nutr Res,* 21, 569-579, 2001. DOI: 10.1016/S0271-5317(01)00281-0

11. Kubo M, Odani T, Nakamura S, Tokumaru S Matsuda H: Pharmacogical study on kefir a fermented milk product in Caucausus I. On antitumor activity. *Yakugaku Zasshi,* 112, 489-495, 1992.

12. Duitschaever CL, Kemp N, Emmons D: Pure culture formulation and procedure for the production of kefir. *Milchwissenschaft,* 42, 80-82, 1987.

13. Neve H: Analysis of kefir grain starter cultures by scanning electron microscopy. *Milchwissenschaft,* 47, 275-278, 1992.

14. Hoolihan LK: Prophylactic and therapeutic use of probiotics: A review. *J Am Diet Assoc,* 101, 220-238, 2001. DOI: 10.1016/S0002-8223(01)00060-8

15. Furukawa N, Matsuoka A, Takahashi T, Yamanaka Y: Effects of orally administered yogurt and kefir on tumor growth in mice. *J Jpn Soc Nutr Food Sci,* 43, 450-453, 1990.

16. Zacconi, C, Parisi MG, Sarra PG, Dallavalle P, Bottazzi V: Competitive exclusion of *Salmonella kedougou* in kefir fed chicks. *Microbiol Alim Nutr,* 12, 387-390, 1995.

17. St-Onge MP, Farnworth ER, Jones PJ: Consumption of fermented and nonfermented dairy products: Effects on cholesterol concentrations and metabolism. *Am J Clin Nutr,* 71, 674-681, 2000.

18. Matsuu M, Shichijo K, Okaichi K: The protective effect of fermented milk kefir on radiation-induced apoptosis in colonic crypt cell of rats. *J Radiat Res,* 44, 111-115, 2003. DOI: 10.1269/jrr.44.111

19. Cohen GM: Caspases: The executioners of apoptosis. *Biochem J*, 326, 1-16, 1997. DOI: 10.1042/bj3260001

20. Everett H, McFadden G: Apoptosis: An innate immune response to virus infection. *Trends Microbiol*, 7, 160-165, 1999. DOI: 10.1016/S0966-842X(99)01487-0

21. Green DR: Apoptotic pathways: The roads to ruin. *Cell,* 94, 695-698, 1988. DOI: 10.1016/S0092-8674(00)81728-6

22. Patel T, Bronk, SF, Gores, GJ: Increases of intracellular magnesium promote glycodeoxycholate-induced apoptosis in rat hepatocytes. *J Clin Invest,* 94, 2183-2192, 1994. DOI: 10.1172/JCI117579

23. Luna LG: Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. McGraw-Hill Book Co., NewYork. p.32, 1968.

24. Bruck RR, Hershkoviz O,Lider, H Aeed, L Zaidel, Z Matas, J Berg, Helpern Z: Inhibition of experimentally induced liver cirrhosis in rats by a nonpeptidic mimetic of the extracellular matrix associated Arg-Gly-Asp epitope. *J Hepatol*, 24, 731-738, 1996.

25. Anand BS: Cirrhosis of liver. *West L Med*, 171, 110-115, 1999.

26. Mutlu N, Ersan Y, Nur G, Koç E: Protective effect of caffeic acid phenethyl ester against lead acetate-induced hepatotoxicity in mice. *Kafkas Univ Vet Fak Derg,* 17 (Suppl. A): S1-S5, 2011. DOI: 10.9775/kvfd.2010.2717

27. Seven İ, Gülbaykalır, Tatlıseven P, Dağoğlu G: The ameliorative effects of propolis against cyclosporine A induced hepatotoxicity and nephrotoxicity in rats. *Kafkas Univ Vet Fak Derg,* 20, 641-648, 2014. DOI: 10.9775/kvfd.2013.10643

28. Güven A, Maraşlı N, Kaya N: Karbontetraklorür (CCl4) ve etil alkolün fare eritrosit antioksidan ve plazma lipid peroksidasyonuna etkisi. *Kafkas Univ Vet Fak Derg*, 9 (1): 1-4, 2003.

29. Abraham P, Wilfred G, Catherine SP: Oxidative damage to the lipids and protein in the lungs, testis and kidney of rats during carbon tetrachloride intoxication. *Clin Chim Acta*, 289, 177-179, 1999. DOI: 10.1016/S0009-8981(99)00140-0

30. Eliş Yıldız S, Yiğit F, Duman Aydın B, Karadağ Sarı E, deprem T, Koral Taşçı S: Effects of kefir, koumiss, milk and yoghurt administration on distribution of plasma cells and mast cells in mice spleen. *Kafkas Univ Vet Fak Derg*, 21, 195-201, 2015. DOI: 10.9775/kvfd.2014.12015

31. Lin MY, Change FJ: Antioxidative effect of ıntestinal bacteria *Bifidobacterium longum* ATCC 15708 and *Lactobacillus acidophilus* ATCC 4356. *Dig Dis Sci*, 45, 1617-1622, 2000.

32. Ho JN, Jae-Woo C, Won-Chul L, Mi-Kyoung K: Kefir inhibits 3T3-L1 adipocyte differentiation through down-regulation of adipogenic transcription factor expression. *J Sci Food and Agricult,* 93, 485-490, 2013. DOI: 10.1002/jsfa.5792

33. Kiessling G, Schneider J, Jahreis G: Long-term consumption of fermented dairy products over 6 months increases HDL cholesterol. *Eur*

J Clin Nutr, 56, 843-849, 2002. DOI: 10.1038/sj.ejcn.1601399

34. Xiao JZ, Kondo S, Takahashi N, Miyaji K, Oshida K, Hiramatsu A, Iwatsuki K, Kokubo S, Hosono A: Effects of milk products fermented by *Bifidobacterium longum* on blood lipids in rats and healthy adult male volunteers. *J Dairy Sci,* 86, 2452-2461, 2003. DOI: 10.3168/jds.S0022-0302(03)73839-9

35. Güven A, Güven A: Hiperkolesterolemi oluşturulmuş tavşanlarda kefirin total kolesterol, trigliserit, HDL-kolesterol, LDL-kolesterol ve lipit peroksidasyonu üzerine etkisi. *Kafkas Univ Vet Fak Derg,* 11 (2): 127-131, 2005.

36. Guicciardi ME, Gores: Apoptosis: A mechanism of acute and chronic liver injury. *Gut*, 54, 1024-1033, 2005. DOI: 10.1136/gut.2004.053850

Kafkas Univ Vet Fak Derg
22 (3): 409-415, 2016
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Research Article

Konya Bölümü Folklorik Veteriner Hekimliğinde Ruminantlarda Doğum Bilgisi ve Jinekoloji [1] [2]

Çağrı Çağlar SİNMEZ [1] Aşkın YAŞAR [2]

[1] *Bu çalışma, "İç Anadolu Bölgesi Konya Bölümünde (Aksaray, Karaman ve Konya) Folklorik Veteriner Hekimliği ve Hayvancılık Üzerine Araştırma" başlıklı ve 112O428 numaralı TÜBİTAK-TOVAG araştırma projesinden yararlanılarak hazırlandı*
[2] *Bu çalışma, 21-23 Mayıs 2014 tarihlerinde Samsun'da düzenlenen IV. Veteriner Hekimliği Tarihi ve Mesleki Etik Sempozyumu'nda sözlü bildiri olarak sunuldu ve Bildiri Özetleri Kitabı'nın 119-120'nci sayfalarında özet olarak yayınlandı*

[1] Erciyes Üniversitesi, Veteriner Fakültesi, Veteriner Hekimliği Tarihi ve Deontoloji Anabilim Dalı, TR-38039 Kayseri - TÜRKİYE
[2] Selçuk Üniversitesi, Veteriner Fakültesi, Veteriner Hekimliği Tarihi ve Deontoloji Anabilim Dalı, TR-42003 Konya - TÜRKİYE

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Özet

Çalışmada, Konya Bölümü'nde ruminantlarda geçmişten günümüze ulaşan doğum bilgisi ve jinekoloji hakkında tedavi, yöntem ve tekniklerin ve bu alandaki somut olmayan kültür mirasının ortaya çıkarılması amaçlandı. Çalışmanın materyalini, doğum bilgisi ve jinekoloji hakkında bilgi sahibi olan toplam 237 kaynak kişiden bilgi derleme formu yoluyla elde edilen yazılı, sözlü ve görsel veriler oluşturdu. Çalışmada, ruminantların doğum ve jinekolojik hastalıklar ve sorunları, üreme-doğum bilgisi ve isimlendirmelerine ilişkin veriler elde edildi. Sonuç olarak, Konya Bölümü'nde ruminantlarda doğum bilgisi ve jinekoloji alanındaki folklorik uygulamaların eski uygarlıklardan beri halk tıbbında kullanılan dinî-sihrî, ampirik ve rasyonel tedavi modellerine örnek gösterilebileceği; ruminantların cinsiyet ve üreme özelliklerine göre Türkçe isimlendirilmelerinin, Türk dilinin ve Türk halk kültürünün yaşadığını ve korunduğunu göstermesi bakımından önem arz ettiği söylenebilir.

Anahtar sözcükler: *Doğum bilgisi, Folklorik veteriner hekimliği, Konya Bölümü, Ruminant, Veteriner jinekoloji*

Obstetrics and Gynecology of Ruminants at Folkloric Veterinary Medicine in Konya Region

Abstract

In this study, the aim was that to uncover to treat, methods, technicals and intangible cultural heritage in this field about obstetrics and gynecology in folklore of Konya Region. The study materials have been occured number of 237 people in Konya Region. Also written, visual and oral informations and documents have been given by people who have knowledge about obstetrics and gynecology. The study has given us those data such as obstetrics and gynecological diseases-problems, knowledge of reproduction and delivery, and naming in ruminants. In conclusion, folkloric methods have been used since the old civilization around Konya Region as a part of practice for the sake of religion, magic, empirical and rational treatments. According to gender and reproduction features of ruminants, their importance can be submitted with regard to naming in Turkish, their adaptation and maintance to Turkish and Turkish culture.

Keywords: *Folkloric veterinary medicine, Konya Region, Obstetrics, Ruminant, Veterinary gynecology*

GİRİŞ

Hayvan tarımı ile buğday tarımının birleşip bütünleştiği Türkiye coğrafyasında Türk kültürünün oluşturduğu sözel-görsel zenginlik, tıpkı on bin yıllık toprak altı zenginliği gibi, henüz işlenmemiş, keşfedilmemiş ve yeniden üretilerek çağımız insanına aktarılamamıştır [1].

Bu zengin kültür mirası içinde Türkiye folklorunun coğrafi konumu ve tarihsel bağlarıyla kendine özgü bir durumu vardır [2]. Folklor ürünlerinin halkın ortak duygu ve düşüncelerini dile getirmeleri bakımından Türk kültürünün korunmasında, yaşatılmasında önemli işlevleri bulunmaktadır. Halk kültürü ürünleriyle yaşadıkları yöre arasında bir bağ vardır [3]. Bu bağlamda, eski çağlardan beri Anadolu'nun sayılı kültür merkezlerinden olan Konya Bölümü, Hititlerden, Roma ve Bizans devirlerine kadar yaşamış ve bu devirler içinde kendine has kültür çeşitliliği olan bir bölgedir [4]. Konya İli, Anadolu'da Türklerin kurduğu ilk büyük devlet olan Selçuklulara başkentlik etmiş, bilimde

İletişim (Correspondence)
+90 532 7697225
cagribey6038@hotmail.com

ve sanatta yüzlerce yıl bütün dünya uluslarını etkileyen bir kültür iklimine sahip olmuştur [5].

Çalışmada, Konya Bölümü folklorunda ruminantlarda geçmişten günümüze ulaşan doğum bilgisi ve jinekoloji hakkında tedavi, yöntem ve tekniklerin ve bu alandaki somut olmayan kültür mirasının ortaya çıkarılması amaçlandı.

MATERYAL ve METOT

Çalışmanın materyalini, Konya Bölümü (Aksaray, Karaman ve Konya)'nde doğum bilgisi ve jinekoloji alanında bilgi sahibi olan toplam 237 kaynak kişiden (KK - halk hekimliği uygulayıcıları, hayvan sahipleri, hayvancılıkla uğraşan kişiler) bilgi derleme formu yoluyla yazılı, ses kayıt yolu ile sözlü ve fotoğraf çekimi ile elde edilen görsel veriler oluşturdu. Görüşmeler 06.11.2012-08.07.2013 tarihleri arasında gerçekleştirildi. Kaynak kişiler, bulgular bölümünde metin içinde yer alma sıralarına göre belirtildi.

Kaynak kişilerden elde edilen veriler ile arşiv, dokümantasyon merkezleri ve kütüphanelerden elde edilen bulgular, folklor ve tarih araştırmalarında kullanılan retrospektif yöntemle değerlendirmeye tabi tutularak, verilerin analiz ve sentezine gidildi. Bulgular "doğum ve jinekolojik hastalıklar ve sorunları", "üreme ve doğum bilgisi" ve "isimlendirme" ana başlıkları altında sunuldu.

BULGULAR

Doğum ve Jinekolojik Hastalıklar ve Sorunları

Hipokalsemi (Hypocalcemia): "*Doğum felci*", "*felç*" olarak bilinir. İnek doğumdan sonra ayağa kalkamaz ve yerde yatalak kalır (KK 1-3) [a]. Hayvanın başına ve vücuduna soğuk su dökülür (KK 4-5). Kuyruğundan çekilerek kaldırılmaya çalışılır. Karın altından uzun bir tahta geçirilir ve tahtanın uçlarından tutularak hayvan kaldırılır. Hayvana yeşil ot yedirilir. Şerbet veya pekmez içirilir. Sıcak su buharı koklatılır. İnek ayağa kalksın diye burnunun içine bir bardak sirke dökülür, altına sıcak battaniye ya da sıcak koyun gaitası konulur (KK 6). İnek meşe külüne yatırılır (KK 7). İneğe bir avuç göktaşı (bakır sülfat) ve kendi ağız sütü (kolostrum) içirilir (KK 8-12).

Prolapsus Vagina ve Prolapsus Uteri: "*Rahim*", "*buzağılık*", "*geyni*" ve "*elmacık çıkması*" olarak tanımlanır (KK 13-16). Tedavide, hayvanın arka tarafı yukarıda kalacak şekilde yükseltilerek bir yere bağlanır (KK 1, KK 17). Prolabe olan uterus soğuk su ile yıkanır. Ezilmiş, berelenmiş yerler sabunlu ılık su ile temizlenerek, yavru zarları uzaklaştırılır. Uterus bir bezin arasına alınarak elle basınç yapılır, bir yandan da soğuk su dökülür. Doğum kanalına girecek hale geldikten sonra itilerek karın boşluğuna reddedilir. Uterusun yerine yerleştirilmesini takiben hayvanın vulvasına *(geyni)* çuvaldız veya yorgan iğnesine geçirilen keçi kılından yapılmış iplerle ayrı dikişler atılır. Vulvanın alt ve üstüne odunlardan (meşe, söğüt dalları) yararlanarak kıskaç yapılır. Buraya keçe, koyun yünü veya odun parçaları tampon maksadıyla tespit edilir (KK 18-29). Rahimin iltihap kapmaması için ardıç *(Juniperus oxycedrus* L.) yaprağı katran karışımı yakılarak tütsüsü vulvaya tutulur (KK 30-31).

Retensiyo Sekundinarum (Retentio Secundinarum): Yavru zarlarına "*eş*" veya "*son*" denir (KK 9, KK 17, KK 32-38). Tedavide, sarkan yavru zarlarına ağır bir cisim (taş, kum torbası, tahta, kömür, eski ayakkabı vb) bağlanır. Sallanan yavru zarlarının altında katran ve ardıç yakılarak oluşan duman ile yavru zarları düşürülmeye çalışılır (KK 39-43). Soğan *(Allium cepa* L.) yedirilerek yavru zarlarının düşürüleceğine inanılır. İneğe kendi ağız sütü içirilir (KK 44). Aynı amaçla ineğe yumurta veya demli çay içirilir (KK 45-46). Rahimdeki yaraların iyileşmesi amacıyla şekerli su veya pekmez içirilir; lahana *(Brassica oleracea* L.) yaprağı ve buğday yedirilir (KK 9, KK 17, KK 32-38).

Güç Doğum (Dystocia): Sağ açlık çukurluğundan buzağının geliş pozisyonu tespit edilir. Eller yıkanır, kollara kadar sıvanır ve doğum kanalına girilir. Yavru suları patladıktan sonra doğum kanalını *(hazne)* kayganlaştırsın diye bir litre ayçiçeği yağı dökülür. Buzağının gelişi düzgün değilse baş, ayak ve gövdesi elle itme, döndürme ve çekme işlemleri uygulanarak düzeltilir. Buzağının ayakları yakalanarak dışarı doğru çekilir ve bukağılık çukurluğuna ipler bağlanır. Doğuma yardımcı olmak için komşulardan destek istenir. Yardımcı komşularla beraber, ayaklara veya kafaya bağlanan iplere ya da urgana bağlanmış sopaya asılarak buzağı çekip çıkarılır. Çekme işlemi zor olursa buzağıya bağlanan iplerin bir ucu da traktöre bağlanır ve traktörün hareketiyle buzağı çıkarılır (KK 15, KK 47-51).

Meme Yangısı (Mastitis): "*Yilimsek*", "*yilimseğe*", "*kara yilimseği*", "*boççа*" olarak bilinir. Mal gelinciği *(geçemen, arap tavşanı)*, "*carbık*" (tarla faresi, *gelengi)*, yılan veya "*kaplankaba*"nın (tosbağa) ineğin memelerinden süt emmesi sonucu oluşur (KK 13, KK 45, KK 47, KK 52-60). Memelerdeki *(bicik)* süt damarlarından süt gelmez, tıkanır ve peynirleşme olur (KK 61-73).

Köstebek *(kösnü)* toprağı ile yoğurt karıştırılarak memeye sürülür. Memeler, kil, ispirto ve ılık suyla yıkanır (KK 74-80). Bir hocaya tuz götürülür ve bu tuza dua okutulur. Okunan tuz hasta olan hayvana yedirilir (KK 81-82). Memelere sirke ve buzlu su ile masaj yapılır. Limon tuzu suda eritilir ve boz toprakla karıştırılarak memelere sürülür (KK 83-86). Meme lobları arasındaki damardan *(Vena subcutanea abdominis)* kan akıtılır (KK 87-95). Hastalığın, ahırı terk etmesi için ahır içine "*avula tası*" konulur (KK 96).

Meme Ödemi: Meme lobları, arka bacakların arasına doğru genişlemiş, bazen yere kadar sarkmış görünümdedir. Şişlik, karın altına doğru yayılım da gösterebilir. Bu şişliğe "*alazlama*", "*yel*", "*halavıza*", "*nuzla*" veya "*mundağlama*" adı verilir (KK 30, KK 97-100).

[a] *Kaynak kişi bilgileri istenildiği takdirde yazar/lardan temin edilebilir*

Tedavide, meme dokusu soğuk suyla masaj yapılarak yıkanır. Şiş olan yerlere sirke, yoğurt, ispirto, sumak *(Rhus coriaria* L.), tahin, turşu suyu, kerpiç suyu, ak toprak veya çamurlu çim sürülür (KK 101-107). Yumuşatıcı olarak memelere vazelin ve çam katranı *(Pix liquida)* sürülür. Şok tedavisi adı verilen yöntemle memelere bir bez yardımıyla bir soğuk bir sıcak su masajı yapılır (KK 8, KK 108-113). Domuzun azı dişi meme loblarına sürülür (KK 114). Meme dokusuna sarımsak *(Allium sativum* L.) yakı edilir (KK 46).

Agalaksi Enfeksiyonu (Mycoplasma agalactiae): "*Süt gidikliği*", "*süt kesen*", "*yel kesen*" olarak bilinen bu hastalıkta, hayvanlar yatır (dede, evliya türbesi) ziyaretine götürülür ve yatırın etrafında döndürülür (KK 80, KK 115-117).

Meme Başı ve Dokusu Çatlakları: Meme başı çatlaklarında, vazelin, bal, katran ve tereyağı sürülür. Meme yaralanması ve çatlaklarında ayva *(Cydonia oblonga)* çekirdeği ezilerek sürülür (KK 27, KK 67, KK 71, KK 118).

Üreme ve Doğum Bilgisi

Gebelik Teşhisi: Gebe ineklerin vulvaları şişkin ve içi kırmızı renktedir. Vulva içi (mukozası) beyaz renkte ise hayvan kısırdır (gebe değil) (KK 51, KK 119). Gebe ineğin çarası direk yere akar, asla kuyruğa bulaşmaz (KK 120 122). İneğin belini indirmesi (pelvis ligamentlerinin gevşemesi) gebeliğin son dönemlerine geldiğinin işaretidir. Bu dönemde, memeler dolgun, büyümüş ve sarkık hale gelmektedir (KK 123-124).

"*İneğin karınlaşması*" da gebelik alametidir. Suni tohumlamadan *(aşı, aşundura)* veya tabii aşımdan sonra ineğin vulvasından akan kan (metöstrus kanaması), tohumun tuttuğunun, ineğin gebe kaldığını işaret eder. İneğin idrarı bir tasa konur ve içine arpa, buğday atılır. Bu tahıllarda çimlenme gözlenirse hayvanın gebe olmadığı, çimlenme gözlenmez ise hayvanın gebe olduğu anlaşılır. Gebeliğin ilk 45-50 günlük döneminde bu uygulamadan sonuç alınır (KK 125). İnek tüylerini dikine (önden arkaya) yalarsa buzağısı erkek olur (KK 84, KK 126).

Koyunlarda ise karın duvarı boyunca elle yoklamak suretiyle gebelik muayenesi yapılır (KK 127). Koyunların kuyruğu kaldırıldığında vulvası aşağıya doğru sarkık ise gebelik teşhisi konulur (KK 47, KK 128-129). Gebe koyun güzelleşir, etlenir, yağlanır, tüy atar ve tüyleri parlaklaşır (KK 130).

Dişi koyunların genital bölgelerinde idrarın yol açtığı ıslaklık, zemindeki toprak, dışkı vb ile bulaşarak kuyrukta kirli görünüme neden olur. Bu ıslak görünüme "*çöpel*", "*siğe*", "*sağsak*" adı verilir. Cinsiyet tayininde bu görünüme göre hayvanın dişi ya da erkek olduğu anlaşılır (KK 8, KK 122, KK 131-132).

Fertilite ve İnfertilite: Kızgınlık gösteren ineğin vajinasından *(teninden*) "*çara*" (servikal mukus) adı verilen bir akıntı gelir. Çara, hayvanın vulvasından akarak kuyruğuna veya yere bulaşır. Böylece aşım zamanının geldiği anlaşılır. Kızgın inek yanındaki hayvanın üzerine çıkar veya altında durur. Ahırda bulunan veya komşudan getirilen bir boğa ineğin yanına getirilir. Eğer inek boğanın altında zıpkın gibi duruyorsa "*öğürsektir*" ya da "*keleğe gelmiş*" denilir (KK 125, KK 133-136). Kışın kapalı ahırlarda bulunan inekler kızgınlık halinde bazı sesler *(höörümek, böğürmek)* çıkarır; hayvan sahiplerinin üzerine atlarlar *(abrışır)* (KK 32, KK 137-139). İneğin kızgınlığının çabuk geçmesi için klitorisine kireç sürülür ve beline soğuk su dökülür (KK 140).

"*Öğürsemeyen*, *boğasamayan*" ineklerin önüne hemen kızgınlık göstersin diye tuz konulur. Gebe ineklere ise ilk 15-20 gün tuz verilmez, yeşil otlarla besleme yapılır (KK 141). Boğalara aşımı çok yapması için tuzsuz tereyağı ve yumurta içirilir. İkiz olarak dünyaya gelen dişi buzağılar çiftleşme dönemlerine geldiklerinde boğa aşımı (*keleğe çekmek*) sonucu döl tutmazlar (KK 142-144).

İnek eğer suni tohumlama veya tabii aşım sonunda döl tutmuyorsa "*ten*", "*soğukluk*", "*ala*" adı verilen klitorisi jiletle ya da bıçakla kesilir. Oluşan yaraya tuz basılır. Bu işleme "*kesme*" adı verilir. Kesme işleminden başka klitoris ateşte kızdırılmış demir, maşa, gibi aletlerle yakılır (KK 6, KK 10, KK 23, KK 50, KK 61, KK 63, KK 81, KK 106, KK 145-164). Her aşımdan sonra klitorise sönmüş kireç sürülür (KK 165).

Damızlıkta kullanılacak koçu seçerken testisleri büyük olanlar seçilir, çünkü döllerinin sütü bol, kuzusu iri olur (KK 47). Koçsayan (östrustaki) koyuna döl tutmaz korkusuyla tuz verilmez (KK 166). Koyunların aynı zamanda kızgınlık geçirmesi (senkronizasyon) için anason veya 20 gün süreyle arpa yedirilir (KK 9, KK 167). Gebe koyunlara verilen arpa miktarı azaltılır. Arpa azaltılmazsa koyunun sütü kesilir ve kuzuya zarar verir (KK 168-169).

Danaların birbirlerinin üzerine çıkarak yaralanmalarını engellemek için penisleri yaylaya çıkmadan önce uzun bir bez ile sarılıp bellerine bağlanır. Aynı amaçla tosunların boynuzlarından birinden geçirilen ip ayaklarından birine bağlanır. Bu uygulamaya "*dabındırık*" denilir (KK 60, KK 170).

Doğum Sonrası Bakım ve Beslenme: Doğumdan sonra ineğin kafasına, irkilmesi için bir kova su dökülür. İneğin yatacağı zemine saman serpilir. Evden tuz getirilerek yavrusunun üzerine serpilir. Böylece inek hemen yavrusunu yalamaya başlayarak yavru zarları ve sularını temizleyip buzağısını kurutmuş olur. Doğum sonrasında ineğin süt damarları açılsın diye pekmez veya şekerli su içirilir (KK 120, KK 142). Kepek, tuz ve ılık su karıştırılarak hazırlanan "*yal*" veya "*bulamaç*", ineğin içindeki yarayı iyileştirmesi amacıyla içirilir (KK 134, KK 171-177). İneğe enerji vermesi amacıyla un ve kırmızı pul biber *(Capsicum annuum* L.) su ile karıştırılarak içirilir (KK 45).

İnek veya koyun doğurduktan sonra ilk gelen süte "*ağız, "ağın*" veya "*burun ağzı*" denilir. Ağız sütünü ilk defa içen yavrularda "*ağız vurgunu*" denilen hastalık oluşur. Bu nedenle bu süt buzağılara ilk gün belli aralıklarla içirilir

(KK 178-181). Kendi sütünü emen inekleri bu alışkanlıktan vazgeçirmek için burunlarına sac, çivi, demir, kirpi derisinden yapılmış "*burunsalık*" takılır (KK 60).

Buzağı doğar doğmaz soluğunun açılması için kafasına ve vücuduna soğuk su dökülür. Sumak kaynatılır ve suyu içirilir (KK 182). Buzağılara ve malaklara daha iyi gelişmeleri ve solucan düşürmeleri için doğumu takiben iki ay boyunca her gün çiğ yumurta içirilir (KK 35, KK 171, KK 175, KK 183-186). Aynı amaçla "*palimez*" (un ve su karışımı) içirilir (KK 103).

Doğum *(buzağılama, kuzulama)* mevsimi Şubat-Mart ayları arasında olup, bu döneme "*döl ayı*", "*döllük zamanı*" denilir. Çobanlar, emzirmede çıkabilecek bazı sorunları gidermek için doğum yapan koyunları ve yeni doğan kuzuları takip ederler. Anasını ememeyen yavrulara biberonla süt içirilir. Kuzusunu emzirmek istemeyen koyun-keçinin -bunlara "*almaz*", "*almamış*" denilir- vajinasına bir çubukla tuz sokulur ve vajinasından gelen yavru suları yavrunun üzerine sürülür. Sonra, koyun "*almazlık*" adı verilen yere kazılan dar bir kuyuya ya da bölmeye kuzuyla beraber konulur ve kuzunun üzerindeki tuzla bulaşık akıntıları yalayarak kuzuyu emzirir (KK 65, KK 146, KK 187-193). Doğumda anasını kaybeden kuzu başka bir koyundan emzirilmek istendiğinde emzirmek istemeyen koyun kendi etrafında döndürülerek sersemletilir ve kuzunun üzerine koyunun yavru suları sürülür, sonra bir köpek veya kedi getirilerek koyunun üzerine salınır. Köpekten ve kediden korkan koyun kuzuyu kendi kuzusu zannederek emzirir, bu olaya "*ığındırık*" ismi verilir (KK 194-201). Kuzusunu almayan koyuna muska da yazılır (KK 130, KK 202).

Buzağısı ölen ineklerin sütten kesilmemesi için ölen buzağının derisi yüzülerek alıştırılacak buzağının sırtına bağlanır ya da ölen buzağının üzerindeki yavru suları ve yavru zarları alıştırılacak buzağının üzerine sürülerek ineğin buzağıyı kabul edip sağdırması sağlanır (KK 168, KK 203-207). Bir başka uygulamada ise ölen buzağının kafası üzerinde kalacak şekilde derisi yüzülür ve şişirilir, derisinin içi samanla doldurulur ve ayaklarından kasığa kadar değneklerle yere sabitlenerek dikiş iğnesiyle postun arkası dikilir. Hazırlanan yapma buzağının üstüne tuz dökülerek sağım sırasında ineğin altına konulur ve inek deriyi yalarken buzağıyı gerçek yavrusu zannederek sağdırır. Bu uygulamalara "*yakma*" denilir (KK 65, KK 132, KK 157, KK 174, KK 195, KK 208-214).

Buzağı ve "*emlik kuzular*" (süt emen kuzu) doğumdan sonra ilk iki gün analarının yanında bırakılarak kolostrum içmeleri sağlanır. Kuzu ve buzağıların analarını emmeden önce süt salınışını uyarmak için kafalarıyla analarının memelerine vurmalarına "*emişmek*" denilir. "*Kuzuluk*" adı verilen bölmelere alınan kuzular, günde sabah ve akşam olmak üzere iki kez emzirilir (KK 215-218).

İsimlendirme

Koyunlarda: "*Emlik*", yeni doğmuş süt emen kuzu; "*kuzu*", 25 gün - 6 aylık koyun yavrusu; "*toklu*", 1 yaşındaki kuzu; "*şişek*", 2 yaşında dişi koyun; "*koç*", 2 yaşında, bükülmemiş, damızlık erkek koyun; "*eke*", 2 yaşından yukarı koyun; "*kart*", 3 yaşını dolduran erkek koyun; "*öveç*", "*kızıl*" enenmiş erkek koyuna verilen isimlerdir (KK 219-220). Döl tutmayan koyuna "*yoz*", gebe koyuna ise "*yozlak*" koyun denilir (KK 127, KK 151, KK 186, KK 188, KK 221-227). Koyunun ergenlik dönemine "*çilenti*" denilir (KK 187). Birden çok kuzuyu emziren koyuna "*hondu*" ismi takılır (KK 228-229). Bir kuzu doğurmuş, ikinciye gebe olan koyuna "*gez*"; iki kuzu doğurmuş, üçüncüye gebe olan koyuna "*gezden çıkma*"; dördüncü kuzusunu doğuracak koyuna da "*ağzını düzmüş*" koyun adı verilir (KK 230-231).

Keçilerde: "*Körpe*", süt emen yavru keçi; "*oğlak*", "*gidik*", erkek yavru keçi; "*çebiş*", altı aylık keçi yavrusu; "*körüt*", bir yaşından üç yaşına kadar olan erkek keçi; "*yazmış*", 2 yaşındaki keçi; "*seyis*", 3 yaşındaki keçi; "*erkeç*", 3 yaşından büyük erkek keçi; "*teke*", tohumluk erkek keçi olarak isimlendirilir (KK 131, KK 221, KK 225, KK 232-235).

Sığırlarda: "*Boduk*", "*malak*", "*balak*", manda yavrusu; "*buzağı*", 0-6 ay arasındaki sığır yavrusu; "*dana*", 06-1 yaş arasındaki sığır; "*düve*", boğaya gelmemiş 2 yaştaki dişi sığır; "*boğa*", 2 yaş üzerindeki damızlık erkek sığır; "*öküz*", enenmiş erkek sığır olarak adlandırılır. İkiz doğan dişi ve erkek buzağılardan dişi olanın büyüdüğünde kısır olmasına "*ekiz eşi*" denilir. Gebe kalmış hayvanlara "*avunmuş*", "*tutmuş*" adı verilir (KK 62, KK 94, KK 127-128, KK 143, KK 225, KK 236-237).

TARTIŞMA ve SONUÇ

Acıpayamlı'ya [6] göre, Türkiye'de halk hekimliğindeki hastalık adlarının birçoğu Türkçe kökenlidir. Türkçenin, Arapça ve Farsça sözcük ve kurallarca işgaline karşı, halk hekimliği hastalık adlarının bu direnişi, halk kültürünün bu alanda güçlü olduğunu göstermektedir. Hayvan ehlileştirmesini ve yetiştiriciliğini en iyi şekilde yapabilen Türkler, aynı zamanda hayvan tedavisinde de başarılı olmuşlar ve dillerinde de çeşitli hastalıklara özel isimler vermişlerdir [7]. Nitekim, Kaşgarlı Mahmut Divanü Lûgat-İt-Türk'te [8,9], Orta Asya Türklerinin inek memesine ve bütün tırnaklı hayvanların memelerine "*yilin*" dediklerini; Dinçer [10], bu kelimeye "*siye*" bağlanmasıyla oluşan "*yilin-siye*" sözcüğünün memenin hastalığı anlamında kullanıldığını, Sinmez [11] ise bu hastalık isminin bazı değişikliklere uğramasıyla birlikte temelde öz Türkçe oluşu ve günümüze kadar fazla bir değişikliğe uğramadan gelişinin, Türk halk kültürünün dış etkenlere karşı direncini gösteren önemli bir kanıt olduğunu bildirmiştir. Akalın'a [12] göre, Türk kültüründe göçebe yaşayış uzun bir süre egemen olduğu için Türk halkı hayvanın erkeğine ayrı, dişisine ayrı, yavrusuna ayrı ad yakıştırmada çok ustadır. Özellikle tarihî süreç içerisinde yaşanılan din ve coğrafya değişiklikleri kültürün taşıyıcısı olan dili önemli oranda etkilemiş ve bu durum hayvan adlarında da kendini göstermiştir. Türkçede belirgin olarak karşılaşılan bu durum hayvanlarla ilgili kelime sayısını artırmış ve aynı cins içerisinde aynı kavram aralığında farklı

kelimelerin ortaya çıkmasına sebep olmuştur [13]. Çalışma verilerine göre, Konya Bölümü halk veteriner hekimliğinde mastitis hastalığına "*yilimsek*", "*yilimseğe*", "*boçça*"; agalaksi hastalığına "*süt gidikliği*", "*süt kesen*", "*yel kesen*"; yavru zarlarına "*eş*", "*son*"; dişi genital organına "*buzağılık*", "*elmacık*", "*geyni*" gibi adlar verilmesinin ve hayvanların yaşına, cinsiyetine, seksual dönemlerine ve gebelik durumuna göre farklı isimlendirilmelerinin yukarıdaki literatürlere benzer şekilde Türkçenin halk veteriner hekimliği içinde yaşadığını ve korunduğunu aynı zamanda hayvanlara yönelik özel isimlendirmelerin yapılmasının da onlara verilen bir değer ölçütü olarak kabul edilebileceği şeklinde yorumlanabilir.

Özen [14], 14'üncü yüzyıla ait bir baytarname incelemesinde bir hayvanın hamile kalması için klitorisinin keskin bir makasla kesildiğini; bazı folklorik veteriner hekimliği araştırıcıları [11,15-19] Anadolu'da döl tutmayan ineklerin klitorisinin demir, tava, maşa ile dağlandığı veya jiletle kesilip oluşan yaraya tuz, soğuk su, sarımsak sürüldüğünü; Kalkan [20], araştırmasında döl tutmayan düvelerde kliteridektominin ve bilhassa klitoris koterizasyonunun kan progesteron profilleri dengesini düzelterek gebe kalma oranını artırdığını; Gül [21], Eski Mısır Medeniyetinde bir kadının gebe olup olmadığının bir adet arpa, bir adet buğday dolu iki torbaya iki kadının idrar yapmasıyla ortaya çıktığını, bu uygulamada hamilelik şüphesi taşıyan kadının idrarla sulamış olduğu torbaların, diğer kadının sulamış olduğu torbalardan daha önce çimlenirse, kadının hamile olduğunun anlaşıldığını, Erk [22], bir baytarname incelemesinde aynı yöntemin atların gebe olup olmadıklarını tespit için kullanıldığını, Özen [14], buradaki teşhis yönteminin gebe hayvanın idrarındaki östrojen hormonunun yoğunluğu ile ilgili olabileceğini; Güler [23], gebe ineğin vulvasının ödemli ve hiperemik olduğunu, pelvis ligamentlerinin östrojen ve relaksin hormonları sonucu gevşediğini; Kalkan ve Horoz [24], ineklerde östrus bittikten 2-3 gün sonra görülen metöstrus kanamasının gebelikle bir ilişkisi olmadığı bildirmektedirler. Çalışmada, suni tohumlama veya tabii aşım sonunda ineklerin gebelik şansını artırmak için "*ten*", "*soğukluk*", "*ala*" adı verilen klitorisin jiletle ya da bıçakla kesildiği, oluşan yaraya tuz, kızdırılmış demir, maşa gibi aletlerle koterizasyon yapılmasının 14' üncü yüzyıla ait bir baytarname [14] ve Anadolu'daki folklor çalışma sonuçları [11,17,19] ile benzerlik gösterdiği; gebelik teşhisinin arpa veya buğdayın üzerine dökülen inek idrarının bu tahıllarda çimlenme yapıp yapmamasına göre belirlendiği; çiftleşmeden sonra ineğin vulvasından akan kanın gebelik bulgusu olduğu; gebe ineğin vulvasının şişkin, vulva mukozasının kırmızı renkte göründüğü, ineğin belini indirmesinin ve memelerinin büyümesinin ve koyunların vulvasının aşağıya doğru sarkık görünümünün gebelik belirtisi olduğu tespit edildi. Özen [14], Kalkan [20], Gül [21], Erk [22], Güler [23], Kalkan ve Horoz'un [24] tespitlerine benzer şekilde Konya Bölümü folklorunda gebelik konusunda yapılan uygulamaların halkın gebelik fizyolojisini iyi yorumladığı ve bazı pratiklerin baytarnameler ve eski Mısır Uygarlığından günümüze kadar süregeldiği sonucu çıkarılabilir.

İnsanlar, şifa dilemek, zor zamanlarında sığınılacak bir güce teslim olmak amacıyla Allah'a dua ederek kendilerini daha güvende hissetmişlerdir. İşte şifa tasları, bu tip ruhsal ve moral tedaviler için kullanılan kutsal taslardır [25]. Türklerde atalar kültüne göre, çok yaşayan, bilgili, yönetici insanların öldüğünde ruhlarının, ailesine ve toplumuna yardım ederek onları koruduğuna inanılan şaman geleneğinin izleri görülmektedir [26,27]. Ataların ve büyük şamanların mezarları, günümüzde İslami renge bürünmüş ve dua ederek ya da o mekânda bulunan bir nesneyle temas ederek ziyaret edilen evliya türbelerine dönüşmüştür [28]. İnsanlar tarafından bu türbelere ümitsiz hastalıklara karşı psikolojik tedavi gerçekleştirmesi amacıyla ziyaretler yapılmaktadır [29]. Çalışmada, Konya Bölümünde agalaksi hastalığının tedavisinde hayvanların evliya veya dede adı verilen kişilerin yattığı türbeye götürülmesinin İzgi [26], Ocak [27] ve Karaaslan'ın [28] belirttiği gibi kökeninin atalar kültüne dayandığı ve günümüzde İslami bir görünümle yaşatılmaya çalışıldığı, mastitis tedavisinde bir çeşit şifa tası olan "*avula tası*" gibi dini sembollerin kullanılmasının İşcan [25] ve Coşkun'un [29] ifadelerine benzer şekilde hayvan sahiplerinin hayvanlarının hastalıktan kurtulacağı ümidiyle olağanüstü ve kutsallık yüklediği bu tür ziyaret ve nesnelere başvurarak bir tür psikolojik rahatlama sağladığı şeklinde yorumlanabilir.

Anadolu'da halk arasında, "*yataklık çıkması*, *rahmin dışarı çıkması*, *iç çıkması*, *buzağılık* ve *elmacık çıkması*" olarak adlandırılan prolapsus uteri tedavisinde uterusun dışarı çıkan kısmının ılık su ve sabunla yıkanarak reddedildiği ve vulvanın çuvaldızla dikildiği [17-19]; Bizans Veteriner Hekimi Apsyrtus tarafından uterusun reddedildiği ve vulva dudaklarının tellerle dikildiği [30]; Dokuzuncu yüzyıla ait "*Kitab al-Hayl val-Baytara*" üzerine bir incelemede organların önce ılık suyla sonra şarap ve mazı ile yıkanıp reddedildiği, vulvaya keten iplikle dikiş konulup kavuk bağlanmak suretiyle vulvaya tazyik edildiği [31]; Ebu Bekr'in "*Naseri*" adlı eserinin incelenmesinde uterusun papatya suyu ile yıkanıp, yumuşayınca red edildiği [32] bildirilmektedir. Çalışma verilerine göre, prolapsus uteri ve prolapsus vagina tedavisinin Bizans Dönemi, baytarnameler ve Anadolu'daki folklor çalışmalarında yer alan tedavi yöntemlerine benzer özellikte olduğu ayrıca prooperatif tedavi açısından rasyonel uygulamalar içerdiği ileri sürülebilir.

Üçer [33], "*bazı eş, düşmek için toprak ister*" şeklindeki inanışa göre, doğum yapan kadınların göbek kordonlarına eşin düşürülmesi için hafif ağırlık ya da toprakla ilgili oldukları için çarık ya da eski ayakkabı bağlandığını bildirmektedir. Çalışma bulgularına göre, hayvanların sarkan yavru zarlarına ağır bir cisim (taş, kum torbası, tahta, eski ayakkabı vb) bağlanması, insanların kendilerine uyguladıkları tedavi ve yöntemleri hayvanları için de uyguladıklarının bir göstergesi olarak yorumlanabilir.

Baştan'a [34] göre, rasyondaki tuz ve bikarbonat içeriği yüksek ise meme ödemi riski artmakta ve sonuçta mastitise duyarlılık şekillenmektedir. Mastitis tedavisinde memeye

sık sık masaj yapılması ve günde 3-4 defa dönüşümlü soğuk-sıcak kompres uygulaması dolaşımı artırarak ödemin rezorpsiyonuna yol açmaktadır. Çalışma verilerine göre, memelere soğuk su veya şok tedavisi adı altında soğuk-sıcak masaj yapılmasının Baştan'ın [34] bulgularıyla örtüşen nitelikte rasyonel bir tedavi örneği olduğu söylenebilir.

Doğum döneminde işletmede sıklıkla karşılaşılan ve işgücünü artıran problemlerden biri öksüz veya anası tarafından reddedilen kuzuların bakımıdır. Yeni doğan kuzuların yaklaşık %10'u açlıktan ölebilmektedir. Belirtilen yavru kaybının nedenleri; anası tarafından kabul edilmeyenler, çoklu doğumlarda ana sütünün yeterli olmaması, meme fonksiyonlarında problem olan anaların yavruları, öksüz olanlar ve zayıf doğanlar olarak sıralanabilir. Eğer ana doğum esnasında ölürse veya yavrusu için yeterli süt üretemiyorsa bu durumda kuzu elden beslenebilir (yapay büyütme), yavrusunu kaybetmiş bir anaya alıştırılabilir (yakma işlemi, kuzunun başka bir koyuna alıştırılarak beslenmesinin sağlanması) veya tek bir yavrusu için fazla miktarda sütü olan bir ananın bakımına bırakılabilir [35]. Ancak, yetiştiriciler, bakım-besleme, yüksek ölüm oranı, artan işgücü ve süt ikame yeminin pahalı olması gibi nedenlerle yapay büyütmeyi pek tercih etmemektedirler [36]. Bu bağlamda, İzmir yöresinde küçükbaş hayvancılık işletmelerinde yapılan bir araştırmaya göre [37], öksüz yavruların işletmelerin %94.1'inde yakma yöntemiyle büyütüldüğü tespit edilmiştir. Yukarıdaki literatürler dikkate alındığında Konya Bölümü halk veteriner hekimliğinde tespit edilen bir koyunun bakıma muhtaç bir kuzu ile kapalı bir bölmede tutulması ve korkutularak öksüz bir koyunu kabul etmesi; koyunun yavrusu doğar doğmaz ölmüş ise koyunun yavru sularının öksüz kuzuya sürülmesi; ölen buzağının derisi yüzülerek, öksüz buzağının üstüne konması ve bu şekilde yavrusu ölen ineklerin alıştırılması gibi geleneksel kuzu/buzağı besleme yöntemleriyle işletmelerde kuzu kayıplarının önlenebileceği, işgücünün azaltılabileceği ve karlılığın artırılabileceği ileri sürülebilir.

Birçok kültürel miras, küreselleşmeyle birlikte kültür tek tipleşmesi, savaş, turizm, sanayileşme, kırsal göç, toplu göç ve doğa dengesinin bozulması gibi nedenlerle yok olma tehlikesi altındadır [1]. Bu tehlikeye karşı, UNESCO tarafından halkbilimi, etnoloji ve antropoloji disiplinlerinin ilgilendiği *gelenek* alanındaki kültürün korunması amacıyla *Somut Olmayan Kültürel Mirasın Korunması Sözleşmesi* 17 Ekim 2003 tarihinde Paris'te kabul edilmiştir. Bu çalışmayla, ruminantlarda doğum bilgisi ve jinekoloji alanındaki folklorik verilerin, Somut Olmayan Kültürel Miras Sözleşmesi' nde belirtilen *Halk Bilimi Kadroları* arasında görülen *Doğa ve Evrenle İlgili Bilgi ve Uygulamalar* bölümündeki *Halk Veteriner Hekimliği ve Zoolojisi*'ne [1] konu ve materyal desteği olabilecek nitelikte, yaşayan geleneksel kültür mirası olarak değerlendirilmesi ve eğitim programlarında yer alarak gelecek kuşaklara aktarılması önerilebilir.

Sonuç olarak, Konya Bölümünde ruminantlarda doğum bilgisi ve jinekoloji alanındaki folklorik uygulamaların eski uygarlıklardan beri halk tıbbında kullanılan dinî-sihrî, ampirik ve rasyonel tedavi modellerine örnek gösterilebileceği; ruminantların cinsiyet ve üreme özelliklerine göre Türkçe isimlendirilmelerinin, Türk dilinin ve Türk halk kültürünün yaşadığını ve korunduğunu göstermesi bakımından önem arz ettiği söylenebilir.

KAYNAKLAR

1. Oğuz MÖ: Somut Olmayan Kültürel Miras Nedir? II. Baskı. 62-63, Geleneksel Yayıncılık, Ankara, 2013.

2. Artun E: Günümüzde Adana Âşıklık Geleneği ve Âşık Feymani. *5. Milletlerarası Türk Halk Kültürü Kongresi* Bildirileri, Cilt I, Ankara, 41-52, 1997.

3. Günay U: Osmanlı İmparatorluğu ve Türk Halk Kültürü. Osmanlı Kültür ve Sanat, Yeni Türkiye Yayınları, Ankara, 1999.

4. Önder M: Konya Maarifi Tarihi. Ülkü Basımevi, Konya, 1952.

5. Küçükbezirci S: Konya Halkbilimi Folklor Güldestesi. 11-30, T.C. Konya Valiliği İl Kültür ve Turizm Müdürlüğü Yayınları, No: 115, Konya, 2006.

6. Acıpayamlı O: Türkiye folklorunda halk hekimliğinin morfolojik ve fonksiyonel yönden incelenmesi. *Türk Halk Hekimliği Sempozyumu* Bildirileri (23-25 Kasım 1988), Kültür Bakanlığı Milli Folklor Araştırma Dairesi Yayınları: 110, Seminer, Kongre Bildirileri Dizisi: 27, Ankara Üniversitesi Basımevi, Ankara, 1-9, 1989.

7. Dilgimen H: Veteriner Hekimliği Tarihi. 63-127, Bozkurt Matbaası, İstanbul, 1947.

8. Kaşgarlı Mahmut: Divanü Lûgat-İt-Türk (Çev. Besim Atalay). C.I, T.T.K. Basımevi, Ankara, 1985.

9. Kaşgarlı Mahmut: Divanü Lûgat-İt-Türk (Çev. Besim Atalay). C.III, T.T. K. Basımevi, Ankara, 1986.

10. Dinçer F: Hastalık adlarının halk dilindeki Türkçe karşılıkları. *Türk Dili*, 211, 62-63, 1969.

11. Sinmez ÇÇ: Bozlak Kültüründe Folklorik Veteriner Hekimliği ve Hayvancılık Üzerine Araştırma. *Doktora Tezi*, Selçuk Üniv. Sağlık Bil. Enst., 2011.

12. Akalın LS: Türk Folklorunda Kuşlar. 77, Kültür Bakanlığı Halk Kültürlerini Araştırma ve Geliştirme Genel Müdürlüğü Yayınları: 191, Gelenek-Görenek-İnançlar Dizisi: 19, Ersa Matbaası, Ankara, 1993.

13. Uçar İ: Türkiye Türkçesinde hayvan adlarından türetilmiş bitki adları. *Uluslararası Türkçe Edeb Kültür Eğit Derg*, 2 (1): 1-19, 2013.

14. Özen A: Milli Kütüphanedeki Yazma Baytarnameler Üzerinde Tarihsel İncelemeler. *Doktora Tezi*, Ankara Üniv. Sağlık Bil. Ensti., 1999.

15. Arslan ES: Ege Bölgesi Folklorunda Veteriner Hekimliği ve Hayvancılık Üzerine Araştırmalar. *Doktora Tezi*. Ankara Üniv. Sağlık Bil. Ensti., 1998.

16. Dinçer F: Türk Folklorunda Veteriner Hekimliği Üzerine Araştırmalar. *Doktora Tezi*. Ankara Üniversitesi, Ankara, 1967.

17. Sinmez ÇÇ: Sivas Yöresinde Folklorik Veteriner Hekimliği ve Hayvancılık Üzerine Araştırma. Cumhuriyet Üniversitesi Bilimsel Araştırma Projesi No: V-006, Sivas, 2013.

18. Yerlikaya H: Elazığ ve çevresinde hayvan hastalıklarında halk hekimliği üzerine araştırmalar. *Kafkas Univ Vet Fak Derg*, 8 (2): 133-136, 2002.

19. Yüksel E: Aşağı Fırat Havzasında Veteriner Hekimliği Folkloru Üzerine Araştırmalar. *Doktora Tezi*, Fırat Üniv. Sağlık Bil. Enst., 2012.

20. Kalkan C: Döl Tutmayan Düvelerde, Klitorisin Çıkarılması ve Koterizasyonunun, Kan Progesteron ve Östrojen Seviyeleri ile Gebe Kalma Üzerine Etkisi. *Doktora Tezi*, Fırat Üniv. Sağlık Bil. Ensti., 1991.

21. Gül E: Eski Mısır Medeniyetinde Hamilelik Testi. 2013. http://www.bilgiustam.com/eski-misir-medeniyetinde-gebelik-testi-cinsiyet-tespiti-ve-dogum-kontrolu, *Erişim tarihi:* 19.10.2013.

22. Erk N: Bir baytarname incelemesi. *Ankara Üniv Vet Fak Derg*, 6 (1-2): 126-135, 1959.

23. Güler M: Gebelik fizyolojisi. **In,** Alaçam E (Ed): Evcil Hayvanlarda

Doğum ve Jinekoloji. 106-107, Medisan Yayın Serisi: 40, Ankara, 2002.

24. Kalkan C, Horoz H: Pubertas ve seksüel sikluslar. **In**, Alaçam E (Ed): Evcil Hayvanlarda Doğum ve Jinekoloji. 26, Medisan Yayın Serisi: 40, Ankara, 2002.

25. İşcan A: Anadolu halk hekimliği örneği şifa tası. Anadolu Şehrinin Günlük Hayatında Su Kültürü. 144, Ankara Büyükşehir Belediyesi, Aski Genel Müdürlüğü Yayınları, Ankara, 2013.

26. İzgi MC: Şamanizm ve şamanlara genel bakış. *J Lokman Hek*, 2 (1): 31-38, 2012.

27. Ocak AY: Alevi ve Bektaşi İnançlarının İslam Öncesi Temleri. 53-135, İstanbul, 2000.

28. Karaaslan M: Kaşkay Türklerinde doğum çevresinde gelişen inanç ve pratikler. *Turk Stud*, 6 (3): 1435-1448, 2011.

29. Coşkun NÇ: Yatır ve ziyaretlerin halk kültüründeki rolü bağlamında Mersin'deki Muğdat Dede türbesinin incelenmesi. *Turk Stud*, 8 (1): 1205-1219, 2013.

30. Erk N, Erk H: Veteriner doğum ve jinekoloji tarihine bir bakış. *Ankara Üniv Vet Fak Derg*, 10 (1): 27, 1963.

31. Erk N: Dokuzuncu yüzyıla ait "Kitab al-Hayl val-Baytara" üzerinde bir inceleme. *Ankara Üniv Vet Fak Derg*, 8 (4): 367-386, 1962.

32. Erk N: İslam Medeniyeti Çağında Veteriner Tababette Gelişmeler ve Naseri. 50-68, Ankara Üniversitesi Veteriner Fakültesi Yayınları, Ankara, 1959.

33. Üçer M: Sivas'ta doğum adetleri (doğum sonrası). *Sivas Folkloru*, 29, 1-5, 1975.

34. Baştan A: İneklerde Meme Hastalıkları. II. Baskı, 38, Hatiboğlu Basım ve Yayım, Ankara, 2007.

35. Taşkın T: Yetiştirme pratikleri. *Koyun Keçi Genetik Islah Çalıştayı*. Türkiye Damızlık Koyun Keçi Yetiştiricileri Merkez Birliği Yayınları, 229, Ankara, 2014.

36. Taşkın T: Kuzuların yapay sütle büyütülmesi. Ege Üniversitesi Tarımsal Uygulama ve Araştırma Merkezi. Teknik Bülten: 45, 2003.

37. Kandemir Ç, Alkan İ, Yılmaz Hİ, Ünal HB, Taşkın T, Koşum N, Alçiçek A: İzmir yöresinde küçükbaş hayvancılık işletmelerinin coğrafik konumlarına göre genel durumu ve geliştirilme olanakları. *Hayv Üretim*, 56 (1): 1-17, 2015.

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Research Article

Tumour Necrosis Factor-alpha, Haptoglobin, Serum Amyloid A and Neopterin Levels in Cattle with Lumpy Skin Disease [1][2]

Onur BAŞBUĞ [1] Zahid T. AĞAOĞLU [1] Nevin TUZCU [2]
Alparslan COŞKUN [1] Uğur AYDOĞDU [1] Akın YIĞIN [3]

[1] *This study was supported by "Research Fund of Cumhuriyet University with the grant number V-021"*
[2] *This study was presented as an poster presentation in 11. Veterinary Internal Diseases Congress, May 21-24, 2015, Samsun, Turkey*
[1] Cumhuriyet University, Faculty of Veterinary Medicine, Department of Internal Diseases, TR-58140 Sivas - TURKEY
[2] Cumhuriyet University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, TR-58140 Sivas - TURKEY
[3] Harran University, Faculty of Veterinary Medicine, Depertmant of Genetics, TR-63300 Sanliurfa - TURKEY

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Abstract

Lumpy skin disease (LSD) is a viral disease of cattle, characterised by the formation of nodules in different parts of the body. In this study, it was conducted to assess the pattern of changes of albumin, tumour necrosis factor-alpha (TNF-α), haptoglobin (Hp), serum amyloid A (SAA) and neopterin levels in cattle with LSD, to assess the clinical course of the disease, and to the demonstration of inflammatory process in cattle with LSD. This study was carried out in 30 cattle, including 20 animals naturally infected with LSD and 10 healthy animals. It was determined that, in the cattle infected with LSD, while albumin concentrations had significantly decreased (P=0.004) in comparison to the control group, Hp (P<0.001), SAA (P<0.001) and neopterin (P<0.001) concentrations had significantly increased. Receiver operating characteristic (ROC) curve was used to calculate the sensitivity and specificity of Hp, SAA and neopterin. The cut-off values of the healthy and infected cattle for Hp, SAA and neopterin were determined to be 0.196 mg/mL, 41.38 µg/mL and 23.93 nmol/mL, respectively. At these cut-off values, high levels of sensitivity (85% for Hp, 95% for SAA and 70% for neopterin) and specificity (90%) were detected. It was determined that SAA levels were of higher sensitivity and specificity compared to Hp and neopterin levels with respect to the demonstration of inflammation associated with LSD. Furthermore, the clinical picture of the disease was found to be significantly correlated with the Hp, SAA and neopterin levels.

Keywords: *Lumpy Skin Disease, Acute phase, Neopterin, Cattle*

Lumpy Skin Disease'li Sığırlarda Tümör Nekroz Faktör-alfa, Haptoglobin, Serum Amiloid A ve Neopterin Düzeyleri

Özet

Lumpy Skin Disease (LSD), vücudun çeşitli bölgelerinde nodül oluşumu ile karekterize sığırların viral bir hastalığıdır. Bu çalışmada; LSD'li sığırlarda albümin, tümör nekroz faktör-α (TNF-α), haptoglobin (Hp), serum amyloid A (SAA) ve neopterinin, hastalığın klinik seyrini değerlendirme, inflamatuar sürecin gösterilmesi ve bu testlerin öneminin belirlenmesi amaçlanmıştır. Bu çalışma, doğal olarak LSD'li 20 hasta sığır ile aynı bölgeden 10 sağlıklı sığır olmak üzere toplam 30 hayvan üzerinde yürütüldü. LSD'li sığırların albümin konsantrasyonun önemli düzeyde (P=0.004) düşük olduğu; serum Hp (P<0.001), SAA (P<0.001) ve neopterin (P<0.001) konsantrasyonunun kontrol grubuna göre anlamlı düzeyde yüksek olduğu belirlendi. Hp, SAA ve neopterin değerlerinin sensitivite ve spesifiteyi hesaplamada receiver operating characteristics (ROC) eğrisi kullanıldı. Sağlıklı ve LSD'li sığırların cut off değerleri Hp 0.196 mg/mL, SAA 41.38 µg/mL ve neopterin ise 23.93 nmol/mL olarak belirlendi. Bu değerler baz alındığında yüksek bir sensitivite (Hp %85, SAA %95 ve neopterin %70) ve spesifite (%90) olduğu anlaşıldı. LSD'deki inflamasyonu göstermede SAA düzeylerinin Hp ve neopterin düzeylerine göre daha yüksek sensitivite ve spesifiteye sahip olduğu belirlendi. Ayrıca hastalığın klinik görünümü ile Hp, SAA ve neopterin arasında önemli korelasyonlar bulundu.

Anahtar sözcükler: *Lumpy Skin Disease, Akut faz, Neopterin, Sığır*

INTRODUCTION

In recent years, several studies have been conducted on the establishment of diagnostic and prognostic markers that can be used for infectious diseases [1-5]. Thus, several researchers have reported that biological markers,

İletişim (Correspondence)
+90 346 2191010/2583
onurbasbug@hotmail.com

including haptoglobin (Hp), serum amyloid A (SAA) and neopterin, which demonstrate non-specific immune responses that develop during the course of infectious diseases, can be used for the diagnosis of infections [6-9].

Hp and SAA, which are produced during the acute phase reaction, are glycoproteins of hepatic origin. Veterinary medicine research has shown that, of species-specific acute-phase proteins, serum Hp and SAA provide valuable insight into the clinical picture of ruminant diseases [1,3,10,11]. Hp and SAA levels aid in the clinical diagnosis of the inflammatory diseases of cattle as well as in the differentiation of acute and chronic infections [1,12].

Changes in the levels of acute-phase proteins are induced by proinflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α), and gamma interferon (IFN-γ) [10,13]. The release of these cytokines also causes fever, anorexia, leukocytosis, activation of the coagulation system, and changes in trace elements and the endocrine system [10].

Due to the fact that it can significantly contribute to diagnosis and prognosis and can also be readily measured, neopterin, which is synthesized by monocytes and macrophages stimulated by IFN-γ released from activated T lymphocytes, is commonly used in medical research on human infectious diseases [14,15]. Medical studies have demonstrated that, during the course of acute viral infections, including among others human immunodeficiency virus (HIV), influenza and hepatitis, neopterin levels start to rise before the development of a specific antibody response against the viral agent and display correlation with the clinical course of the disease [16,17].

Lumpy skin disease (LSD) is an infectious disease of cattle, which is characterised by the formation of nodules on the skin and in various parts of the body, and which may be fatal [18,19]. Outbreaks have been reported the morbidity rate of the disease to range between 3-100% and the mortality rate of the disease to range from 0% to 26% [18,20]. The aetiological agent of the disease, the lumpy skin disease virus (LSDV), belongs to the family *Poxviridae* and genus *Capripoxvirus* [21]. The incubation period of LSD is reported to vary between 1-4 weeks. The disease may follow an acute or subclinical course in cattle [19,22]. The clinical symptoms of LSD include fever, reduced food intake, nodules on the skin and mucous membranes as well as in the internal organs, enlargement of the lymph nodes, salivation, lacrimation, nasal discharge, oedema in various regions of the body, and in the case of severe infections, ulcerative lesions of the mucous membranes [18,20,22,23]. LSD causes significant economic losses as a result of reduced milk yield, weight loss, damaged hides due to skin nodules, abortion, infertility and mortality [18,20,24]. Reports indicate that the polymerase chain reaction (PCR) produces more reliable diagnostic results in comparison to other immunological tests [20,25,26].

To the authors' knowledge, no literature report has been published on serum Hp, SAA and neopterin levels in cattle infected with LSD. In this study, the inflammatory potential of Hp, SAA and neopterin levels was investigated in cattle infected with LSD.

MATERIAL and METHODS

Animals and Samples Collection

This study was performed in the years 2014-2015, Sivas province of Turkey. This study was carried out in 30 cattle, including 20 animals naturally infected with LSD and 10 healthy animals, all which were raised in the same region. The cattle included in the infected (LSD) group were of different breed (8 Holstein, 8 Brown Swiss, and 4 Simmental cattle), age (1-4 years) and sex (16 females, 4 males). Similarly, the cattle included in the control group were also of different breed (4 Holstein, 4 Brown Swiss, and 2 Simmental cattle), age (1-4 years) and sex (8 females, 2 males). The cattle in the control group was identified as Infectious Bovine Rhinotracheitis (IBR), Brucellosis, Mucosal Disease-negative. The study protocol was approved by the Ethics Committee of Cumhuriyet University, Turkey (Approval No: 2014/50).

On the basis of the clinical examination of the cattle infected with LSD, clinical scoring was performed with respect to body temperature (>39.4°C, 1 point, >40.0°C, 2 point), the frequency of skin lesions (very few-1 point, moderate-2 points, diffuse-3 points), the presence of oedema (1 point), lacrimation and nasal discharge (1 point), and the enlargement of the lymph nodes (1 point) *(Table 1)*.

Following the clinical examination of the animals, blood samples were taken from the jugular vein for laboratory analyses. For Real-Time PCR and haematological analyses, the blood samples were drawn into tubes coated with tripotassium ethylenediamine tetra-acetate (K_3EDTA), and for biochemical analyses and ELISA, the blood samples were collected into sterile plastic tubes. Haematological analyses were performed within an hour after the collection of the blood samples. For biochemical analyses and ELISA, the blood samples drawn into sterile plastic tubes were centrifuged (10 min; 3.000 × g) for serum extraction.

Table 1. *Clinical scoring of cattle infected with LSD*
Tablo 1. *Lumpy skin disease (LSD)'li sığırların klinik skorlaması*

Parameters	1 point	2 points	3 points
Rectal temperature	>39.4°C	>40.0°C	-
Presence of nodules	few	moderate	diffuse
Enlargement of lymph nodes	+	-	-
Lacrimation	+	-	-
Nasal discharge	+	-	-
Oedema	+	-	-

Until being analysed for TNF-α, Hp, SAA, neopterin and biochemical, the serum samples were stored at -80°C.

DNA Isolation

DNA isolation from whole blood samples was performed using a DNA isolation kit (MagNA Pure Compact Nucleic Acid Isolation Kit I, Roche Cat No: 03730964001) and the samples were stored at -80°C.

Real Time PCR

Virus detection by Real Time PCR was performed using the forward primer 5'-AAA ACG GTA TAT GGA ATA GAG TTG GAA-3, the reverse primers 5'-AAA TGA AAC CAA TGG ATG GGA TA-3' and 5'-6FAM-TGG CTC ATA GAT TTC CT-MGB/NFQ-3' probe [27] and a Real Time PCR device (Light Cycler 480, Roche Diagnostic, GmBh, Germany). The master mix solution was prepared as described below: 9.0 µL of PCR-purity *ddH₂O*, 4.0 µL of TaqMAN Probe Master Kit (Roche Cat No: 04535286001), 0.5 µL of Primer F, 0.5 µL of Primer R, 1.0 µL of Probe, and 5.0 µL of viral DNA were mixed to obtain a total volume of 20 µL. The initial denaturation cycle was performed at 95°C for 600 sec, and was followed by a quantification protocol of 45 denaturation cycles at 95°C for 3 sec, annealing at 60°C for 30 sec, extension at 72°C for 1 sec (single), and a cooling cycle at 40°C for 30 sec.

Haematological and Biochemical Analyses

Haematological analyses (total leukocyte, erythrocyte, thrombocyte, haemoglobin and haematocrit measurements) were performed using an automated hematology cell counter (Mindray BC-2800Vet, PRC). Serum glucose, creatinine, total bilirubin, total protein, albumin, aspartate aminotransferase (AST), ɣ-glutamyl transferase (GGT), alkaline phosphatase (ALP), urea and creatine kinase levels were measured using an auto-analyser (Mindray BS 200, PRC).

Analyses for haptoglobin, SAA (Tridelta LTD, Ireland), TNF-α (SunRed, PRC) and neopterin (Yehua, PRC) were performed with commercial kits. The optical density the samples was measured by use of a micro plate reader (Thermo Multiskan GO, Microplate Spectrophotometer, USA).

Measurement of TNF-a Concentrations

Serum TNF-α concentrations were measured using the sandwich ELISA method, in accordance with the recommendations of the manufacturer (Sunred Biological Technology Co. Ltd., Shanghai, PRC). It has been reported that the measurement limit and sensitivity of the test are 15-4.000 ng/L and 14.155 ng/L, respectively, and the intra- and inter-assay precision (reproducibility) (coefficient of variation, CV, %) are <9% and <11%, respectively.

Hp Measurements

The inhibition of haemoglobin peroxidase activity and the level of Hp in the samples are directly proportional. Therefore, serum Hp levels were measured using commercial kits (Tridelta Development Ltd., Maynooth, County Kildare, Ireland) on the basis of the inhibition of haemoglobin peroxidase activity. It has been reported that the analytic sensitivity, and intra- and inter-assay precision (reproducibility) (CV, %) of this test are 0.005 mg/mL, 5.3-12.1% and 4.1-5.7%, respectively.

SAA Measurements

Serum SAA concentrations were measured using the solid-phase sandwich ELISA method (Tridelta Development Ltd., Maynooth, County Kildare, Ireland). The samples were analysed after being diluted at a proportion of 1:500. The manufacturer has reported the bovine serum or plasma analytic sensitivity of this test as 1.5 ug/mL. The intra- and inter-assay precision (reproducibility) (CV, %) of the test have been indicated as 7.5% and 12.1%, respectively, in cattle.

Neopterin Measurements

Serum neopterin concentrations were measured using commercial kits (Yehua Biological Technology Co., Ltd. Shanghai, China) and by the sandwich ELISA method. The measurement limit, sensitivity and intra- and inter-assay precision (reproducibility) (CV, %) of this test have been reported as 0.2-60 nmol/mL, 0.11 nmol/mL, and <10%, respectively.

Statistical Analysis

Statistical analyses were performed using the 15.0 SPSS package programme (Statistical Package for Social Sciences, Chicago, IL). The variables were tested for normal distribution with the Kolmogorov-Smirnov test. Comparisons between groups were made by use of Student's t test for continuous variables with a normal distribution, whilst variables with non-normal distribution were analysed with the Mann-Whitney U test. Furthermore, correlations between serum albumin, TNF-α, Hp, SAA and neopterin levels were assessed with Pearson's correlation coefficients.

Receiver operating characteristic (ROC) curves and the area under these curves were used to assess the diagnostic potential of serum albumin, TNF-α, Hp, SAA and neopterin levels. ROC analyses were performed for both the cattle infected with LSD and the healthy control animals. To assess the diagnostic potential of serum albumin, TNF-α, Hp, SAA and neopterin levels in the diagnosis of LSD, the area under curve (AUC) and some cut-off values were analysed. The results were assessed at a 95% confidence interval and at a significance level of $P<0.05$.

RESULTS

Clinical examination *(Table 2)* revealed increased body temperature (n=14), excessive salivation and lacrimation

Table 2. *Clinical scores of cattle infected with LSD*
Tablo 2. *Lumpy skin disease (LSD)'li sığırların klinik skorları*

Samples No	Rectal Temperature (°C)	Presence of Nodules	The Enlarged Lymph Nodes	Lacrimation	Nasal Discharge	Oedema
LSD 1	++	++		+	+	
LSD 2	+	+	+			+
LSD 3	++	++				
LSD 4	++	+		+		
LSD 5	++	+	+			+
LSD 6	+	+				
LSD 7	++	+				+
LSD 8	++	++	+			+
LSD 9	++	+++		+	+	
LSD 10	+	+				+
LSD 11	+	+++	+	+	+	
LSD 12		+++		+	+	
LSD 13		+		+	+	
LSD 14	+	++				
LSD 15	++	++			+	
LSD 16		++	+			
LSD 17		++		+	+	
LSD 18	++	+++		+	+	
LSD 19		++	+			+
LSD 20		+		+	+	

Table 3. *Haematological and biochemical parameters of cattle control and with Lumpy Skin Disease (LSD)*
Tablo 3. *Lumpy Skin Disease'li ve kontrol sığırlarının hematolojik ve biyokimyasal parametreleri*

Parameters	Control Group X ± Sx	LSD Group X ± Sx	P value
WBC (X10^{9}L)	9.05±0.70	11.38±8.59	.302
RBC (X10^{12}L)	6.42±0.21	6.1±0.21	.309
HGB (g/dL)	9.65±0.57	8.87±0.38	.272
HCT (%)	29.65±1.24	27.85±0.83	.245
PLT (X10^{9}L)	428.10±26.13	543.65±54.91	.071
Total protein (g/dL)	6.80±0.17	6.97±0.15	.694
Albumin (g/dL)	3.60±0.06	3.30±0.08	.004**
Creatine kinase (mg/dL)	197.78±31.63	645.00±359.27	.699
Creatinine (mg/dL)	1.04±0.04	0.97±0.027	.164
BUN (mg/dL)	16.50±0.69	15.40±1.01	.376
Total bilirubin (mg/dL)	0.08±0.02	0.29±0.14	.104
AST (u/L)	92.60±5.83	95.20±9.76	.821
GGT (u/L)	27.90±2.60	25.75±1.60	.491
ALP (u/L)	77.56±9.29	101.16±15.93	.212

WBC, *White blood cell count;* ***RBC,*** *Red blood cell count;* ***HGB,*** *Hemoglobin;* ***PCV,*** *Packed cell volume;* ***PLT,*** *Platelet;* ***BUN,*** *Blood urea nitrogen;* ***AST,*** *Aspartate aminotransferase;* ***GGT,*** *Gamma-glutamyl transferase;* ***ALP,*** *Alkaline phosphatase; ** Correlation is significant at the 0.01 level*

Table 4. *Tumour necrosis factor-alpha (TNF-α), haptoglobin (Hp), serum amyloid A (SAA) and neopterin levels of cattle control and with Lumpy skin disease (LSD)*
Tablo 4. *Lumpy skin disease'li (LSD) ve kontrol sığırların tümör nekroz faktör-α (TNF-α), haptoglobin (Hp), serum amyloid A (SAA) ve neopterin düzeyleri*

Parameters	Control Group X ± Sx	LSD Group X ± Sx	P value
TNF-α (ng/L)	1079.41±311.72	1502.19±254.13	.307
Hp (mg/mL)	0.13±0.015	1.54±0.25	.001
SAA (µg/mL)	19.80±4.19	275.98±17.13	.001
Neopterin (nmol/mL)	18.55±1.60	31.54±2.43	.001

(n=9), enlargement of the lymph nodes (n=6), oedema in various regions of the body (n=6), and multifocal skin nodules ranging from 1 cm to 5 cm in size (n=20). Although these skin nodules were particularly diffuse in the head, neck, genital region, perineum and legs, they were distributed throughout the body.

The results of the haematological and biochemical analyses for each group are presented in *Table 3*. TNF-α, Hp, SAA and neopterin levels are shown in *Table 4*. It was determined that, when compared to the control group, the albumin concentrations of the cattle infected with LSD were significantly lower (P=0.004), and the serum Hp (P<0.001), SAA (P<0.001) and neopterin (P<0.001) levels were significantly higher *(Table 3, Table 4)*.

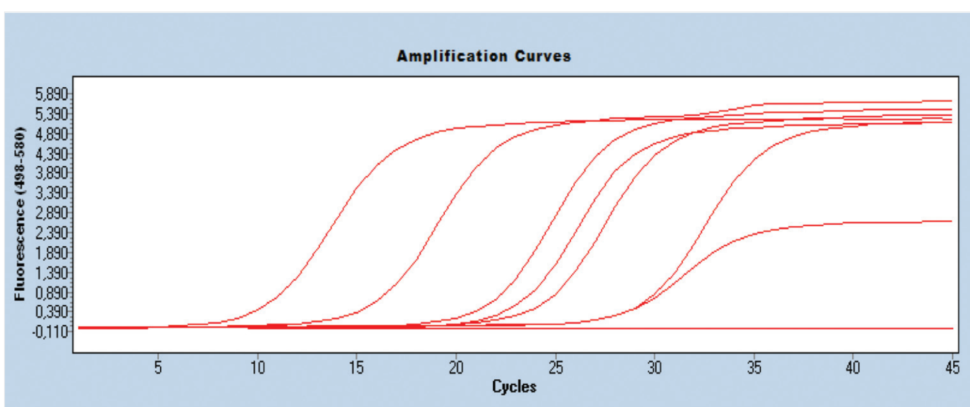

Fig 1. Amplification graph of cattle determined to be LSD-positive by RealTime PCR

Şekil 1. Real-Time PCR ile Lumpy skin disease (LSD) pozitif belirlenen sığırların amplifikasyon grafiği

Table 5. *The areas under the ROC curves (AUC), cut-off values, sensitivity and specificity of Albumin (Alb), Tumour necrosis factor-alpha (TNF-α), haptoglobin (Hp), serum amyloid A (SAA) and neopterin levels*

Tablo 5. *Albümin (Alb), Tümör nekroz faktör-alfa (TNF-α), haptoglobin (Hp), serum amyloid A (SAA) ve neopterin düzeylerinin ROC eğrisi altında kalan alan (AUC), cut off değeri, sensitivitesi ve spesifikliği*

Parameters	Alb (g/dL)	TNF-α (ng/L)	Hp (mg/mL)	SAA (µg/mL)	Neopterin (nmol/mL)
AUC	0.808	0.781	0.950	0.975	0.865
Cut off	3.39	814.03	0.196	41.38	23.93
Sensitivity	60.0	70.0	85.0	95.0	70.0
Specificity	90.0	87.5	90.0	90.0	90.0

Table 6. *Correlations amongs albumin (Alb), Tumour necrosis factor-alpha (TNF-α), haptoglobin (Hp), serum amyloid A (SAA), neopterin and clinical score*

Tablo 6. *Albümin (Alb), Tümör nekroz faktör-alfa (TNF-α), haptoglobin (Hp), serum amyloid A (SAA), neopterin ile klinik skorlama arasındaki korelasyonlar*

Parameters	Alb (g/dL)	TNF (ng/L)	Hp (mg/mL)	SAA (µg/mL)	Neopterin (nmoL/mL)	Clinical Scoring
Alb (g/dL)		-.251	-.563(**)	-.476(**)	-.599(**)	-.617(**)
TNF (ng/L)	-.251		.430(*)	.409(*)	.776(**)	.310
Hp (mg/mL)	-.563(**)	.430(*)		.636(**)	.745(**)	.587(**)
SAA (µg/mL)	-.476(**)	.409(*)	.636(**)		.640(**)	.862(**)
Neopterin (nmol/mL)	-.599(**)	.776(**)	.745(**)	.640(**)		.601(**)
Clinical scoring	-.617(**)	.310	.587(**)	.862(**)	.601(**)	

** Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level*

ROC analysis was performed to ascertain albumin, TNF-α, Hp, SAA and neopterin activity in the control animals and the cattle infected with LSD. The ROC curves, used to assess the diagnostic potential of albumin, TNF-α, Hp, SAA and neopterin levels, are presented in *Fig. 1* and the areas under the curves (AUC) are shown in *Table 5*. The ROC analysis was used to establish the cut-off, sensitivity and specificity values.

When the control animals and cattle infected with LSD were differentiated on the basis of Hp, SAA and neopterin cut-off values of 0.196 mg/mL, 41.38 µg/mL and 23.93 nmol/mL, respectively, the sensitivity rates for serum Hp, SAA and neopterin levels were determined as 85%, 95% and 70%, respectively, and the specificity rate was ascertained as 90% *(Table 5)*.

Correlations between albumin, TNF-α, Hp, SAA and neopterin levels were analysed in both the control group and the cattle infected with LSD. TNF-α, Hp, SAA and neopterin levels were found to be positively correlated with each other. While albumin levels were found to be negative correlate with TNF-α, Hp, SAA and neopterin levels *(Table 6)*.

DISCUSSION

Tests, which demonstrate the diagnostic and prognostic factors in diseases, are of particular significance in both veterinary and human medicine. Reports indicate that the levels of Hp and SAA, which are acute-phase proteins that have important functions in the various phases of inflammation, provide valuable information throughout

the clinical course of infectious diseases [1,4,28,29]. Research carried out in animals infected either experimentally or naturally with viral diseases has yielded results demonstrating the correlation of acute-phase protein levels with the clinical picture. Höfner et al.[30] reported that, in cattle exposed to the foot and mouth disease (FMD) virus, as from the onset of viraemia on the 8. day, clinical symptoms were observed and increased haptoglobin levels were detected.

Heegaard et al.[5] reported that, in calves experimentally infected with bovine respiratory syncytial virus, serum Hp concentrations exceeded 10 mg/mL and SAA levels exceeded 80 µg/mL. These researchers suggested that these increased levels and local pathological changes associated with the disease induced the acute phase response, and indicated that the acute phase response was correlated with the clinical course of the disease.

In the present study, it was determined that the serum Hp and SAA levels were 1.54±0.25 mg/mL and 275±17.3 µg/mL, respectively, in the cattle infected with LSD, and it was ascertained that, when compared to the control group, both markers had significantly increased ($P<0.001$). A statistically significant positive correlation ($P<0.001$) was detected between the clinical scoring, Hp and SAA levels ($r=588$ and $r=862$, respectively). This showed that the disease was associated with a strong acute phase response and that the pathological changes were correlated with the Hp and SAA levels. Also, increase in SAA levels may be a indicator of inflammatory process associated with LSD as evident of high test sensitivity and specificity.

Neopterin is produced in monocytes and macrophages by the guanosine triphosphate (GTP) cyclohydrogenase-I enzyme via γ-interferon (INF-γ), following the activation of Th1 lymphocytes [31,32]. As its secretion depends on the level of activation of T cells, neopterin has been suggested to be a non-specific indicator of the activation of the cellular immune system. Reports indicate that changes resulting in the activation of T lymphocytes during the pathogenesis of viral infections also affect neopterin levels [8,31,32]. Kaleli et al.[17] reported that, in carriers of the hepatitis B virus, macrophage activity, which increases with virus replication, elevates neopterin levels. Plata-Nazar et al.[8] reported to have observed significantly elevated neopterin levels in pediatric gastroenteritis cases caused by adenovirus and rotavirus infections. In rotavirus and adenovirus infections, at a neopterin cut-off value of 11.0 nmol/L, these researchers determined sensitivity and specificity rates of 86.6% and 94.3%, respectively. In their study on the assessment of the potential of serum neopterin levels in the detection of the severity of disease and causative agent in pneumonia patients, Prat et al.[9] observed that serum neopterin levels varied with the aetiology and severity of pneumonia. Furthermore, these researchers detected that the serum neopterin levels of bacteraemic patients with pneumococcal pneumonia were higher than those of non- bacteraemic pneumonia patients. Ercan et al.[33] indicated that in healthy cattle, neopterin levels were significantly higher particularly during the neonatal period, and suggested that this could be related to monocyte activation in new-born animals.

In the present study, the neopterin levels of the animals infected with LSD were determined to within the range of 31.54±2.43 nmol/mL, and it was ascertained that the mean values of the control group and the animals infected with LSD differed significantly ($P<0.001$). A statistically significant positive correlation ($r=640$) was determined to exist between clinical scoring and serum neopterin levels ($P<0.001$). Furthermore, in cattle infected with LSD, at a cut-off value of 23.93 nmol/mL, the sensitivity and specificity rates were determined as 70% and 90%, respectively. The high serum neopterin levels detected in the present study were attributed to the activation of the cellular immune system. Thus, in view of the data obtained, it is suggested that serum neopterin levels can be used as a biochemical parameter indicative of infectious activity in LSD cases.

TNF-α is a cytokine that can be determined in blood at an early stage following the activation of macrophages and other proinflammatory cells. It plays an important role in the regulation of the immune system [34]. Sordillo and Peel [35], reported that, in experimental *Escherichia coli* infections, increased TNF-α levels were observed up to 12-24 h, and suggested that the elevated TNF-α levels could aid in the assessment of the clinical course of the disease. In viral infections, the *in vitro* antiviral effect of TNF-α is observed as the inhibition of virus replication [35]. Indicated that the blood levels of cytokines, including TNF-α increased in cattle infected with the FMDV, and suggested that these increased levels could be involved in the inhibition of the development and growth of the virus in T cells, and thereby, in the prevention of the establishment of the virus in the body [13].

In the present study, the TNF-α levels detected in the cattle infected with LSD (1502.19±254.13 ng/L) were observed to have insignificantly increased in comparison to the levels determined in the control group (1079.41±311.72 ng/L) ($P<0.307$). This increase was attributed to the response of the host immune system to the virus. Clinical scoring and TNF-α levels were determined to be insignificantly correlated ($r=310$).

In study, no statistically significant differences in haematological and biochemical values except of albumin were determined in the animals with LSD and control. Most of the mean of the haematological and biochemical values found in the reference range [36].

Reports indicate that the level of albumin, which is considered to be the most significant negative acute-

phase protein in cattle, decreases in the event of infectious diseases [10]. Furthermore, it has been suggested that albumin levels could be used to assess the clinical picture of patients in human medicine [37]. In the present study, the serum albumin levels of the cattle infected with LSD ranged between 3.30±0.08 g/dL, and were found to significantly differ from the levels detected in the control group (P<0.001). Clinical scoring and albumin levels were ascertained to be negatively correlated with each other (r=-617). When evaluated together with Hp and SAA levels with which they have been determined to be correlated, decreased albumin levels can be interpreted as an indicator of inflammation.

In conclusion, a positive correlation was determined to exist between clinical findings and Hp, SAA and neopterin levels in cattle infected with LSD. Furthermore, it was ascertained that, when compared to Hp and neopterin, SAA showed a higher sensitivity in the detection of the severity of inflammation.

REFERENCES

1. Basbug O, Gul Y: Investigations on hemolysis in Cows with tropical theileriosis. *Kafkas Univ Vet Fak Derg,* 17, 421-427, 2011. DOI: 10.9775/kvfd.2010.3664

2. Beutler B, Cerami A: The biology of cachectin/TNF-α primary mediator of the host response. *Annu Rev Immunol*, 7, 625-655, 1989. DOI: 10.1146/annurev.iy.07.040189.003205

3. Chan JP, Chang C, Hsu W, Liu W, Chen T: Association of increased serum acute-phase protein concentrations with reproductive performance in dairy cows with postpartum metritis. *Vet Clin Pathol,* 39, 72-78, 2010. DOI: 10.1111/j.1939-165X.2009.00182.x

4. Eckersall PD, Bell R: Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine. *Vet J,* 185, 23-27, 2010. DOI: 10.1016/j.tvjl.2010.04.009

5. Heegaard PMH, Godson DL, Toussaint MJM, Tionerhoi K, Larsen LE, Viuff B, Ronsholt L: The acute phase response of haptoglobin and serum amyloid A (SAA) in cattle undergoing experimental infection with bovine respiratory syncytial virus. *Vet Immunol Immunopathol*, 77, 151-159, 2000. DOI: 10.1016/S0165-2427(00)00226-9

6. Jawor P, Steiner S, Stefaniak T, Baumgartner W, Rzasa A: Determination of selected acute phase proteins during the treatment of limb diseases in dairy cows. *Vet Med,* 53, 173-183, 2008.

7. Orro T, Pohjanvirta T, Rikula U, Huovilainen A, Alasuutari S, Sihvonen L, Pelkonen S, Soveri T: Acute phase protein changes in calves during an outbreak of respiratory disease caused by bovine respiratory syncytial virus. *Comp Immunol Microbiol Infect Dis,* 34, 3-29, 2011. DOI: 10.1016/j.cimid.2009.10.005

8. Plata-Nazar K, Luczak G, Gora-Gebka M, Liberek A, Kaminska B: Serum neopterin concentration in children with viral gastroenteritis. *Pteridines,* 21, 11-16, 2010. DOI: 10.1515/pteridines.2010.21.1.11

9. Prat C, Dominguez J, Andreo F, Blanco S, Pallares A, Cuchillo F, Ramil C, Ruiz-Manzano J, Ausina V: Procalcitonin and neopterin correlation with aetiology and severity of pneumonia. *J Infect,* 52, 169-177, 2006. DOI: 10.1016/j.jinf.2005.05.019

10. Gruys E, Toussaint MJM, Niewold TA, Koopmans SJ: Acute phase reaction and acute phase proteins. *J Zhejiang Univ Sci*, 11, 1045-1056, 2005.

11. Guzelbektas H, Sen I, Ok M, Constable PD, Boydak M, Coskun A: Serum amyloid A and haptoglobin concentrations and liver fat percentage in lactating dairy cows with abomasal displacement. *J Vet Intern Med,* 24, 213-219, 2010. DOI: 10.1111/j.1939-1676.2009.0444.x

12. Horadagoda NU, Knox KMG, Gibbs HA, Reid SWJ, HoradagodaA, Edwards SER, Eckersall PD: Acute phase proteins in cattle: Discrimination between acute and chronic inflammation. *Vet Rec*, 144, 437-441, 1999. DOI: 10.1136/vr.144.16.437

13. Nazifi S, Ansari-Lari M, Ghafari N, Mohtarami S, Ghezelbash A, Tabandeh MR: Evaluation of sialic acids, TNF-α, INF-γ, and acute-phase proteins in cattle infected with foot-and-mouth disease. *Comp Clin Pathol,* 21, 23-28, 2012. DOI: 10.1007/s00580-010-1059-5

14. Berdowska A, Zwirska-Korczala K: Neopterin measurement in clinical diagnosis. *J Clin Pharm Ther*, 319-329, 2001. DOI: 10.1046/j.1365-2710.2001.00358.x

15. Hoffmann G, Wirleitner B, Fuchs D: Potential role of immune system activation-associated production of neopterin derivatives in humans. *Inflamm Res,* 52, 313-321. 2003. DOI: 10.1007/s00011-003-1181-9

16. Eisenhut M: Neopterin in diagnosis and monitoring of infectious diseases. *J Biomark,* 1, 1-10. 2013. DOI: 10.1155/2013/196432

17. Kaleli I, Demir M, Cevahir N, Yılmaz M, Demir S: Serum neopterin levels in patients with replicative and nonreplicative HBV carriers. *BMC Infect Dis*, 6, 157, 2006. DOI: 10.1186/1471-2334-6-157

18. Abutarbush SM, Ababneh MM, Al Zoubi IG, Al Sheyab OM, Al Zoubi MG, Alekish MO, Al Gharabat RJ: Lumpy skin disease in Jordan: Disease emergence, clinical signs, complications and preliminary-associated economic losses. *Transbound Emerg Dis*, 62, 549-554, 2015. DOI: 10.1111/tbed.12177

19. Coetzer JAW: Lumpy skin disease. **In,** Coetzer JAW, Tustin RC (Eds): Infectious Diseases of Livestock. 2nd ed., 1268-1276, University Press Southern Africa, Oxford, 2004.

20. Gurcay M, Sait A, Parmaksız A, Kilic A: The detection of Lumpy Skin Disease virus infection by clinical findings and PCR method in Turkey. *Kafkas Univ Vet Fak Derg,* 21, 417-420, 2015. DOI: 10.9775/kvfd.2014.12364

21. Kitching PR, Mellor PS: Insect transmission of Capripox viruses. *Res Vet Sci*, 40, 255-258, 1986.

22. El-Neweshy MS, El-Shemey TM, Youssef SA: Pathologic and immunohistochemical findings of natural lumpy skin disease in Egyptian cattle. *Pak Vet J,* 33, 60-64, 2012.

23. Magori-Cohen R, Louzoun Y, Herziger Y, Oron E, Arazi A, Tuppurainen E, Shpigel NY, Klement E: Mathematical modelling and evaluation of the different routes of transmission of lumpy skin disease virus. *Vet Res,* 43, 1, 2013. DOI: 10.1186/1297-9716-43-1

24. Salib FA, Osman AH: Incidence of lumpy skin disease among Egyptian cattle in Giza Governorate. *Egypt Vet World*, 4, 162-167, 2011.

25. El-Kenawy AA, El-Tholoth MS: Lumpy skin disease virus identification in different tissues of naturally infected cattle and chorioallantoic membrane of embryonated chicken eggs using immunofluorescence, immunoperoxidase techniques and polymerase chain reaction. *Int J Virol,* 7, 158-166. 2011. DOI: 10.3923/ijv.2011.158.166

26. Sharawi SS, Abd ERI: The utility of polymerase chain reaction for diagnosis of lumpy skin disease in cattle and water buffaloes in Egypt. *Rev Sci Tech Off Int Epizoot,* 30, 821-830, 2011.

27. Stubbs S, Oura CAL, Henstocka M, Bowden TR, King DP, Tuppurainen ES: Validation of a high-throughput real-time polymerase chain reaction assay for the detection of capripoxviral DNA. *J Virol Methods,* 179, 419-422, 2012. DOI: 10.1016/j.jviromet.2011.11.015

28. Nielsen BH, Jacobsen S, Andersen PH, Niewold TA, Heegaard PM: Acute phase protein concentrations in serum and milk from healthy cows, cows with clinical mastitis and cows with extramammary inflammatory conditions. *Vet Rec,* 154, 361-365, 2004. DOI: 10.1136/vr.154.12.361

29. Murata H, Shimada N, Yoshioka M: Current research on acute phase proteins in veterinary diagnosis: An overview. *Vet J,* 168, 28-40, 2004. DOI: 10.1016/S1090-0233(03)00119-9

30. Höfner MC, Fosbery MW, Eckersall PD, Donaldson AL: Haptoglobin response of cattle infected with foot and mouth disease virus. *Res Vet Sci,* 57, 125-128, 1994. DOI: 10.1016/0034-5288(94)90093-0

31. Fuchs D, Hausen A, Reibnegger G, Werner ER, Dietrych MP, Wachter H: Neopterin as a marker for activated cell-mediated immunity:

Application in HIV infection. *Immunol Today*, 9, 150-155, 1998. DOI: 10.1016/0167-5699(88)91203-0

32. Watcher H, Fuchs D, Hausen A, Reibnegger G, Werner ER: Neopterin as a marker for activation of cellular immunity: Immunologic basis and clinical application. *Adv Clin Chem*, 27, 81-141, 1989. DOI: 10.1016/S0065-2423(08)60182-1

33. Ercan N, Tuzcu N, Basbug O, Kurtuluş G, Isidan H, Ograk YZ: The evaluation of important biomarkers in healthy cattle. *Kafkas Univ Vet Fak Derg*, 20, 749-755, 2014. DOI: 10.9775/kvfd.2014.11066

34. Francisco NM, Hsu NJ, Keeton R, Randell P, Sebesho B, Allie N, Govender D, Quesniaux V, Ryffel B, Kellaway L, Jacobs M: TNF-dependent regulation and activation of innate immune cells are essential for host protection against cerebral tuberculosis. *J Neuroinflamm*, 12, 1-14, 2015. DOI: 10.1186/s12974-015-0345-1

35. Sordillo LM, Peel JE: Effect of interferon on the production of tumor necrosis factor during acute *Escherichia coli* mastitis. *J Dairy Sci,* 75, 2119-2125, 1992. DOI: 10.3168/jds.S0022-0302(92)77971-5

36. Rodostits OM, Gay CC, Hinhcliff KW, Constable PD: Appendix 2 Reference Laboratory Values. **In,** Veterinary Medicine - A Textbook of the Diseases of Cattle, Sheep, Goats, Pigs and Horses. 10th ed., 2047-2050, WB Saunders, London,2006.

37. Wi YM, Kim JM, Peck KR: Serum albumin level as a predictor of intensive respiratory or vasopressor support in influenza A (H1N1) virus infection. *Int J Clin Pract*, 68, 222-229, 2014. DOI: 10.1111/ijcp.12249

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Research Article

Interdental and Interfragmentary Stabilisation (IAIS) of Mandibular Symphysis Separations and Parasymphyseal Fractures in Cats: A New Technique

[1] Faculty of Veterinary Medicine, University of Istanbul, Department of Surgery, TR-34320 Avcilar, Istanbul - TURKEY
[2] Istanbul Municipality, Cebeci Provisional Animal Shelter, TR-34270 Sultangazi, Istanbul - TURKEY

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Abstract

In veterinary literature, in order to achieve a more rigid stabilisation and complication-free recovery, both methods of circular (interfragmentary) and 8-shaped interdental cerclage wiring around the canine teeth are recommended in the treatment of open, infected and dislocated mandibular symphysis separations in cats. This is the only way to counteract craniocaudal and dorsoventral forces acting on the mandibular symphysis. However, this prolongs intraoperative time for the application and removal of cerclage wire, as well as necessitating deep sedation or general anaesthesia for removal. The aim of this study is to present the application stages and efficacy of a new technique (IAIS–Interdental and Interfragmentary Stabilisation) achieving both interdental and interfragmentary stabilisation in a single operation using only cerclage wire, with no need for extra stabilisation. For this purpose, the authors used the IAIS technique in the treatment of 46 patients presented to the surgery clinic between the years 2007-2015 and diagnosed with mandibular symphysis separation. Weekly post-operative follow-up examinations were carried out and clinical recovery time was evaluated. In conclusion, this new technique may be used effectively in the treatment of dislocated-uninfected and open-infected-dislocated mandibular sympyhsis separations.

Keywords: *Symphysis mandibula seperations, Cat, Cerclage wire, Interdental and interfragmentary stabilisation*

Kedilerde Symphisis Mandibula Ayrılmalarının ve Parasymphiseal Kırıkların Sağaltımında İnterdental ve İnterfragmenter Stabilizasyon (İVİS): Yeni Bir Teknik

Özet

Veteriner literatürde kedilerin, açık enfekte ve dislokasyonlu symphysis mandibula ayrılmalarının sağaltımında, daha rijit bir stabilizasyon ve komplikasyonsuz bir iyileşme elde edebilmek için, hem dairesel (interfragmenter) hem de canin dişler etrafından '8' şeklinde interdental serklaj teli uygulamasının bir arada kullanılması önerilmektedir. Bunun nedeni, symphysis mandibula üzerine etkiyen craniokaudal ve dorsoventral kuvvetlerin etkisinin ancak bu şekilde karşılanabilecek olmasıdır. Bu durum uygulama ve serklaj telinin uzaklaştırılması için geçen intraoperatif süreyi uzatmakta, aynı zamanda uzaklaştırma esnasında derin bir sedasyon ya da genel anesteziyi gerekli kılmaktadır. Bu çalışmanın amacı, ek bir stabilizasyon gerekmeksizin, sadece serklaj teli kullanılarak tek seferde hem interdental hem de interfragmenter stabilizasyon sağlayabilen yeni bir tekniğin (İVİS-interdental ve interfragmenter stabilizasyon) uygulama aşamalarını ve etkinliğini ortaya koymaktır. Bu amaçla 2007-2015 yılları arasında kliniğimize getirilen ve symphysis mandibula ayrılması tanısı konan 46 hastanın sağaltımında İVİS tekniğini kullandık ve postoperatif haftalık kontroller yaparak klinik iyileşme süresini değerlendirdik. Sonuç olarak bu yeni tekniğin kedilerde dislokasyonlu enfekte olmayan, açık enfekte ve dislokasyonlu symphysis mandibula ayrılmalarının sağaltımında etkin olarak kullanılabileceği sonucuna vardık.

Anahtar sözcükler: *Symphysis mandibula ayrılmaları, Kedi, Serklaj teli, Interdental ve interfragmanter stabilizasyon*

INTRODUCTION

The mandible is formed by the rostral median meeting of the two hemimandibulae in the joint known as the mandibular symphysis [1]. The cartilage structure forming the mandibular symphysis both acts as a growth centre and determines the shape and form of the anterior mandible by growing in a specific direction [2].

Clear differences are present between the mandibular symphyseal joint structure of adult mammals. While in some mammals fusion in this joint is never completed

İletişim (Correspondence)
+90 212 4737070/17296
ozer_kursat@yahoo.com

(amphiarthrosis), there is a union in some (synarthrosis), and complete ossification in others (synostosis). In small adult cats, the mandibular symphysis is in the form of a synarthrosis [3,4].

Road traffic accidents occupy first place in the etiology of mandibular symphysis separations, followed by falling from a height, fighting with other animals, firearm injuries and, rarely seen in cats, periodontal diseases and neoplasias [5,6]. Mandibular symphysis separation can easily be diagnosed with inspection and palpation of the free movement of one hemimandibula against the other [7].

The incidence of jaw fractures in cats varies between 15-23% among all fractures [8,9]. Mandibular symphysis separations occur at a rate of 73% of all jaw fractures [8]. Mandibular symphysis separations can be classified as: uninfected with no dislocation; uninfected with dislocation; and open, infected with dislocation [10].

In humans, vertical fractures of the mandibular region between the mandibular symphysis and canine tooth are known as parasymphyseal fractures [11] and are reported to present 19.5%-27% of mandibular fractures [12]. In dogs, the area between two mandibular canine teeth is described as the parasymphyseal region. Mandibular symphysis separations and fractures including the canine tooth are regarded as parasymphyseal fractures and the incidence has been calculated [13]. In this study, the authors considered cases in which the mandibular symphysis separation started rostrally and continued ventrally incorporating a fragment of one hemimandibula, to be cases with a parasymphyseal fracture.

In the treatment of mandibular symphysis separations, while options include; cerclage wiring, transmandibular pin and screw applications, the most prominent method is the straightforward but effective technique of treatment with cerclage wire [1]. However, in treatment using cerclage wire, only one of either interdental or interfragmentary stabilisation can be achieved [7]. In this study, we aimed to demonstrate the application stages and efficiency of a new technique (IAIS) achieving both interdental and interfragmentary stabilization by a single cerclage wire.

MATERIAL and METHODS

The material of the study consisted of cats presented to the surgery clinic with a history of trauma between the years 2007-2015. Following clinical and radiological examinations, cats diagnosed with uninfected-dislocated and open-infected-dislocated mandibular symphysis separation were included in the study. The cats had no other orthopaedic lesions apart from mandibular symphysis separation in the lower jaw and the mandibular canine teeth were intact in all cases. All cats were radiologically assessed, with regard to presence of lung damage due to trauma. Patients with breathing difficulties and epistaxis, together with those with haemathorax, pneumothorax and atelectasis were stabilised before surgery.

Patients deemed to be suitable for surgery following pre-anaesthesia assessment were put under general anaesthesia with 4-6 mg/kg IV propofol (Pofol®, Sandoz) and positioned in ventral recumbency. Beginning ventrally at the third incisive tooth on the medial aspect of the canine teeth and avoiding causing damage to teeth roots, two oblique canals opening into the mouth were formed using a Kirschner wire of 0.8-1mm diameter, depending on the cat's size *(Fig. 1-a)*. A 0.6mm-cerclage wire was threaded through these holes leaving both ends of the wire within the oral cavity *(Fig.1 b1, b2, b3)*. The ends of the cerclage wire within the oral cavity were bent caudo-lateral to the mandibular canine teeth *(Fig. 1c)* and stabilised by tightening above the gingiva on the ventral part of the incisive teeth *(Fig. 1 d1, d2, d3)*. Patient owners were advised to have amoxycilline clavulonic acid 22 mg/kg IM (Synulox®, Pfizer) injections administered to their cats for 5 days post-operatively and feed their cats a soft diet and wet food for 10 days. All cats were stabilised during same-day reanimation, returned to their owners and discharged.

Post-operative weekly follow-up examinations of the cases were carried out and recovery was evaluated. During these examinations, patients displaying no instability upon cranio-caudal and dorso-ventral manipulation of the joint were considered to have recovered and the cerclage wire was removed.

Cats determined to have clinically recovered were sedated using 1 mg/kg IM xylazine HCl (Rompun® 2%, Bayer). The cerclage wire was removed by cutting through two points, one inside the mouth and the other to the cranial of the incisive teeth.

The age, breed, gender, fracture type and recovery times of the patients were recorded. The Kruskal-Wallis test was used to demonstrate the effect of etiology on healing time and the Mann Whitney-U test was used to show the effect of the fracture type and gender on healing time. The Spearman test was used to determine the relationship between age and healing time. Statistical significance level was regarded as $P<0.05$. Analyses were carried out using the SPSS 13.0 programme.

RESULTS

The material of this study comprised a total of 46 cats, of which 30 were male and 16 female, of different breeds and ages presented to the surgery clinic. Clinical examination revealed uninfected-dislocated mandibular symphysis separation in 20 cats, open-infected-dislocated mandibular symphysis separation in 23 cats and open-infected-dislocated parasymphyseal fracture in 3 cats. Breed distribution was; 4 Persian, 3 Van, 2 Scottish Fold, 2 Siamese and 35 mixed breed. History taking revealed road

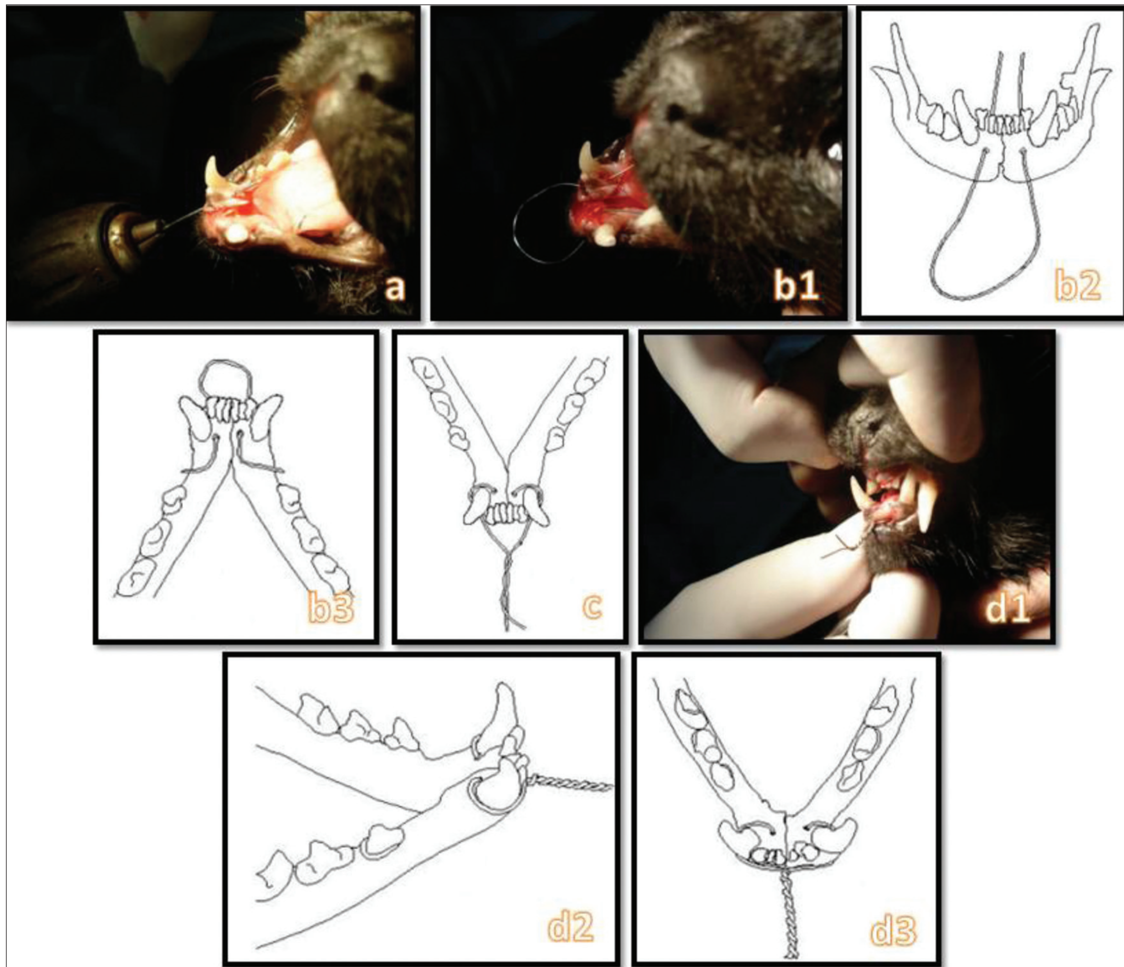

Fig 1. Stages of the IAIS technique for the surgical treatment of mandibular symphysis separations. Establishing canals opening into the mouth using a 0.8-1.0 mm Kirschner wire on the medial aspect of the canine teeth and ventral aspect of the 3rd incisive tooth **(a)**, advancing cerclage wire into oral cavity through canals **(b1)**, cranial aspect **(b2)**, intraoral aspect **(b3)**, bending of cerclage wire ends inside the mouth caudolateral to mandibular canine teeth **(c)**; twisting and securing cerclage wire ends over gingiva ventral to incisive teeth **(d1)**, lateral aspect **(d2)**, rostral aspect **(d3)**

Şekil 1. Symphysis mandibula ayrılmalarının IVIS tekniği kullanılarak yapılan cerrahi tedavisinin aşamaları. Köpek dişlerinin mediali, 3. insisiv dişin ventralinde 0.8-1.0 mm çapında bir kirchner teli ile ağız içine açılan birer kanal oluşturulması **(a)**, açılan kanallardan serklaj telinin ağız boşluğuna doğru ilerletilmesi **(b1)**, kranial görünüm **(b2)**, intraoral görünüm **(b3)**, serklaj telinin ağız içerisinde kalan uçlarının mandibular köpek dişlerinin kaudo-lateraline doğru bükülmesi **(c)**, ve incisiv dişlerin ventralinde, diş etleri üzerinde sıkılarak sabitlenmesi **(d1)**, lateral görünüm **(d2)**, rostral görünüm **(d3)**

traffic accident in 23 cats, falling from a height in 14 cats and unknown etiology in 9 cats. Recovery was observed to take place between 2-5 weeks (average 3 weeks) *(Table 1)*. No complications such as; loosening of the cerclage wire, infection or non-union were seen in any of the patients. Application time of the technique was not longer than 4 minutes in any of the cases.

Three cats were presented to our clinics with breathing difficulties and epistaxis together with those with haemathorax, pneumothorax and atelectasis respectively. Thoracocentesis was performed to haemathorax and pneumothorax patients and the own blood of haemathorax patient which we removed from thorax, was auto-transfused. Oxygen support and symptomatic therapy were performed these three cat before procedure.

Of the three cats with a parasymphyseal fracture, two were 12-months old and one was 24-months old. All three parasymphyseal fractures were open, infected and dislocated. Etiologically, 2 cats had suffered road traffic accidents, while the reason for the fracture in the third cat was unknown.

Due to its fibrocartilagenous structure, since the mandibular symphysis radiologically appeared as a radioluscent line even after recovery, post-operative radiographic assessment was not performed.

Statistical analysis demonstrated that etiology, fracture type or gender had no effect on recovery time (P>0.05). The relationship between age and recovery, however, was found to be significant (P<0.05).

Table 1. *Patient information*
Table 1. *Olgulara ait bilgiler*

Cases		Fracture Type							
		Uninfected with Dislocation		Open, Infected with Dislocation		Open, Infected with Dislocation- Parasymphyseal		Total	
		n	%	n	%	n	%	n	%
		20	**43.4**	**23**	**50.0**	**3**	**6.6**	**46**	**100**
Breed	Persian	3	15	1	4.4	-	-	4	8.7
	Scottish Fold	1	5	1	4.4	-	-	2	4.3
	Siamese	2	10	-	-	-	-	2	4.3
	Turkish Van	1	5	2	8.7	-	-	3	6.6
	Mix	13	65	19	82.5	3	100	35	76.1
Sex	Male	13	65	16	69.5	1	33.3	30	65.2
	Female	7	35	7	30.5	2	66.7	16	34.8
Cause	Traffic Accident	6	30	15	65.2	2	66.6	23	50.0
	High Rise	9	45	5	21.7	-	-	14	30.4
	Unknown	5	25	3	13.1	1	33.4	9	19.6
Other Parameters									
Age	Months	6-60		6-36		12-24		6-60	
	Average	20		15.4		16		17.4	
Healing Time	Weeks	2-5		2-5		3-4		2-5	
	Average	2.95		3.13		3.25		3.065	

DISCUSSION

The most prominent method in the treatment of mandibular symphysis fractures is the simple yet effective technique of cerclage wiring [5]. This is due to the fact that the mandibular bone is almost completely filled with tooth roots at the level of the symphysis and this, in turn, leads to reluctance to employ invasive methods for treatment [1].

In fracture treatment, using cerclage wire alone to achieve stabilisation provides one of either interdental or interfragmentary stability [7]. Particularly in the treatment of open, infected and dislocated mandibular symphysis cases, the combined application of circular and interdental 8-shaped cerclage wiring around the canine teeth has been reported to decrease cranio-caudal and dorso-ventral forces affecting the separation and provide a more rigid stabilization [6,14-16]. With the use of the IAIS technique, it was seen that dorso-ventral and cranio-caudal forces were easily counterbalanced without any need for extra stabilisation.

It has been reported that healing that will allow cerclage wire removal in mandibular symphysis fractures is achieved in 4-6 weeks [16-19]. In the present study, this duration was determined to be 3 weeks. This indicates that, in comparison to previously described methods, the IAIS technique provides shorter healing time.

The growth center, where endochondral type bone formation occurs within the mandibular symphysis, remains active until the age of 60-days when temporary canine teeth eruption is completed. Once cartilage growth is complete, the mandibular symphysis remains a fibrocartilagenous structure [2]. In other words, regardless of age, the chances of mandibular symphysis separation occurring in cats older than 2-months can be considered a high possibility under the same conditions. This demonstrates that, mandibular symphysis separations can be observed in adult cats, and not only in growing cats. At the same time, the fact that the mean age of the cats with mandibular symphysis separation in this study was 17 months is informative.

The oral mucosa and gingiva is highly vascularized and can give a rapid inflammatory response to bacterial infection. Also, saliva and gingival fluid contain numerous antibacterial substances contributing to the non-specific defense system. Properties such as; the bactericidal effect of lysosymes and hypothiocyanites in the saliva, the effect of IgA preventing bacteria from attaching to the oral mucosa, and the lactoferrin within the saliva binding the iron needed by bacteriae, are components of the oral cavity defense mechanism [20]. In this study, the fact that no infection complications were encountered in any of the patients during weekly post-operative follow-ups can be explained by the post-operative antibiotic use in addition to this mechanism.

Desirable features for the technique used in the treatment of mandibular fractures are; quick application,

atraumatic procedure and low cost, anatomical reduction, a rigid fixation and complete occlusion [17]. Anatomical reduction and rigid fixation are essential for bone healing and return to normal function. With the technique developed by the authors, the most rigid fixation possible using cerclage wire is applied using a single method. This technique also has the cerclage wire-related advantages of quick application, low cost, less invasiveness compared to other techniques, less soft tissue damage and rapid return to food consumption [16]. However, the most recommended method in the treatment of mandibular symphysis separations is for both ends of the cerclage wire to be advanced subcutaneously on the lateral side of each hemimandibula, exiting through a medial skin incision made on the ventral aspect of the mandibular symphysis and intertwining the two ends. In this technique, as well as cutting the piece of cerclage wire within the oral cavity, a medial incision on the ventral aspect must be made and the cerclage removed from this position where it has been twisted [5]. This can be performed under deep sedation or anaesthesia and not light sedation. This procedure achieved by light sedation in IAIS technique.

The IAIS technique can also be modified in cats with a single mandibular canine tooth fracture, by drilling a transversal hole in the mandibular segment between the canine tooth and first premolar tooth on the affected side, passing the intra-oral end of the cerclage wire through this hole and securing it on the ventral aspect of the incisive teeth. However, this will only provide interfragmentary stabilisation. All the same, removal of the cerclage wire following healing will be easier compared to circular cerclage wiring on the ventral medial line.

Statistical data obtained at the end of the study demonstrated that clinical healing occurred faster in young cats compared to old cats. This expected outcome was not included in the discussion since it could not be related to either the technique used by the authors or the bone in question.

In conclusion, the IAIS technique is a quick and easy-to-apply technique, provides sufficient stabilisation for bone healing and the cerclage wire can be removed under light sedation. This technique also succeeded in achieving dental occlusion in patients, and the cats were able to consume food shortly after the operation. As a result, it was concluded that the IAIS technique is a successful method in the treatment of mandibular symphysis separations in cats with intact mandibular canine teeth and that it is a first choice option particularly in open, infected and dislocated mandibular symphysis separations.

REFERENCES

1. Verstraete FJM: Maxillofacial fractures. **In,** Slatter D (Ed): Textbook of Small Animal Surgery. 3rd ed., 2190-2207, WB Saunders, Philedelphia, 2003.

2. Schacter RI, Furstman L, Bernick S: Postnatal development of the mandible of the cat. *Am J Orthod,* 56, 354-364, 1969. DOI: 10.1016/S0002-9416(69)80003-5

3. Scott JE, Hogue AS, Ravosa MJ: The adaptive significance of mandibular symphyseal fusion in mammals. *J Evol Biol,* 25, 661-673, 2012. DOI: 10.1111/j.1420-9101.2012.02457.x

4. Yalçın K, Kaya MA, Arslan A: Comparative geometrical morphometries on the mandibles of Anatolian wild sheep *(Ovis gmelini anatolica)* and Akkaraman sheep *(Ovis aries). Kafkas Univ Vet Fak Derg*, 16, 55-61, 2010. DOI: 10.9775/kvfd.2009.385

5. Harasen G: Maxillary and mandibular fractures. *Can Vet J,* 49 (8): 819-820, 2008.

6. Scott HW, McLaughlin R: Fractures and disorders of the skull and mandible. **In,** Scott HW, McLaughlin R (Eds): Feline Orthopedics. 261-271, Manson Publishing, London, 2006.

7. Johnson AL, Hulse DA: Management of specific fractures. **In,** Fossum TW (Ed): Small Animal Surgery. 2nd ed., 901-913, Mosby, Missouri, 2002.

8. Piermattei DL, Flo GL, DeCamp CE: Fractures and luxations of the mandible and maxilla. **In,** Piermattei DL, Flo GL, DeCamp CE (Eds): Handbook of Small Animal Orthopedics and Fracture Repair. 4th ed., 717-736, Saunders Elsevier, St. Louis Missouri, 2006.

9. Owen MR, Langley Hobbs SJ, Moores AP, Bennett D, Carmichael S: Mandibular fracture repair in dogs and cats using epoxy resin and acrylic external skeletal fixation. *Vet Comp Orthop Traumatol,* 4, 189-197, 2004.

10. Dingman RO, Natvig P: Occlusion and intermaxillary fixation. **In,** Dingman RO, Natvig P (Eds): Surgery of Facial Fractures. 2nd ed., 143, WB Saunders, Philadelphia, 1964.

11. Farwell DG: Management of symphyseal and parasympheal mandibular fractures. Operative techniques in otolaryngology, 19, 108-112, 2008. DOI: 10.1016/j.otot.2008.06.001

12. Boole JS, Holtel M, Amoroso P, Yore M: 5196 mandible fractures among 4381 active duty army soldiers, 1980 to 1998. *Laryngoscope,* 111, 1691-1696, 2001. DOI: 10.1097/00005537-200110000-00004

13. Lopes FM, Gioso MA, Ferro DG, Leon-Roman MA, Venturini MAFA, Correa HL: Oral fractures in dogs of Brasil - A retrospective study. *J Vet Dent,* 22 (2): 86-90, 2015.

14. Wiggs RB, Lobprise HB: Oral fracture repair. **In,** Wiggs RB, Lobprise HB (Eds): Veterinary Dentistry: Princibles and Practice. 259-279, Lippincott-Raven Publishers, Philadelphia, 1997.

15. Saglam M, Cetinkaya MA: Clinical studies of orthopaedic treatments of maxillar and mandibular traumatic lesions in cats. *Vet Cerrahi Derg,* 9 (1-2): 5-10, 2003.

16. Legendre LF: Management of facial fractures in cats. *Can Vet J,* 40 (11): 821-825, 1999.

17. Taylor RA: Surgical repair of mandibular fractures. **In,** Bojrab MJ (Ed): Current Techniques in Small Animal Surgery, 4th ed., 977-984, Lippincott Williams & Wilkins, Maryland, 1997.

18. Umphlet RC, Johnson AL: Mandibular fractures in the cat: A retrospective study. *Vet Surg,* 17, 333-337, 1988. DOI: 10.1111/j.1532-950X.1988.tb01028.x

19. Woodbridge N, Owen M: Feline mandibular fractures a significant surgical challenge. *J Feline Med Surg,* 15, 211-218, 2013. DOI: 10.1177/1098612X13477541

20. Ozer K: Ağız boşluğunun savunma mekanizmaları. **In,** Ozer K (Ed): Küçük Hayvan Diş Hekimliği. 13, Teknik Yayınevi, İstanbul, 1999.

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Research Article

Textural Properties of Fat - Reduced Sucuk with Orange Fiber

Barış YALINKILIÇ [1] Şeyma ŞİŞİK OĞRAŞ [2] Güzin KABAN [2]
M. Murat KARAOĞLU [2] Mükerrem KAYA [2]

[1] Department of Food Engineering, Faculty of Engineering, Iğdır University, TR-76000 Iğdır - TURKEY
[2] Department of Food Engineering, Faculty of Agriculture, Atatürk University, TR-25240 Erzurum - TURKEY

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Abstract

In this study, sucuk samples with different orange fiber (0%, 2% and 4%) and fat (sheep tail fat) levels (10%, 15% and 20%) were produced and textural parameters were investigated during ripening time. Use of orange fiber significantly affected hardness, chewiness, gumminess and resilience parameters in all groups (P<0.01). The highest hardness score was detected in samples containing 4% orange fiber. In contrast, no statistically significant effect of fat usage was observed on hardness (P>0.05). While textural parameters, adhesiveness, springiness, chewiness, gumminess and resilience, were affected (P<0.01) by fat level, cohesiveness was not significantly affected by fat level (P>0.05). However, ripening time was the most effective factor on all textural parameters (P<0.01). While a permanent increase was observed in hardness, adhesiveness, gumminess and chewiness values of all samples during ripening, the highest value (increase) was determined in hardness.

Keywords: *Sucuk, Orange fiber, Fat level, Texture*

Portakal Lifli Yağı - Azaltılmış Sucuğun Tekstürel Özellikleri

Özet

Bu çalışmada, farklı oranlarda portakal lifi (%0, %2 ve %4) ve yağ (koyun kuyruk yağı) (%10, %15 ve %20) kullanılarak sucuk üretilmiş ve tekstürel parametreler olgunlaştırma süresince incelenmiştir. Portakal lifi kullanımı tüm gruplarda sertlik, çiğnenebilirlik, sakızımsılık ve esneklik parametrelerini önemli seviyede (P<0.01) etkilemiştir. En yüksek sertlik değeri %4 portakal lifi içeren örneklerde belirlenmiştir. Buna karşın, yağ kullanımının sertlik üzerine istatiksel olarak önemli bir etkisi (P>0.05) belirlenmemiştir. Yapışkanlık, elastikiyet, çiğnenebilirlik, sakızımsılık ve esneklik yağ seviyesinden etkilenen (P<0.01) tekstürel parametreler iken, bağlayıcılık yağ seviyesinden (P>0.05) etkilenmemiştir. Bununla birlikte olgunlaştırma süresi tüm tekstürel parametreler üzerinde en etkili faktördür (P<0.01). Olgunlaştırma sırasında örneklerin sertlik, yapışkanlık, sakızımsılık ve çiğnenebilirlik değerlerinde sürekli bir artış gözlenirken, en yüksek değer (artış) sertlikte belirlenmiştir.

Anahtar sözcükler: *Sucuk, Portakal lifi, Yağ seviyesi, Tekstür*

INTRODUCTION

Textural properties of dry-fermented sausages play an important role on consumer preferences. In order to obtain best results for textural properties, all processing stages and ingredients should be controlled. However, attempts to bring functional properties to dry-fermented sausages with addition of dietary fibers bring new questions in controlling texture development. Because of their technological properties such as gel forming ability and water holding capacity, dietary fibers can strongly effect the textural properties during production and eventually causes a new product having different textural properties than the traditional one [1,2].

Fat is important for flavor, texture and aroma formation in meat products. However, to satisfy consumer health concerns, it is required to decrease the fat ratio in dry-fermented sausage formulation. But, decreasing fat in dry-fermented sausage formulation could cause some technological and sensorial problems. Hence, for minimizing the negative effects of fat reduction, plantal fibers have been used in dry fermented sausages [3,4].

Sucuk is a traditional dry fermented sausage produced and consumed in Turkey. Many important changes occur in biochemical and textural parameters of sucuk during processing. Changes in pH and moisture level during ripening are particularly important for sucuk production [5,6].

İletişim (Correspondence)
+90 442 2311623 Fax: +90 442 2360958
seymasisik@atauni.edu.tr

In our scan of research conducted on sucuk, there is only one study dealing with the textural properties of sucuk [5]. On the other hand, there are only a few studies on the use of fruit fibers in sucuk manufacture [7-9]. However, there is no information about the effects of different fat and fiber levels on sucuk's textural attributes. Thus, the aim of the study was to investigate the effects of different levels of fat and orange fiber on textural properties of sucuk.

MATERIAL and METHODS

Production of Orange Fiber and Sucuk Manufacturing

Cooked and dried orange fiber was obtained according to a method offered by Fernandez-Gines et al.[10]. Two replicates (Experiment I and Experiment II) were carried out for the study. Nine sucuk batters were prepared for each experiment according to fat level (10% sheep tail fat + 90% lean meat, 15% sheep tail fat + 85% lean meat, and 20% sheep tail fat + 80% lean meat) and orange fiber level (0%, 2% and 4%). As a parallel research project to that of Yalınkılıç et al.[9], the ingredients (g/kg) and ripening conditions were used. *Staphylococcus xylosus* GM92 and *Lactobacillus plantarum* GM77 strains were used as starter culture [11]. Sucuk mixture was prepared in a laboratory-type cutter (MADO MTK 662, Schwarzwald) by mincing and mixing. Prepared mixture were filled into collagen casings (38 mm, Naturin Darm, Germany) using a laboratory-type stuffing machine (MADO MTK 591,Schwarzwald). Fermentation and ripening of sucuk samples were carried out in an automatic climate unit (Reich, Stuttgart).

Moisture Content and Texture Profile Analysis

Sampling was carried out by randomly selecting two sucuk samples of each group at certain days (1, 3, 5, 7 and 9 days) of fermentation and ripening. Moisture content of samples was measured according to Gökalp et al.[12]. Sucuk samples were evaluated using a texture analyser (TA-XTplus, Stable Micro Systems, Godalming, Surrey, UK) equipped with a cylindrical metal probe (50 mm) (P/25) using a 50 kg load cell. Five slices of each sample (17 mm height and 25 mm diameter) were compressed to 50% of their original height in two cycles. The TPA method was carried out under these conditions: pre-test speed: 1 mm/s, test speed: 2 mm/s, post-test speed: 3 mm/s, trigger type: outo-20 g and time: 5 s. The data obtained were processed by Texture Expert Software (Stable Micro System, London, United Kingdom) and expressed as hardness, adhesiveness, cohesiveness, springiness, gumminess, chewiness and resilience.

Statistical Analysis

All data from each experiment were subjected to variance analysis (two replications, complete randomized design) and differences between means were assessed by Duncan's multiple range test using the SPSS 13.0.0.246 for Windows (SPSS, Inc., Chicago, Ill., USA).

RESULTS

Overall effect of orange fiber, fat and ripening time on the moisture values of sucuk is shown in *Table 1*. No significant differences were observed between groups containing orange fiber (P>0.05). In contrast, increase in fat level had a very significant effect on the decrease of final moisture content (P<0.01) of the product. In the other hand, ripening time also had a very significant effect on moisture content (P<0.01) *(Table 1)*.

The effects of different levels of orange fiber and fat on textural parameters (hardness, adhesiveness, cohesiveness, springiness, chewiness, resilience and gumminess) were observed during ripening and results are given in *Table 1*. Significant differences in some textural parameters (hardness, gumminess, chewiness, and resilience) were observed between sucuk samples containing different fiber levels (P<0.01). In contrast, fiber level had no significance (P>0.05) on adhesiveness, cohesiveness and springiness values of samples *(Table 1)*. An increase in the amount of fiber in sucuk formulation increased the gumminess and chewiness parameters of samples and the highest mean scores were obtained in samples containing 4% fiber. Although the addition of orange fiber increased the resilience values, no statistically significant difference was found between 2% and 4% fiber levels. The interaction of fiber level and ripening time had a significant (P<0.01) effect on resilience values *(Fig. 1-A)*. Similarly, the interaction of fiber level and ripening time had very significant effect on springiness values of samples (P<0.01) *(Fig. 1-B)*.

In samples containing different fat levels, significant differences were observed in adhesiveness, springiness, gumminess, chewiness, and resilience values (P<0.01). The another factor, ripening time, had very significant effects on all textural parameters of sucuk (P<0.01) *(Table 1)*. Although reduced fat content resulted in increased hardness and cohesiveness, the differences were not statistically significant (P>0.05). The interactions of fat level×fiber level (P<0.05) and fat level×ripening time (P<0.01) had significant effects on springiness values of samples *(Table 1)*.

DISCUSSION

Moisture content in all groups were lower than 40% at the end of ripening time which is compatible with Communique of Meat and Meat Products of Turkish Food Codex (No: 2012/74) [13]. There are no significant differences between sucuk samples with or without fiber. Garcia et al.[14] stated that moisture loss during ripening in low fat dry fermented sausages containing fruit fiber slightly lower than those containing cereal fiber.

Dietary plant fibers are well-known ingredients used

Table 1. *Overall effect of orange fiber, fat and ripening time on the textural parameters and moisture values of sucuk*[1]

Tablo 1. *Portakal lifi, yağ ve olgunlaştırma süresinin sucuğun tekstürel parametreleri ve nem değerlerine etkileri*[1]

Parameter	Textural Parameters							
	Hardness	Adhesiveness	Cohesiveness	Springiness	Gumminess	Chewiness	Resilience	Moisture
Orange Fiber (O)								
0%	211.43±116.39a	-1.98±1.74a	0.46±0.1a	0.65±0.09a	99.50±49.46c	68.03±33.79c	0.14±0.05b	51.50±9.39a
2%	262.46±124.26b	-1.76±1.34a	0.45±0.08a	0.64±0.08a	123.42±57.78b	82.96±39.85b	0.15±0.05a	51.43±8.44a
4%	295.33±129.78c	-1.59±1.05a	0.45±0.06a	0.64±0.06a	134.65±58.07a	89.75±40.13a	0.15±0.04a	51.37±8.07a
Significance	**	NS	NS	NS	**	**	**	NS
Ripening Time (R)								
0	NA	NA	NA	NA	NA	NA	NA	61.81±2.68a
1	54.59±17.51e	-4.10±1.12c	0.34±0.03e	0.51±0.04d	19.22±7.49e	10.08±4.56e	0.06±0.01c	59.40±2.82b
3	221.38±43.95d	-1.62±0.48b	0.55±0.04a	0.71±0.03a	120.62±18.48d	85.10±11.36d	0.18±0.0a	54.70±3.38c
5	276.19±59.47c	-1.24±0.85ab	0.50±0.06b	0.69±0.03b	135.48±19.43c	93.81±12.6c	0.17±0.01a	48.05±3.27d
7	326.02±60.15b	-1.14±0.69ab	0.46±0.06c	0.66±0.02c	149.14±27.67b	99.61±18.04b	0.16±0.02b	45.05±4.83e
9	403.83±46.02a	-0.80±0.31a	0.42±0.03d	0.65±0.02c	171.47±22.56a	112.65±16.25a	0.16±0.01b	39.58±2.27f
Significance	**	**	**	**	**	**	**	**
Fat (F)								
10%	263.89±137.34a	-1.54±1.24a	0.47±0.09a	0.66±0.08a	126.17±61.07a	86.86±42.18a	0.158±0.05a	54.13±8.63a
15%	250.83±125.63a	-1.68±1.3a	0.45±0.08ab	0.65±0.08b	115.55±55.34b	78.21±38.5b	0.15±0.04b	51.57±8.52b
20%	254.49±121.61a	-2.12±1.61b	0.44±0.07b	0.63±0.06c	115.85±54.37b	75.68±35.54b	0.149±0.04b	48.60±7.85c
Significance	NS	**	NS	**	**	**	**	**
OxR	NS	NS	NS	**	NS	NS	**	NS
OxF	NS	NS	NS	*	NS	NS	NS	NS
RxF	NS	NS	NS	**	NS	NS	NS	NS

[1] *Presented values are means ±SD;* ***a-e*** *Any two means in the same column having the same letters in the same section are not significantly different (*P<0.05, **P<0.01);* ***NS:*** *not significant,* ***SD:*** *standard deviation,* ***NA:*** *not analyzed*

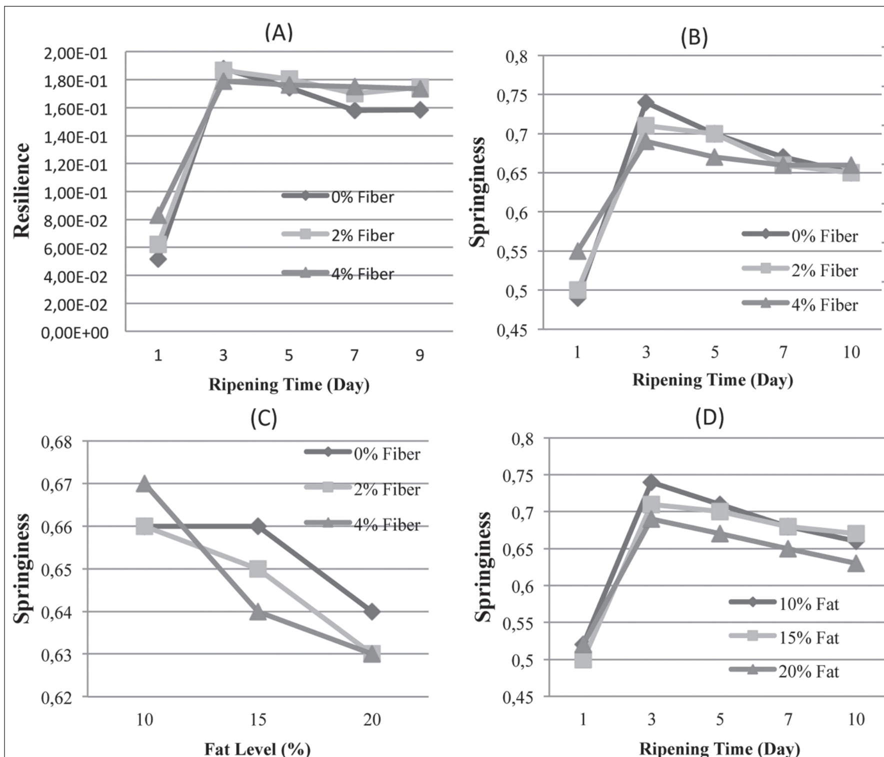

Fig 1. The effects of interactions between treatments on the textural parameters of sucuks

A- Fiber level × ripening time, **B-** Fiber level × ripening time, **C-** Fiber level ×f at level, **D-** Fat level × ripening time

Şekil 1. Sucuğun tekstürel parametrelerine muameleler arasındaki interaksiyonun etkisi

A- Lif seviyesi × olgunlaştırma süresi, **B-** Lif seviyesi × olgunlaştıma süresi, **C-** Lif seviyesi × yağ seviyesi, **D-** Yağ seviyesi x olgunlaştırma süresi

for improving technological properties of meat products with their health benefits in the last decade. Particularly, their properties such as water holding capacity and gel forming ability makes it important to clarify dietary fiber's impact on textural parameters [2]. The highest mean value for hardness was determined in samples containing 4% orange fiber. Similarly, Aleson-Carbonell et al.[1] reported that albedo type and content, significantly affected the textural characteristics of dry cured sausages and, in both types of albedo, the sausages with 5% added albedo showed the highest hardness value. In another study, it was reported that cereal fiber (3%) added sausages were harder, particularly in presence of wheat fiber. On the other hand, it was shown that addition of 1.5% orange fiber decreased hardness scores in both sausage containing 6% and 10% fat [14].

The level of fiber in sucuk formulation affected gumminess and chewiness values. The highest mean scores were determined in sucuk samples with 4% fiber. In a partly similar study, Aleson-Carbonell et al.[1] found that use of orange fiber in low fat (6-10%) dry fermented sausages decreased the gumminess values. In the same study, orange fiber slightly increased chewiness value in samples containing 6% fat, but a sharp decrease was observed in chewiness when the fat level reached to 10%. Springiness value was not affected by fiber level. However, the interaction of fiber level and ripening time was very significant on springiness. The highest springiness value was obtained in control group (fiber-free) on day 3 *(Fig. 1-B)*. In contrast, control group showed higher values than samples with 2% and 4% fiber in days 7 and 9 *(Fig. 1-A)*.

In the present study it was determined that fat level has an important effect on textural parameters (adhesiveness, springiness, gumminess, chewiness, and resilience). As indicated by Garcia et al.[14] decreasing fat to lower levels significantly affects the textural parameters. The effect of different fat levels on hardness value of sucuk samples was found close to those observed in chorizo de Pamplona by Gimeno et al.[15] and for cohesiveness in low-fat dry-fermented sausages [16]. Adhesiveness was directly related to fat content. This parameter decreased with a reduction in fat content and this difference was highest ($P<0.05$) between sucuk samples containing 10 and 20% fat. This also had been observed by Mendoza et al.[17]. The highest gumminess value was determined in samples containing 10% fat and no statistically significant difference was observed in samples containing 15 and 20% fat. The highest mean chewiness value was obtained in samples containing 10% fat. As amount of fat increased, score decreased in all samples. Salazar et al.[18] and Mendoza et al.[17] reported similar increases in gumminess and chewiness values when lower levels of fat were added to dry fermented sausage formulations. The increase in chewiness values could be explained by level of moisture loss during production [19]. The highest mean springiness value was observed in samples containing 10% fat. Increased levels of fat (15 and 20%) resulted in a decrease in springiness value *(Table 1)*. Similar results were found in breakfast sausages for springiness [20]. The highest springiness value was obtained in group 4% fiber and 10% fat *(Fig. 1-C)*. Springiness value increased until 3rd day and the highest value was observed in samples containing 10% fat. After 3rd day, the springiness value decreased until end of ripening time *(Fig. 1-D)*. As stated by Olivares et al.[21], fat reduction in dry fermented sausages causes significant changes in textural parameters.

Ripening time is one of the key steps in sucuk production with fermentation where significant moisture loss and changes in acidification, protein and fat level are observed. The major changes in hardness, chewiness and gumminess took place in the first three days. Hardness score which is the peak force of the first compression [20], increased from 54.59 to 221.38. This case may be explained by coagulation of protein at low pH and moisture loss, which took place during ripening [5]. Also, an increase in protein and decrease in moisture level during ripening can make product more denser, which results in higher hardness [20]. The same relationship between the ripening time and hardness was observed by Bozkurt and Bayram [5] in sucuk and by Lorenzo et al.[22] in dry-cured foal salchichon. For gumminess and chewiness scores of samples, a significant increase was observed during the progress of ripening. Similarly, Bozkurt and Bayram [5] reported that gumminess and chewiness increased during ripening of sucuk. Moreover, Lorenzo et al.[22] stated an increase in gumminess and chewiness scores during ripening of dry-cured foal salchichon. Cohesiveness and springiness values of samples showed a different pattern during ripening time. In the first three days, an increase was observed but after day 3, a regular decrease was detected in both parameters. However, final values of both parameters were higher than initial values *(Table 1)*. Increase in cohesiveness during ripening can be explained by pH decrease to isoelectrical point during ripening which favors gelification of proteins [23]. In contrast to our findings, Bozkurt and Bayram [5] detected a statistically insignificant decrease in cohesiveness and springiness values of sucuk samples during ripening. In another study, cohesiveness and springiness values was decreased during ripening significantly for cohesiveness and insignificantly for springiness [22]. During ripening, a strong decrease was observed in adhesiveness on day 1 and this decrease slightly proceeded for the following days similar to those obtained by Bozkurt and Bayram [5] in sucuk. The resilience values of samples changed by ripening. The lowest mean resilience value was observed in day 1 and the highest was in day 3. But just after that, a slight decrease was detected during the rest of the days of ripening *(Table 1)*. The values of springiness observed by Lorenzo et al.[22] in salchichon during ripening are very similar to ours. Decrease in adhesiveness score is good for cutting scores

of sucuk samples and makes it more sliceable. Moreover, an increase in springiness value which is related to elastic properties of sucuk shows a rise in elasticity probably due to moisture loss during ripening [5].

The main findings of the present study are proper for understanding the texture evolution of sucuk during ripening with different fat and fiber levels. Both fiber and fat levels significantly affected the many textural parameters which are important for consumer approval. Also, the importance of ripening time and moisture content on textural parameters were determined in detail. As can be understood from current study, its necessary to evaluate the textural parameters of sucuk when new ingredients are added to formulation for obtaining best results.

REFERENCES

1. Aleson-Carbonell L, Fernandez-Lopez J, Sayas-Barbera E, Sendra E, Perez-Alvarez JA: Utilization of lemon albedo in dry-cured sausages. *J Food Sci,* 68, 1826-1830, 2003. DOI: 10.1111/j.1365-2621.2003.tb12337.x

2. Kim HJ, Paik HD: Functionality and application of dietary fiber in meat products. *Korean J Food Sci An*, 32, 695-705, 2012. DOI: 10.5851/kosfa.2012.32.6.695

3. Campagnol PCB, Santos BAD, Wagner R, Terra NN, Pollonio MAR: The effect of soy fiber addition on the quality of fermented sausages at low-fat content. *J Food Qual,* 36, 41-50, 2013. DOI: 10.1111/jfq.12013

4. Vasilev D, Saicic S, Vasilijevic N: Quality and nutritive value of fermented sausages produced with inulin and pea fibre is fat reaplacers. *Fleischwirtschaft,* 93 (3): 123-127, 2013.

5. Bozkurt H, Bayram M: Colour and textural attributes of sucuk during ripening. *Meat Sci,* 73, 344-350, 2006. DOI: 10.1016/j.meatsci.2006.01.001

6. Özdemir H, Soyer A, Kurt E: Meyve lifi ilavesinin sucuğun kalite özelliklerine etkisi. *Dünya Gıda,* 5, 79-84, 2009.

7. Çoksever E, Sarıçoban C: Effects of bitter orange albedo addition on the quality characteristics of naturally fermented Turkish style sausages (sucuks). *J Food Agr Environ*, 8 (1): 34-37, 2010.

8. Yalınkılıç B, Kaban G, Ertekin Ö, Kaya M: Determination of volatile compounds of sucuk in different orange fiber and fat level. *Kafkas Univ Vet Fak Derg,* 21, 233-239, 2015. DOI: 10.9775/kvfd.2014.12197

9. Yalınkılıç B, Kaban G, Kaya M: The effects of different levels of orange fiber and fat on microbiological, physical, chemical and sensorial properties of sucuk. *Food Microbiol*, 29, 255-259, 2012. DOI: 10.1016/j.fm.2011.07.013

10. Fernandez-Gines JM, Fernandez-Lopez J, Sayas-Barbera E, Sendra E, Perez-Alvarez JA: Effect of storage conditions on quality characteristics of Bologna sausages made with citrus fiber. *J Food Sci,* 68, 710-715, 2003. DOI: 10.1111/j.1365-2621.2003.tb05737.x

11. Kaban G, Kaya M: Identification of lactic acid bacteria and Gram-positive catalase-positive cocci isolated from naturally fermented sausage (Sucuk). *J Food Sci,* 73, M385-M388, 2008. DOI: 10.1111/j.1750-3841.2008.00906.x

12. Gökalp HY, Kaya M, Tülek Y, Zorba Ö: Determination of moisture level in meat products. **In,** Practical Laboratory Guide and Quality Control in Meat and Meat Products. 4th ed., 72, Atatürk Üniv. Publ. No: 786, Faculty of Agric. No: 69, Erzurum, Turkey, 2001.

13. FAOLEX-legislative Database of FAO Legal Office: Notification of Meat and Meat Products of Turkish Food Codex (No: 2012/74). *Resmi Gazete*, Number: 28.888, 2012.

14. Garcia ML, Dominguez R, Galvez MD, Casas C, Selgas MD: Utilization of cereal fiber and fruit fibres in low fat dry fermented sausages. *Meat Sci,* 60, 227-236, 2002. DOI: 10.1016/S0309-1740(01)00125-5

15. Gimeno O, Ansorena D, Astiasaran I, Bello J: Characterization of chorizo de Pamplona: Instrumental measurements of colour and texture. *Food Chem,* 69, 195-200, 2000. DOI: 10.1016/S0308-8146(99)00239-3

16. Liaros NG, Katsanidis E, Bloukas JG: Effect of ripening time under vacuum and packaging film permeability on processing and quality of low-fat fermented sausages. *Meat Sci,* 83, 589-598, 2009. DOI: 10.1016/j.meatsci.2009.07.006

17. Mendoza E, Garcia ML, Casas C, Selgas MD: Inulin as fat substitute in low fat, dry fermented sausages. *Meat Sci,* 57, 387-393, 2001. DOI: 10.1016/S0309-1740(00)00116-9

18. Salazar P, Garcia ML, Selgas MD: Short-chain fructooligosaccharides as potential functional ingredient in dry fermented sausages with different fat levels. *Int J Food Sci Tech,* 44, 1100-1107, 2009. DOI: 10.1111/j.1365-2621.2009.01923.x

19. Ruiz-Capillas C, Triki M, Herrero AM, Rodriguez-Salas L, Jimenez-Colmenero F: Konjac jel as pork backfat replacer in dry fermented sausages: Processing and quality characteristics. *Meat Sci,* 92, 144-150, 2012. DOI: 10.1016/j.meatsci.2012.04.028

20. Tobin BD, O' sullivan MG, Hamill RM, Kerry JP: The impact of salt and fat level variation on the physiochemical properties and sensory quality of pork breakfast sausages. *Meat Sci,* 93, 145-152, 2013. DOI: 10.1016/j.meatsci.2012.08.008

21. Olivares A, Navarro JL, Salvador A, Flores M: Sensory acceptability of slow fermented sausages based on fat content and ripening time. *Meat Sci,* 86, 251-257, 2010. DOI: 10.1016/j.meatsci.2010.04.005

22. Lorenzo JM, Temperan S, Bermudez R, Cobas N, Purrinos L: Changes in physic-chemical, microbiological, textural and sensory attributes during ripening of dry-cured foal salchichon. *Meat Sci,* 90, 194-198, 2012. DOI: 10.1016/j.meatsci.2011.06.025

23. Gonzalez-Fernandez C, Santos EM, Rovira J, Jaime I: The effect of sugar concentration and starter culture on instrumental and sensory textural properties of chorizo-Spanish dry-cured sausage. *Meat Sci,* 74, 467-475, 2006. DOI: 10.1016/j.meatsci.2006.04.019

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Research Article

The Effect of Borax on Some Energy Metabolites in Dairy Cows during the Transition Period

Metin ÖĞÜN [1] Oğuz MERHAN [1] Abdulsamed KÜKÜRT [1]
Mushap KURU [2] Mahmut KARAPEHLİVAN [3]

[1] Department of Biochemistry, Faculty of Veterinary Medicine, University of Kafkas, TR-36100 Kars - TURKEY
[2] Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, University of Kafkas, TR-36100 Kars - TURKEY
[3] Department of Medical Biochemistry, Faculty of Medicine, University of Kafkas, TR-36100 Kars - TURKEY

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Abstract

The purpose of this study is to investigate the effects of sodium borate ($Na_2B_4O_7 \cdot 5H_2O$) addition to dairy cow rations starting from prepartum period on serum cortisol, glucose, β-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), triglyceride (TG), blood urea nitrogen (BUN), aspartate aminotransferase (AST), alanine aminotransferase (ALT) levels. Clinically healthy eighty pregnant cows were randomly divided into two groups. Sodium borate (30 g/day) was added to the rations of the borax group (n=40) until day 21 postpartum. Blood samples were taken from all cows (n=40) on days 21, 14 and 7 before parturition, at parturition and on days 7, 14 and 21 after parturition. Serum cortisol levels in the borax group were lower (P<0.05) than those in the control group, there was a decrease (P<0.05) in serum BHB, NEFA and TG levels before, at parturition and after parturition, serum BUN concentrations increased (P<0.05) in prepartum and postpartum samples in the borax group, except for prepartum days 21 and 14, AST concentrations were higher (P>0.05), on all other sampling days, and ALT levels were not affected (P>0.05). It was concluded that adding sodium borate to rations especially in the transition period in highly productive dairy breeds might be an alternative to protect against negative energy imbalances.

Keywords: *Borax, Cortisol, Glucose, NEFA BHB, Dairy Cows*

Geçiş Dönemi Süt İneklerinde Bazı Enerji Metabolitlerinin Üzerine Boraksın Etkisi

Özet

Bu çalışmanın amacı, prepartum dönemden başlanarak sütçü sığırların rasyonlarına sodyum borat ($Na_2B_4O_7 \cdot 5H_2O$) ilavesinin serum kortizol, glukoz, β-hidroksibütirat (BHB), esterleşmemiş yağ asitleri (NEFA), trigliserid (TG), kan üre nitrojen (BUN), aspartat aminotransferaz (AST), alanin aminotransferaz (ALT) düzeylerine etkisinin araştırılmasıdır. Klinik olarak sağlıklı 80 adet gebe inek rastgele iki gruba ayrıldı. Borax grubuna (n=40) doğumdan 30 gün önce başlanarak postpartum 21. güne kadar rasyonlarına ek olarak sodyum borat (30 g/gün) eklendi. Hem çalışma hem de kontrol grubundan (n=40) doğum öncesi 21, 14 ve 7. gün, doğumda ve doğum sonrası 7, 14 ve 21. günlerde kan örnekleri alındı. Analizler sonrasında borax grubu serum kortizol düzeyinin kontrol grubuna göre daha düşme (P<0.05), doğum öncesi, doğum ve doğum sonrasında serum BHB, NEFA ve TG düzeyinde azalma (P<0.05), serum BUN konsantrasyonunda doğum öncesi ve doğum sonrası artış (P<0.05), AST konsantrasyonunun doğum öncesi 21 ve 14. günleri hariç diğer günlerde daha yüksek (P>0.05) olduğu ve ALT düzeyinin etkilenmediği (P>0.05) belirlendi. Sonuç olarak, yüksek süt verimli ırklarda özellikle geçiş döneminde rasyona sodyum borat ilavesinin negatif enerji dengesizliklerinden korunmada bir seçenek olabileceği belirlendi.

Anahtar sözcükler: *Boraks, Kortizol, Glukoz, NEFA, BHB, Sütçü İnek*

INTRODUCTION

The periodic table of elements represents Boron as B. It has both metal and nonmetal characteristics [1-3]. This dynamic trace element enters the body in small amounts via food and drink [4,5]. Boron can affect at least 26 different enzyme activities required for energy substrate metabolism [6,7]. After inorganic borates are absorbed through mucosal membranes, they are transformed into boric acid [8]. Boric acid is an essential element with biological significance in animal and human nutrition, metabolic, hormonal and physiological events [9]. Its effect

İletişim (Correspondence)
+90 474 2426807/5143
metinogun@hotmail.com

on mineral metabolism, the endocrine system and immune response is also recognized [3,10,11]. Even though studies have been conducted, the effects of boric acid in metabolic events in animals have not been explained in detail [3].

Basoglu et al.[11] have determined that supplementing rations with sodium borate can be effective in preventing fatty liver in cow. Another study found that sodium borate added to the rations of dairy cow during the transition period did not affect blood urea nitrogen (BUN) and alanine aminotransferase (ALT) levels, but that aspartate aminotransferase (AST) and β-hydroxybutyrate (BHB), triglyceride (TG) and non-esterified fatty acids (NEFA) levels decreased and glucose levels increased [12]. The transition period is a period of approximately 6 weeks encompassing the three weeks before parturition and three weeks after parturition, during which important endocrine and metabolic changes take place in dairy cows. During this time there is a greater need for nutrients for the fetus, development of the mammary glands and synthesis of milk. In this situation, the body is unable to meet the demand for glucose required for energy metabolism, and so it satisfies the need for energy by metabolizing NEFA from adipose tissues. The energy deficit results in insulin sensitivity and loss of appetite by causing an increase in circulating NEFA. All of these metabolic and hormonal changes give rise to metabolic syndromes such as fatty liver in dairy cows that produce large quantities of milk [13-19]. Compensating mechanisms play an important role in minimizing the changes that occur in metabolic activities during the transition period in cows. These changes in particularly energy metabolism cause a certain amount of stress in the body. All types of environmental and care conditions that could create stress on the animal need to be removed [16,20-23]. Therefore, substances that provide energy in addition to rations or that reduce the mobilization of triglycerides can be provided for nutrition during this time [12,19,24].

The purpose of this study was to investigate the effects of sodium borate on cortisol levels that can occur due to the negative energy balance and the stress of parturition, and its effects on the consequently varying energy metabolites such as serum glucose, BHB, NEFA, TG, BUN, AST and ALT when added to rations 30 days prepartum and 21 days postpartum in Red Holstein cows.

MATERIAL and METHODS

This study was conducted after obtaining approval from the Kafkas University Animal Experiments Local Ethics Committee (KAÜ HADYEK - Study code: 2015/111, Meeting number: 2015/13, Edition no: 2015/133).

The study was conducted at the Niğtaş Farm in the province of Niğde, which practices intensive farming. The study material consisted of 80 pregnant Red Holstein cows ranging from 3-5 years of age. The cows' body condition scores were between 3.00-3.50 before parturition and 2.50-3.00 after parturition on a 5-point scale with increments of 0.25 [25,26]. Postpartum milk production varied between 24-28 liters per day. The study included cows which had at least one normal parturition. Cows that had difficult parturitions were not included in the study.

Eighty cows that were clinically healthy and which had been given anti-parasite medications and vaccinations prior to pregnancy were randomly divided into two groups. Beginning 30 days before parturition, sodium borate ($Na_2B_4O_7{\cdot}5H_2O$, 30 g/day, Merck) was added to the rations of the experimental group (Borax group, n=40) until day 21 postpartum. It has been shown that ration contain were given to animals at the *Table 1*. Blood samples were taken from both the experimental and the control group (n=40) on days 21, 14 and 7 prepartum, at parturition and on days 7, 14 and 21 postpartum. Blood was taken from the *vena coccygea* and placed in 10-ml vacuum vials (BD Vakutainer®, Tıpkimsan, Turkey) using sterile holder needles. The blood was brought to a laboratory on the farm within one hour of being drawn and centrifuged for 10 min at 3.000 rpm (Hettich Universal 320®, Hettich, Germany). The serums were stored at -20°C until biochemical tests were performed.

The serum samples from the study were analyzed for cortisol, glucose, BHB, NEFA, TG, BUN, ALT and AST. Cortisol measurements were performed using the Radio-immunoassay (RIA, Beckman Coulter®, USA) method with a commercial kit (Access® Cortisol, Unicel DxI 600, Beckman Coulter, USA). Spectrophotometric measurements (Epoch®, Biotek, USA) were performed using commercial kits for glucose, TG, BUN, ALT and AST levels (DDS, Turkey), BHB (Ranbut®, Randox, UK) and NEFA (Wako Diognostics, VA).

Statistical analyses of the serum cortisol, glucose, BHB,

Table 1. *Ingredient and nutrient composition of prepartum and postpartum diets*

Tablo 1. *Prepartum ve postpartum diyetlerin bileşen ve besin kompozisyonu*

Ingredients (%DM)	Prepartum	Postpartum
Corn silage	11.17	19.64
Hay	28.49	2.78
Alfalfa hay	23.46	17.67
Barley	0.00	5.89
Dairy cattle feed	0.00	25.92
Heifer feed	16.39	0.00
Barley pulp	20.11	23.79
Soypass	0.00	4.13
Yeast	0.372	0.181
NEL (cal/g)	1.28	1.52

DM: *Dry matter;* ***NEL:*** *Net energy lactation*

NEFA, TG, BUN, ALT and AST levels were performed using the SPSS® (SPSS 20, IL, USA). The change in biochemical parameter levels in the groups by days was analyzed with the Anova, Tukey HSD test. Statistical comparison of the groups by days was performed using the Student-t test. The results are provided as mean ± SD (SD: Standard deviation). Values of P<0.05 were considered statistically significant.

RESULTS

Prepartum and postpartum serum cortisol levels did not change (P>0.05). During parturition, however, serum cortisol concentrations increased in both the control and the borax group (P<0.001). When the groups were compared, serum cortisol levels in the Borax group were significantly lower (P<0.05) than those of the control group.

Serum glucose concentrations were lower (P<0.001) during parturition than in both prepartum and postpartum samples in both groups However, the administration of sodium borate affected the serum glucose levels. The glucose concentration in the borax group was significantly higher than that in the control group on the sampling days (P<0.05).

It was determined that serum BHB levels were affected after parturition and that there was a trend higher (P<0.001) in both groups after parturition. In addition, supplementing rations with sodium borate resulted in a statistically significant decline (P<0.05) in serum BHB levels prepartum, at parturition and postpartum compared to the control group.

The NEFA concentration increased in both groups in the time leading up to parturition and fell postpartum (P<0.001). NEFA levels on all sampling days in the borax group were significantly lower than in the control group (P<0.05).

Serum TG levels were higher until close to the time of parturition in the control group and later the serum concentrations fell again (P<0.001). However, serum TG levels in the borax group were similar on sampling days (P>0.05). Furthermore, TG levels during parturition and on postpartum days 7 and 14 were significantly lower than those of the control group (P<0.05).

Serum BUN concentrations increased in the borax group compared to the control group (P<0.05). However, administration of sodium borate in the first week prepartum and during parturition did not affect serum BUN levels (P>0.05). Furthermore, there was a tendency for the BUN levels to rise in both groups as it came closer to time to give parturition (P<0.001).

Measurements showed that serum ALT levels were similar (P>0.05) in both groups, but that concentrations were significantly lower (P<0.05) in serum samples from the borax group taken on postpartum day 21.

AST concentrations in serum samples on days -21 and -14 prepartum were similar between the two groups (P>0.05). On the other days, however, the AST concentration in the borax group was significantly higher. In addition, AST levels were highest in both groups in the first post-partum week (P<0.001).

Changes in levels of serum cortisol, glucose, BHB, NEFA, TG, BUN, ALT and AST in blood samples taken at days -21, -14, -7, parturition, 7, 14 and 21 after administration of sodium borate for both the control group and the borax group are summarized in *Table 2*.

DISCUSSION

The changes that occur in connection with energy metabolism in the transition period in cows cause a certain amount of stress on the body [16,17,27-29]. The increasing cortisol level in particular plays a role in the beginning of labor during parturition. Some diseases that may occur during this period are also thought to be an indicator of stress [30-32]. Serum cortisol levels in cows during a normal parturitions are higher than in the prepartum period [29]. Similarly, we found that serum cortisol levels are higher during parturition than they are in the prepartum or postpartum period (P<0.001). However, serum cortisol was lower in the group whose rations where supplemented with sodium borate (P<0.05). This suggests that adding sodium borate to rations could be a way to reduce stress during parturition. It is thought that this might be due to the positive effect of sodium borate on energy metabolism.

Vannucchi et al.[29] found an inverse relationship between the level of serum cortisol and serum glucose concentrations during parturition. In our study, serum glucose concentrations showed a tendency to decline as parturition approached, which was inversely proportional to serum cortisol. However, it was found that administration of sodium borate caused an increase in serum glucose levels compared to the control group (P>0.05). Studies have reported that adding borax to rations causes an increase in serum glucose levels during parturition compared to prepartum and postpartum periods [12,23]. In our study, on the other hand, glucose was significantly higher in the control group, but tended to decline at parturition compared to the prepartum and postpartum periods. The results in some studies report variations depending on the rate of borax administered to the cows, the source from which the borax was obtained and borax absorption in the body.

It has been reported that BHB levels, which are characterized as a response to the negative energy balance during peripartum, generally rise during parturition [12,33].

Table 2. *Changes in levels of serum cortisol, glucose, BHB, NEFA, TG, BUN, ALT and AST at days -21, -14, -7, Parturition, 7, 14 and 21 for the control group and the borax group*

Tablo 2. *Sodyum borat eklenen rasyonla beslenen geçiş dönemi süt ineklerinde serum kortizol, glukoz, BHB, NEFA, TG, BUN, ALT ve AST düzeylerinin değişimi*

Parameters	Groups	Days							P value
		-21	-14	-7	Parturition	+7	+14	+21	
Cortisol (nmol/L)	C	9.8±0.75^{b}	12.3±0.21^{b}	18.8±0.54^{c}	42.8±11.6^{d}	4.18±0.34^{a}	2.68±0.06^{a}	2.45±0.14^{a}	**
	B	8.65±0.6^{b}	11.9±0.34^{b}	17.6±0.07^{c}	35.9±7.5^{d}	3.96±0.23^{a}	2.56±0.37^{a}	2.44±0.06^{a}	**
	P value	NS	NS	NS	*	NS	NS	NS	
Glucose (mg/dL)	C	58.2±1.2^{c}	56.4±0.9^{abc}	53.7±1.3^{abc}	51.2±0.9^{a}	52.7±0.6^{ab}	56.4±0.8^{abc}	56.9±1.1^{bc}	**
	B	62.7±1.2^{d}	59.5±1.1^{bcd}	56.7±0.7^{ab}	54.8±1.1^{a}	57.9±1.3^{abc}	61.6±0.9^{cd}	63.8±1.1^{d}	**
	P value	*	*	*	*	*	*	*	
BHB (mmol/L)	C	0.52±0.21^{a}	0.58±0.18^{b}	0.67±0.25^{c}	0.84±0.32^{e}	0.75±0.27^{d}	0.72±0.12^{d}	0.66±0.18^{c}	**
	B	0.47±0.24^{a}	0.53±0.31^{b}	0.61±0.15^{c}	0.76±0.09^{e}	0.69±0.14^{d}	0.68±0.12^{d}	0.60±0.08^{c}	**
	P value	*	*	*	*	*	*	*	
NEFA (mmol/L)	C	0.27±0.05^{a}	0.33±0.04^{a}	0.45±0.12^{b}	0.82±0.23^{e}	0.61±0.15^{d}	0.58±0.28^{cd}	0.52±0.14^{c}	**
	B	0.21±0.18^{a}	0.28±0.09^{b}	0.36±0.28c	0.76±0.18^{f}	0.55±0.19^{e}	0.54±0.16^{e}	0.46±0.31^{d}	**
	P value	*	*	*	*	*	*	*	
TG (mg/dL)	C	19.4±2.1^{a}	21.8±3.4^{ab}	22.2±1.9^{ab}	26.8±1.5^{c}	25.8±1.7^{bc}	23.9±2.8^{abc}	19.8±2.7^{a}	**
	B	18.2±2.3	19.2±1.8	20.2±2.3	21.6±1.9	20.11±1.6	19.7±1.8	17.6±0.8	NS
	P value	NS	NS	NS	*	*	*	NS	
BUN (mg/L)	C	98.7±14.7^{a}	105.6±21.3^{bc}	118.3±15.6^{d}	153.9±21.6^{e}	121.6±12.4^{d}	109.8±19.7^{c}	102.8±10.5^{ab}	**
	B	109.6±21.2^{a}	118.7±13.6^{c}	125.8±12.2^{d}	160.4±21.5^{f}	136.8±17.5^{e}	123.9±20.7^{d}	114.6±18.9^{b}	**
	P value	*	*	NS	NS	*	*	*	
ALT (U/L)	C	28.6±3.2^{c}	24.7±4.26^{abc}	22.8±8.7^{ab}	20.9±3.9^{a}	22.5±5.4^{a}	23.7±6.2^{ab}	26.8±3.6^{bc}	**
	B	27.6±4.6^{b}	23.9±5.2^{ab}	21.8±3.7^{a}	19.8±6.4^{a}	21.6±2.8^{a}	22.7±2.7^{a}	23.8±4.7^{ab}	**
	P value	NS	NS	NS	NS	NS	NS	*	
AST (U/L)	C	71.3±5.6^{a}	75.9±11.6^{b}	78.4±12.4^{bc}	80.6±5.6^{c}	98.4±8.7^{e}	89.6±6.8^{d}	80.7±3.6^{c}	**
	B	69.5±7.4^{a}	78.6±9.4^{b}	84.6±11.8^{c}	93.8±15.6^{d}	102.6±13.8^{e}	95.3±8.6^{d}	86.7±7.2^{c}	**
	P value	NS	NS	*	*	*	*	*	

^{a,b,c,d,e,f} *The difference between values with different letters on the same line is significant at the P value, * P<0.05, ** P<0.001,* ***NS:*** *Not significant,* ***C:*** *Control group,* ***B:*** *Borax group,* ***BHB:*** *β-Hydroxybutyrate,* ***NEFA:*** *Non-Esterified Fatty Acids,* ***TG:*** *Triglyceride,* ***BUN:*** *Blood Urea Nitrogen,* ***ALT:*** *Alanine Aminotransferase,* ***AST:*** *Aspartate Aminotransferase*

In this study, BHB concentrations changed in both groups during the peripartum period. It was particularly remarkable that BHB levels increased in both group during parturition. However, administration of sodium borate caused a decrease in serum BHB levels compared to the control group. Kabu and Civelek [12] argued in their study that administration of borax did not affect BHB levels in prepartum and post-partum weeks. In the same study, they reported that serum NEFA levels rise during parturition and that the administration of borax did not affect NEFA concentration during parturition. In the present study, however, we found a statistically significant rise in NEFA levels during parturition in both the control group and the borax group. Furthermore, the administration of borax reduced the formation of NEFA compared to the control group. The rise in epinephrine and norepinephrine levels during parturition in cows contributes to the rise in plasma NEFA and TG concentrations. In particular, it reportedly increases the rate of adipose tissue lipolysis through adrenergic stimulation in the transition from the dry period to lactation in primiparous cows [13,34]. In our study, TG levels in the control group increased (P<0.001) in the period leading up to parturition, which is consistent with the literature. However, there was no statistically significant increase (P>0.05) in TG levels in the borax group in the period leading up to parturition In fact, TG levels in the control group were significantly higher (P<0.05) than those in the borax group. These data suggest that borax could be used to protect dairy cows from the formation of TG in the periparturient period. It is thought that borax might lower serum BHB, NEFA and TG concentrations because it raised serum glucagon levels [12], and our study did find this occurred in connection with its effect of raising glucose levels, which might mean that it would mitigate liver damage.

It has been reported that BUN concentrations in cow can vary between 78-250 mg/L and reach peak values

during calving [12,33]. One study demonstrated that BUN values reach their peak on the 21st day postpartum [35]. This study found that serum BUN levels varied between 98 to 160 mg/L and the peak BUN level was measured during calving. Serum BUN levels were significantly higher during parturition in the group given sodium borate. In the study conducted by Kabu and Civelek [12], they found that serum BUN levels were highest on day 7 postpartum in the group given borax. It is thought that the reason for the increase in BUN levels, especially postpartum, may be due to the fact that sodium borate reduces lipid infiltration and increases protein anabolism.

In studies conducted during the periparturient period, researchers have reported that ALT and AST level may rise during calving [12] or remain the same [33]. There are reports of a significant correlation between AST activity and the concentration of glucose, NEFA and BHB [35]. Using borax supplements in rations not affect to the ALT and AST levels. AST levels are reportedly affected by parturition in both the borax and control group and rise in the postpartum period [12]. In our study, AST levels increased in both groups during parturition and the postpartum period. It is thought that these increases may be due to cellular damage that can occur in the liver due to lipid mobilization that happens in connection with the negative energy balance that occurs the closer the cows get to having parturition

In conclusion, the addition of sodium borate to rations starting in the peripartum period caused maternal cortisol levels to fall during parturition compared to the control group, raised serum glucose and BUN levels, reduced BHB, NEFA and TG concentrations, raised AST levels during and after parturition and did not affect ALT levels. It was concluded that adding sodium borate to rations especially in the transition period might be an alternative to protect against negative energy imbalances, especially in highly productive dairy breeds.

REFERENCES

1. Murray FJ: A human health risk assessment of boron (boric acid and borax) in drinking water. *Regul Toxicol Pharmacol*, 22, 221-230, 1995. DOI: 10.1006/rtph.1995.0004

2. Zhai HJ, Kiran B, Li J, Wang LS: Hydrocarbon analogues of boron clusters-planarity, aromaticity and antiaromaticity. *Nat Mater*, 2, 827-833, 2003. DOI: 10.1038/nmat1012

3. Kabu M, Akosman MS: Biological effects of boron. *Rev Environ Contam Toxicol*, 225, 57-75, 2013. DOI: 10.1007/978-1-4614-6470-9_2

4. Sabuncuoglu BT, Kocaturk PA, Yaman Ö, Kavas GO, Tekelioglu M: Effects of subacute boric acid administration on rat kidney tissue. *Clin Toxicol (Phila)*, 44, 249-253, 2006. DOI: 10.1080/15563650600584386

5. Xu RJ, Xing XR, Zhou QF, Jiang GB, Wei FS: Investigations on boron levels in drinking water sources in China. *Environ Monit Assess*, 165, 15-25, 2010. DOI: 10.1007/s10661-009-0923-8

6. Hunt CD: Regulation of enzymatic activity. One possible role of dietary boron in higher animals and humans. *Biol Trace Elem Res*, 66, 205-225, 1998. DOI: 10.1007/BF02783139

7. Bakken NA, Hunt CD: Dietary boron decreases peak pancreatic in situ insulin release in chicks and plasma insulin concentrations in rats regardless of vitamin D or magnesium status. *J Nutr*, 133 (11): 3577-3583, 2003.

8. Bolaños L, Lukaszewski K, Bonilla I, Blevins D: Why boron? *Plant Physiol Biochem*, 42, 907-912, 2004. DOI: 10.1016/j.plaphy.2004.11.002

9. Blevins DG, Lukaszewski KM: Boron in plant structure and function. *Annu Rev Plant Physiol Plant Mol Biol*, 49, 481-500, 1998. DOI: 10.1146/annurev.arplant.49.1.481

10. Nielsen FH: Boron in human and animal nutrition. *Plant Soil*, 193, 199-208, 1997. DOI: 10.1023/A:1004276311956

11. Basoglu A, Sevinc M, Birdane FM, Boydak M: Efficacy of sodium borate in the prevention of fatty liver in dairy cows. *J Vet Intern Med*, 16, 732-735, 2002. DOI: 10.1111/j.1939-1676.2002.tb02416.x

12. Kabu M, Civelek T: Effects of propylene glycol, methionine and sodium borate on metabolic profile in dairy cattle during periparturient period. *Rev Méd Vét*, 163, 419-430, 2012.

13. Grummer RR: Etiology of lipid-related metabolic disorders in periparturient dairy cows. *J Dairy Sci*, 76, 3882-3896, 1993. DOI: 10.3168/jds.S0022-0302(93)77729-2

14. Vazquez-Añon M, Bertics S, Luck M, Grummer RR, Pinheiro J: Peripartum liver triglyceride and plasma metabolites in dairy cows. *J Dairy Sci*, 77, 1521-1528, 1994. DOI: 10.3168/jds.S0022-0302(94)77092-2

15. Grummer RR: Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. *J Anim Sci*, 73, 2820-2833, 1995.

16. Drackley JK: Biology of dairy cows during the transition period: the final frontier? *J Dairy Sci*, 82, 2259-2273, 1999. DOI: 10.3168/jds.S0022-0302(99)75474-3

17. Drackley JK, Overton TR, Douglas GN: Adaptations of glucose and long-chain fatty acid metabolism in liver of dairy cows during the periparturient period. *J Dairy Sci*, 84, E100-112E, 2001. DOI: 10.3168/jds.S0022-0302(01)70204-4

18. Overton TR: Transition cow programs. The good, the bad, and how to keep them from getting ugly. *Adv Dairy Tech*, 13, 17-26, 2001.

19. Kabu M: Bor, propilen glikol ve methioninin süt sığırlarında metabolik profil üzerine etkisi. *Kacatepe Vet J*, 5, 37-44, 2012.

20. Knight CH, Beever DE, Sorensen A: Metabolic loads to be expected from different genotypes under different systems. **In,** Oldham JD, Simm G, Groen AF, Nielsen BL, Pryce JE, Lawrence TJL (Eds): Metabolic Stress in Dairy Cows. *Br Soc Anim Sci*, Occasional Publication, 24, 27-35, 1999.

21. Kessel S, Stroehl M, Meyer HHD, Hiss S, Sauerwein H, Schwarz FJ, Bruckmaier RM: Individual variability in physiological adaptation to metabolic stress during early lactation in dairy cows kept under equal conditions. *J Anim Sci*, 86, 2903-2912, 2008. DOI: 10.2527/jas.2008-1016

22. Arslan C, Tufan T: Geçiş dönemindeki süt ineklerinin beslenmesi I. Bu dönemde görülen fizyolojik, hormonal, metabolik ve immunolojik değişiklikler ile beslenme ihtiyaçları. *Kafkas Univ Vet Fak Derg*, 16, 151-158, 2010. DOI: 10.9775/kvfd.2009.442

23. Sundrum A: Metabolic disorders in the transition period indicate that the dairy cows' ability to adapt is overstressed. *Animals*, 5, 978-1020, 2015. DOI: 10.3390/ani5040395

24. Stockdale CR, Roche JR: A review of the energy and protein nutrition of dairy cows through their dry period and its impact on early lactation performance. *Aust J of Agric Res,* 53, 737-753, 2002. DOI: 10.1071/AR01019

25. Edmonson AJ, Lean IJ, Weaver LD, Farver T, Webster G: A body condition scoring chart for holstein dairy cows. *J Dairy Sci*, 72, 68-78, 1989. DOI: 10.3168/jds.S0022-0302(89)79081-0

26. Kuru M, Merhan O, Kaya S, Oral H, Kukurt A: The effect of short term progesterone-releasing intravaginal device treatment on acute inflamation markers for Holstein heifers. *Rev Méd Vét,* 166, 336-340, 2015.

27. Serbester U, Çinar M, Hayırlı A: Sütçü ineklerde negatif enerji dengesi ve metabolik indikatörleri. *Kafkas Univ Vet Fak Derg,* 18, 705-711, 2012. DOI: 10.9775/kvfd.2012.6559

28. Kaçar C, Pancarci ŞM, Karapehlivan M, Kaya S, Kuru M, Çitil M, Gürbulak K: Peripartum dönemdeki ineklerde subkutan L-karnitin uygulamalarının enerji metabolizmasının bazı biyokimyasal parametrelerine etkisi. *Harran Üniv Vet Fak Derg*, 2, 67-74, 2013

29. Vannucchi CI, Rodrigues JA, Silva LC, Lúcio CF, Veiga GA, Furtado PV, Oliveira CA, Nichi M: Association between parturition conditions and glucose and cortisol profiles of periparturient dairy cows and neonatal calves. *Vet Rec*, 4, 176, 358, 2015. DOI: 10.1136/vr.102862

30. Leon JB, Smith BB, Timm KI, LeCren G: Endocrine changes during pregnancy, parturition and the early post-partum period in the llama *(Lama glama)*. *J Reprod Fertil*, 88, 503-511, 1990. DOI: 10.1530/jrf.0.0880503

31. Forslund KB, Ljungvall OA, Jones BV: Low cortisol levels in blood from dairy cows with ketosis: A field study. *Acta Vet Scand*, 20, 52, 31, 2010. DOI: 10.1186/1751-0147-52-31

32. Esposito G, Irons PC, Webb EC, Chapwanya A: Interactions between negative energy balance, metabolic diseases, uterine health and immune response in transition dairy cows. *Anim Reprod Sci*, 144, 60-71, 2014. DOI: 10.1016/j.anireprosci.2013.11.007

33. Civelek T, Birdane F, Kabu M, Cıngı CÇ, Acar A: Effects of methionine and lysine on metabolic profile in dairy cattle during periparturient period. *Kafkas Univ Vet Fak Derg* 19, 423-432, 2013. DOI: 10.9775/kvfd.2012.7968

34. McNamara JP, Hillers JK: Regulation of bovine adipose tissue metabolism during lactation. 2. Lipolysis response to milk production and energy intake. *J Dairy Sci*, 69, 3042-3050, 1986. DOI: 10.3168/jds.S0022-0302(86)80767-6

35. Seifi HA, Gorji-Dooz M, Mohri M, Dalir-Naghadeh B, Farzaneh N: Variations of energy-related biochemical metabolites during transition period in dairy cows. *Comp Clin Pathol*, 16, 253-258, 2007. DOI: 10.1007/s00580-007-0682-2

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Research Article

Characterization and Distribution of *Salmonella* spp. Isolated from Poultry Slaughterhouse-processingin Shandong Province [1]

Wenyan GAI [1] Junwei WANG [1] Juan WANG [1] Zhigang CUI [2]
Zhina QU [1] Jun HONG [1] Jinghua CUI [2] Xiaoli DU [2]
Xiumei HUANG [1] Xumin CAO [1] Jianmei ZHAO 1

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[1] China Animal Health and Epidemiology Center, Laboratory of Quality & Safety Risk Assessment for Animal Products of Ministry of Agriculture, 266032 Qingdao - CHINA
[2] Chinese Centerfor Disease Control and Prevention, PulseNetChina, 102206 Beijing - CHINA

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Abstract

Salmonella spp.is the most important food-borne pathogens of public health interest incriminated in poultry meat worldwide. The purpose of this study to estimate the prevalence *Salmonella* spp. contamination in poultry products from 12 different located geographical areas from among the 12 poultry slaughterhouses authorized in China and to characterize all the isolates by serotypes, PFGE patterns, MLST patterns and antimicrobial susceptibility. The prevalence of *Salmonella* spp. in the poultry slaughterhouse was 10.4%. All 100 strains of *Salmonella* spp. comprising 13 majority serotypes were identified. *S. Enteritidis* was the most frequently isolated from samples. The isolates displayed resistance to sulfisoxazole (SF) (79.0%), doxycycline (DOX) (68.0%), tetracycline (TE) (65.0%), florfenicol (FFC) (64.0%), ampicillin (AM) (50.0%), gentamicin (GM) (48.0%), trimethoprim-sulfamethoxazole (SXT) (30.0%), spectinomycin (SPT) (30.0%), enrofloxacin (ENR) (10.0%), ofloxacin (NOR) (10.0%), amoxicillin potassium clavulanat (AC) (1.0%) and polymyxin (PME) (4.0%). The *Salmonella* spp. isolates were not resistant to cefotaxime (EFT). Each isolates were multi-drug resistant as they were resistant to at least 2 groups of antimicrobials. Four clusters and 38 fingerprint-patterns generated by PFGE were identified among strains recovered from various locations, providing information on associations among the strains as well as evidence of the existence of persistent strains in some areas. MLST analysis of isolates identified the 10 STs, the 7 housekeeping genes had the different variation.

Keywords: *Salmonella spp., Poultry slaughterhouse-processing, Serotyping, PFGE, MLST, Antimicrobial susceptibility*

Shandong Eyaletindeki Kanatlı Hayvan Kesimhanelerinden İzole Edilen *Salmonella* spp.'nin Karakterizasyonu ve Yaygınlığı

Özet

Salmonella spp. dünya çapında kanatlı hayvan etlerinden köken alan halk sağlığı bakımından en önemli gıda kaynaklı patojendir. Bu çalışmanın amacı Çin'de 12 değişik bölgede yer alan kesimhanelerde kesilen kanatlı hayvanlara ait ürünlerde *Salmonella* spp. prevalansını tespit etmek ve tüm izolatları serotipleri, PFGE ve MLST görüntüleri ile antimikrobiyal duyarlılıklarına göre karakterize etmektir. Çalışmada *Salmonella* spp. prevalansı kanatlı kesimhanelerinde %10.4 olarak belirlendi. 13 serotipi içeren 100 *Salmonella* suşu tespit edildi. Örneklerden en çok izole edilen etken *S. Enteritidis* olarak kaydedildi. İzolatlar belirtilen antibiyotiklere direnç gösterdi; sulfisozaksol (SF) (%79.0), doksisilin (DOX) (%68.0), tetrasiklin (TE) (%65.0), florfenikol (FFC) (%64.0), ampisillin (AM) (%50.0), gentamisin (GM) (%48.0), trimethoprim-sulfamethoksazol (SXT) (%30.0), spektinomisin (SPT) (%30.0), enrofloksasin (ENR) (%10.0), ofloksasin (NOR) (%10.0), amoksisilin potasyum klavulanat (AC) (%1.0) ve polimiksin (PME) (%4.0). *Salmonella* spp. izolatları cefotaxime (EFT) karşı dirençli değildi. Her bir izolat en az 3 grup antimikrobiyal madde olmak üzere çoklu ilaç direnci gösterdi. PFGE ile elde edilen dört küme ve 38 parmakizi-görüntüsü değişik bölgelerden toplanan suçların arasından belirlendi. Suşlar arası asosiasyon ve bazı bölgelerde persiste suşların varlığı tespit edildi. İzolatların MLST analizi 10 ST belirledi. Yedi housekeeping gen farklı varyasyonlara sahipti.

Anahtar sözcükler: *Salmonella spp., Kanatlı hayvan kesimhane-işleme, Serotiplendirme, PFGE, MLST, Antimikrobiyal duyarlılık*

İletişim (Correspondence)
+86 0532 85633936
yffs2000@sina.com

INTRODUCTION

Salmonella spp. is an important group of bacterial zoonotic pathogens which can cause acute food-borne diseases in humans. Each year, approximately 90 million cases of gastroenteritis due to *Salmonella* spp. occur globally [1]. It multiplies mainly in poutry intestinal tract where it can be detected within two hours after infection [2]. A great increase inhuman food-borne infections caused by *Salmonella* spp. including *Salmonella* Enteritidis and *Salmonella* Typhimurium has been noted in the United States, Europe [3,4] *Salmonella* spp. infections in humans often result from the ingestion of contaminated foods, such as poultry, beef, pork, eggs and produce. Estimates from the Centers for Disease Control and Prevention (CDC) reported that more than a million people have *Salmonella* spp. poisoning every year from a variety of causes. Poultry products have consistently been identified as important sources of *Salmonella* spp. infection in humans, because vertical transfer of infection from breeding hens to progeny is an important aspect of the epidemiology of *Salmonella* spp. infection within the poultry industry [5]. Other study showed that a great number of *Salmonella* spp. contaminate poultry products and have somewhat different genetic types according to the origin of the integrated broiler operation [6]. Nevertheless, there have been no studies following the dissemination of *Salmonella* spp. in the entire poultry industry in China.

The aim of this study was to estimate the prevalence *Salmonella* spp. contamination in poultry products from 12 different located geographical areas from among the 12 poultry slaughterhouses authorized in China and to characterize all the isolates by serotypes, pulse field gel electrophoresis (PFGE) patterns, multi-locus sequence typing (MLST) patterns and antimicrobial susceptibility to investigate possible sources of infection and to provide information which could help strengthen salmonellosis control programs, and minimize the risk of *Salmonella* spp. exposure in the slaughtering line, which can reduce the contamination pressure downstream at retail shops as well as for end consumers.

MATERIAL and METHODS

The Sample Collection and Isolation

The present study was performed in 12 representative slaughterhouses (A ~ L) in 12 different areas in Shandong province from 2014 to 2015. A total of 960 swabs samples were randomly collected from poultry slaughterhouse-processing, every location the sample number is 80. *Salmonella* spp. contamination samples were collected in five critical steps in slaughterhouse-processing.

All samples were shipped in an icebox to the Microbiology Laboratory of Quality and Safety Risk Assessment for Animal Products of Ministry of Agriculture (CAHEC) in Qingdao, China, for *Salmonella* spp. isolation within 24 h. The procedure for the detection and isolation of *Salmonella* spp. was carried out according to the techniques recommended by the International Organization for Standardization [7]. All suspicious *Salmonella* colonies with black center were confirmed biochemically using lysine and triple sugar iron agars and API 20E (bioMérieux, France). The *invA* (F: 5′-GAATCCTCAGTTTTCAGTTTC-3′; R: 5′-TAGCCGTAACAACCAATACAAATG-3′) gene of *Salmonella* was used to further confirm the identity of the presumptive *Salmonella* spp.[8].

Serotyping

Salmonella-positive isolates were randomly serotyped according to the White-Kauffmann-Le Minor scheme by slide agglutination with O and H antigen specific sera (Sifin Diagnostics, Berlin, Germany) [9].

Pulsed-field Gel Electrophoresis

PFGE was performed for *Salmonella enterica* using the Pulse Net protocol procedures described previously [10]. The bacteria genomic DNA was digested with 50 U of *Xba* I (Takara, China) at 37°C for 3 h. The Pulse Net “Universal” standard strain *Salmonella enterica* serovar Braenderup H9812 was used as a reference marker and *Xba* I (Takara, China) was used as the digestion enzyme. PFGE was repeated twice to determine reproducibility. For untypable isolates, 50 μ Mthiourea (Sigma, USA) was added to the 0. 5 XTBE buffer prior to PFGE run as described by Römling and Tümmler [11]. CHEF-Mapper (Bio-Rad, USA) was used for electrophoresis (AutoAlgorithm: 30 kb-low MW, 700 kb-high MW, 19 h). The gel was stained with Gelred (Biofilm) and visualized using a gel imaging system (Bio-Rad, Gel DocXR, USA). PFGE patterns were analyzed using BioNumerics version 5.10; and a dendrogram was constructed using the Dice coefficient and un-weighted pair group methods with the arithmetic mean algorithm (UPGMA). PFGE banding patterns with a similarity index >80% were grouped in the same genotype cluster.

Multi-locus Sequence Typing

The multilocus sequence typing method was performed according to the recommendations of the *Salmonella enterica* MLST website *(http://mlst.warwick.ac.uk/mlst/dbs/Senterica)*. Seven housekeeping genes, *aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA* and *thrA*, were amplified using the recommended primers. DNA Taq premix was used with the amplification procedure: 94°C 5 min; followed by 30 cycles: 94°C 1 min, 55°C 30 s, 72°C 1 min; and a final extension at 72°C 10 min. The amplification products were sent for bidirectional sequencing to Takara, China; and the sequences were analyzed using DNASTAR and MEGA4 software. We submitted the sequences to the UCC database for their allele and ST assignments. A minimum

spanning tree based on these STs was generated with BioNumerics version 5.10.

Antimicrobial Susceptibility Tests

Susceptibility of the isolates to antimicrobial agents was evaluated according to Clinical Laboratory Standards Institute (CLSI, 2012) guidelines using the disc diffusion method on Mueller-Hinton agar (Becton Dickinson, USA) [12]. Following 13 antimicrobial agents were tested, including chloramphenicol (florfenicol), penicillins (ampicillin and amoxicillin potassium clavulanat), sulfonamides (sulfisoxazole and trimethoprim-sulfamethoxazole), cephems (cefotaxime), aminoglycosides (gentamicin and spectinomycin), tetracyclines (tetracycline and doxycycline), fluoroquinolones (enrofloxacin and ofloxacin), and other (polymyxin). Results were interpreted using the Clinical and Laboratory Standards Institute (CLSI, 2012) breakpoints when available *E. coli* ATCC 25922 was used as quality control [13].

RESULTS

The prevalence of *Salmonella* spp. from each of the 12 poultry slaughterhouses is presented in *Table 1*. In this study, 100 (10.42%) *Salmonell*a spp. was isolated from poultry slaughterhouses, the 12 (12.0%) *Salmonella* isolated from duck slaughterhouses and the 88 (88.0%) isolated from chicken slaughterhouses. All of the samples from 2 duck slaughterhouses were contaminated with *Salmonella* spp.. The major prevalent serotypes in the duck slaughterhouses were *Salmonell*a Thompson, and 5 different serotypes (*Salmonell*a Agona, *Salmonell*a Montevideo, *S.* Typhimurium, *Salmonell*a Indiana and *Salmonell*a Albany). In the 10 chicken slaughterhouses, the major of *Salmonella* spp. serotypes were *S.* Enteritidis and *S.* Indiana. Other different serotypes,also were found in chicken slaughterhouses *(Fig.1)*. The characteristics of 100 *Salmonella* spp. strains comprising thirteen majority serotypes were identified. *S.* Enteritidis was the most frequently isolated serotype from samples (43.0%, 43 of 100) *(Fig.1)*. *Salmonella* group *S.* Enteritidis (n = 43), *S.* Indinan (n = 14), *Salmonella* Derby (n = 11), *Salmonella* Infantis (n = 8), *S.* Agona (n = 8), *S. Salmonell*a Agona, *Salmonell*a Montevideo, *S.* Typhimurium, *Salmonell*a Indiana and *Salmonell*a Albany (n = 7), *Salmonella* Essen (n = 2), *S.* Typhimurium (n = 2), *S.* Albany (n = 1), *Salmonella* Othmarschen (n = 1), *Salmonella* Schwarzengrund (n = 1), *Salmonella* Abortus equi (n = 1) and *S.* Montevideo (n = 1) were identified during this study *(Fig.1)*.

The results of the antimicrobial susceptibility analysis of the *Salmonella* spp. isolates were summarized in *Fig. 2*. All 100 *Salmonella spp.* isolates were observed to be susceptible to the 13 antimicrobial agents tested in this

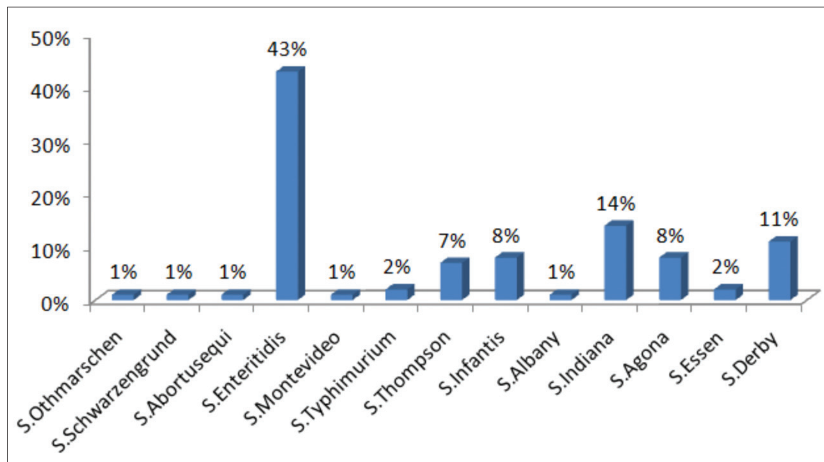


Fig 1. The distinction of serotype of 100 *Salmonella* spp. Isolate

Şekil 1. 100 adet *Salmonella* spp. izolatının serotiplendirmesi

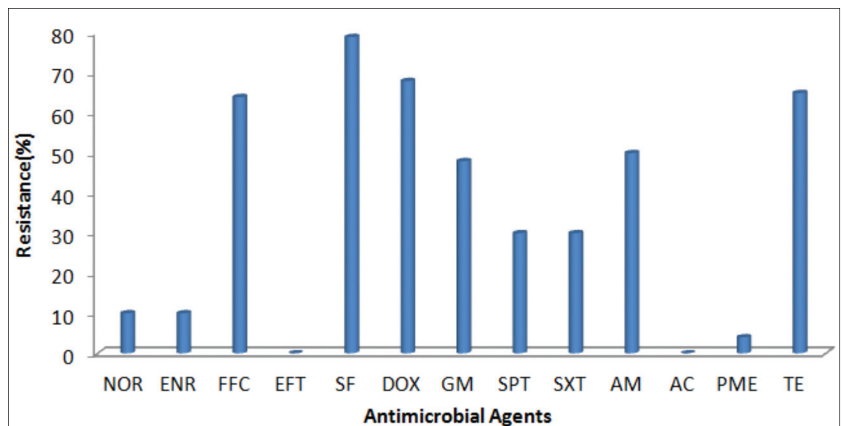


Fig 2. The drug resistance rate of all isolates for 13 kind of antimicrobial agents. Statistical test was only performed in all isolated. Antibiotics abbreviations are: SF: Sulfisoxazole, DOX: Doxycycline, TE: Tetracycline, FFC: Florfenicol, AM: Ampicillin, GM: Gentamicin, SPT; Spectinomycin, SXT: Trimethoprim-sulfamethoxazole, ENR: Enrofloxacin, NOR: Ofloxacin, PME: Polymyxin, EFT: Cefotaxime, AC: Amoxicillin potassium clavulanat

Şekil 2. Tüm izolatların 13 antimikrobiyal madde için ilaç direnci oranları. İstattistiksel test bütün izolatlar için uygulandı. Antibiyotiklerin kısaltmaları; SF: Sulfisozaksol, DOX: Doksisilin, TE: Tetrasiklin, FFC: Florfenikol, AM: Ampisillin, GM: Gentamisin, SPT; Spektinomisin, SXT: Trimethoprim-sulfamethoksazol, ENR: Enrofloksasin, NOR: Ofloksasin, PME: Polymiksin, EFT: Sefotaksim, AC: Amoksisilin potasyum klavulanat

Number of Isolates (n)	Serotype	Location	Animal	MLST Pattern	PFGE Pattern	Prevalence (%)
Table 1. *Origin and characterization of Salmonella isolated from twelve poultry slaughterhouse* **Tablo 1.** *Oniki kanatlı hayvan kesimhanesinden izole edilen Salmonellaların orjin ve karakterizasyonu*						
A(6)	Enteritidis(3) Enteritidis(3)	A A	Chicken Chicken	ST-11 ST-11	0031 0032	7.5
B (6)	Agona(3) Indiana(1) Indiana(1) Enteritidis(1)	B B B B	Chicken Chicken Chicken Chicken	ST-13 ST-17 ST-17 ST-11	0003 0005 0017 0031	7.5
C(19)	Abortusequi(1) Schwarzengrund(1) Enteritidis(2) Othmarschen(1) Enteritidis(1) Derby(8) Essen(2) Derby(3)	C C C C C C C C	Chicken Chicken Chicken Chicken Chicken Chicken Chicken Chicken	ND ND ST-11 ND ST-11 ST-40 ND ND	0018 0024 0031 0031 0032 0037 0031 0024	23.8
D(9)	Indiana(2) Indiana(1) Indiana(1) Indiana(2) Enteritidis(1) Indiana(2)	D D D D D D	Chicken Chicken Chicken Chicken Chicken Chicken	ST-17 ST-17 ST-17 ST-17 ST-17 ST-17	0004 0005 0014 0025 0028 0030	11.3
E(13)	Enteritidis(1) Enteritidis(1) Enteritidis(1) Enteritidis(1) Indiana(1) Agona(1) Enteritidis(1) Agona(1) Agona(1) Indiana(1) Enteritidis(3)	E E E E E E E E E E E	Chicken Chicken Chicken Chicken Chicken Chicken Chicken Chicken Chicken Chicken Chicken	ST-11 ST-11 ST-17 ST-11 ST-17 ST-13 ST-11 ST-13 ST-13 ST-17 ST-11	0008 0009 0010 0011 0012 0012 0013 0015 0016 0028 0031	16.3
F(7)	Agona(1) Infantis(2) Indiana(1) Enteritidis(2) Indiana(1)	F F F F F	Chicken Chicken Chicken Chicken Chicken	ST-13 ST-32 ST-17 ST-11 ST-17	0001 0022 0031 0033 0027	8.8
G(9)	Typhimurium(1) Infantis(1) Infantis(1) Enteritidis(3) Enteritidis(2) Enteritidis(1)	G G G G G G	Chicken Chicken Chicken Chicken Chicken Chicken	ST-34 ST-32 ST-32 ST-11 ST-11 ST-11	0006 0021 0023 0032 0034 0036	11.3
H(7)	Infantis(1) Infantis(2) Enteritidis(1) Enteritidis(3)	H H H H	Chicken Chicken Chicken Chicken	ST-32 ST-32 ST-11 ST-11	0021 0022 0029 0032	8.8
I(4)	Agona(1) Montevideo(1) Typhimurium(1) Indiana(1)	I I I I	Duck Duck Duck Duck	ST-13 ST-305 ST-19 ST-17	0003 0007 0019 0020	5.0
J(6)	Enteritidis(2) Enteritidis(3) Enteritidis(1)	J J J	Chicken Chicken Chicken	ST-11 ST-11 ST-11	0026 0031 0032	7.5
K(6)	Enteritidis(5) Enteritidis(1)	K K	Chicken Chicken	ST-11 ND	0033 0035	7.5
L(8)	Thompson(7) Albany(1)	L L	Duck Duck	ST-26 ST-45	0002 0038	10.0

***Table 2.** Sequence types (STs)and allele profile of each isolate*
***Tablo 2.** Herbir izolatın sekans tipi ve allel profili*

STs	*acroC*	*dnaN*	*hemD*	*hisD*	*purE*	*SucA*	*thrE*	Number of Isolates
11	5	2	3	7	6	6	11	41
13	3	3	7	3	3	3	7	8
17	8	8	11	11	5	11	15	16
19	10	7	12	9	5	9	2	1
26	14	13	18	12	5	18	1	7
32	17	18	22	17	5	21	19	7
34	10	19	12	9	5	9	2	1
40	19	20	3	20	5	22	22	8
45	104	100	54	78	104	1	48	1
305	43	41	16	42	34	13	23	1

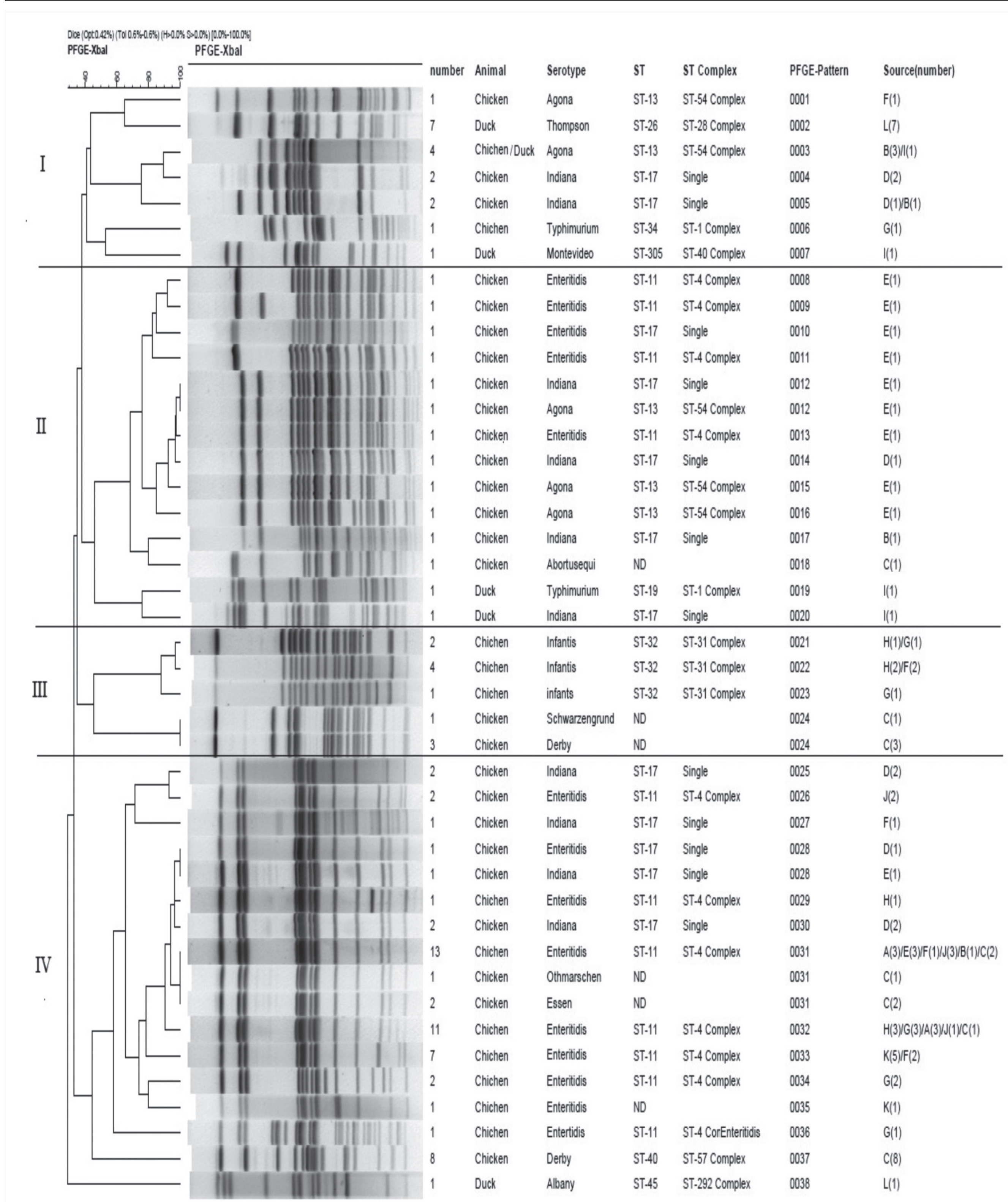
study. The isolates were resistantto sulfisoxazole (SF) (79.0%), doxycycline (DOX) (68.0%), tetracycline (TE) (65.0%), florfenicol (FFC) (64.0%), ampicillin (AM) (50.0%), gentamicin (GM) (48.0%), trimethoprim-sulfamethoxazole (SXT) (30.0%), spectinomycin (SPT) (30.0%), enrofloxacin (ENR) (10.0%), ofloxacin (NOR) (10.0%), amoxicillin potassium clavulanat (AC) (1.0%) and polymyxin (PME) (4.0%). The *Salmonella* spp. isolates were not resistant to cefotaxime (EFT). Each isolates were multi-drug resistant as they were resistant to at least 2 groups of antimicrobials.

The 100 *Salmonella* spp. isolates were analyzed by PFGE using enzymes *Xba*I resulted in 38 distinguishable patterns demonstrating a high level of genetic diversity among the isolates. An UPGMA dendrogram was constructed *(Fig. 3)*, and described in *Table 1*. Ten *Salmonella* spp. isolates were untypeable by PFGE. After the addition of thiourea (Sigma, USA)to the running buffer, all isolates remained typeable. The 100 isolates could be divided into four clusters. All serotypes were divided into groups based on their PFGE patterns. In this study,the most common pattern was 0031, which included 13 strains of *S.* Enteritidis, followed by 0032 which was composed of 11 strains of *S.* Enteritidis, and by 0037 which was composed of 8 strains of *S.* Derby. The origins and characteristics of *Salmonella* spp. Isolates identified in this study are outlined in *(Table 1)*. Looking at the strains in more detail, most pattern were within a single serotype and single source except 0003-pattern, 0005-pattern, 0021-pattern, 0022-pattern, 0031-pattern, 0032-pattern and 0033-pattern. The 0031-pattern was found to be composed of 13 *S.* Enteritidis, all recovered from various processing steps at six slaughterhouse, which were A(3), B(1), C(2), E(3), F(1), and J(3); the 0032-pattern was found to be composed of 11 *S.* Enteritidis, which were from H(3), G(3), A(3), J(1) and C(1) five slaughterhouse. The results showed that different places had the same isolates, these strains maybe come from the same farms, or the slaughterhouse circulation caused by cross contamination.

Ten discrete STs were identified among the 91*Salmonella* spp. isolates, indicating a degree of genotypic diversity, other 9 isolates were not done. Of these 10 STs, 4 were represented by single isolates, 6 were represented by more than one isolate (n = 7 to 41). The predominant STs were ST11, ST17, ST13 and ST26 containing 41 (45.1%), 16 (17.6%), 8 (8.8%) and 8 (8.8%) isolates respectively *(Table 2)*. Isolates characterized as the same STs did not necessarily have the same PFGE pattern. For example, the 41 isolates characterized as ST11 had 11 distinct PFGE patterns *(Fig. 3)*. All of the isolates that shared a PFGE pattern had the same STs. Meanwhile, isolates that shared a PFGE pattern had the same STs *(Fig. 3)*. Showing the detail of the strains in more detail, most STs were within a single serotype.

DISCUSSION

Poultry products have consistently been identified as important sources of *Salmonella* infection in humans, because vertical transfer of infection from breeding hens to progeny is an important aspect of the epidemiology of *Salmonella* spp. infection within the poultry industry. In many countries all over the world including USA, Europe, Korea and China, a wide range of different *Salmonella* serotypes have been found to contaminate the broiler houses, flocks and carcasses of the poultry industry [14]. In this study, 10.4% of the carcasses sampled from 12 poultry slaughterhouses were contaminated with *Salmonella* spp. and showed a lower prevalence than poultry carcasses originating from other countries like Korea (42.1%), Spain (17.9%), Canada (21.2%) and Ireland (26.4%) [15-18]. Although different sampling procedures, sample sizes and bacterial isolation and identification methods could affect the prevalences of *Salmonella* spp., this elevated level of contamination indicates a potential breakdown of hygiene at various stages at poultry farms and processing plants [19]. *S.* Enteritidis, which is responsible for most Salmonella infections in humans, is the major serotypes in this study, and this result supports that contaminated carcasses are the major source of infection in human salmonellosis [20].



number	Animal	Serotype	ST	ST Complex	PFGE-Pattern	Source(number)
1	Chicken	Agona	ST-13	ST-54 Complex	0001	F(1)
7	Duck	Thompson	ST-26	ST-28 Complex	0002	L(7)
4	Chichen / Duck	Agona	ST-13	ST-54 Complex	0003	B(3)/I(1)
2	Chicken	Indiana	ST-17	Single	0004	D(2)
2	Chicken	Indiana	ST-17	Single	0005	D(1)/B(1)
1	Chichen	Typhimurium	ST-34	ST-1 Complex	0006	G(1)
1	Duck	Montevideo	ST-305	ST-40 Complex	0007	I(1)
1	Chicken	Enteritidis	ST-11	ST-4 Complex	0008	E(1)
1	Chicken	Enteritidis	ST-11	ST-4 Complex	0009	E(1)
1	Chicken	Enteritidis	ST-17	Single	0010	E(1)
1	Chicken	Enteritidis	ST-11	ST-4 Complex	0011	E(1)
1	Chicken	Indiana	ST-17	Single	0012	E(1)
1	Chicken	Agona	ST-13	ST-54 Complex	0012	E(1)
1	Chicken	Enteritidis	ST-11	ST-4 Complex	0013	E(1)
1	Chicken	Indiana	ST-17	Single	0014	D(1)
1	Chicken	Agona	ST-13	ST-54 Complex	0015	E(1)
1	Chicken	Agona	ST-13	ST-54 Complex	0016	E(1)
1	Chicken	Indiana	ST-17	Single	0017	B(1)
1	Chicken	Abortusequi	ND		0018	C(1)
1	Duck	Typhimurium	ST-19	ST-1 Complex	0019	I(1)
1	Duck	Indiana	ST-17	Single	0020	I(1)
2	Chichen	Infantis	ST-32	ST-31 Complex	0021	H(1)/G(1)
4	Chichen	Infantis	ST-32	ST-31 Complex	0022	H(2)/F(2)
1	Chichen	infants	ST-32	ST-31 Complex	0023	G(1)
1	Chicken	Schwarzengrund	ND		0024	C(1)
3	Chicken	Derby	ND		0024	C(3)
2	Chicken	Indiana	ST-17	Single	0025	D(2)
2	Chicken	Enteritidis	ST-11	ST-4 Complex	0026	J(2)
1	Chicken	Indiana	ST-17	Single	0027	F(1)
1	Chicken	Enteritidis	ST-17	Single	0028	D(1)
1	Chicken	Indiana	ST-17	Single	0028	E(1)
1	Chichen	Enteritidis	ST-11	ST-4 Complex	0029	H(1)
2	Chichen	Indiana	ST-17	Single	0030	D(2)
13	Chichen	Enteritidis	ST-11	ST-4 Complex	0031	A(3)/E(3)/F(1)/J(3)/B(1)/C(2)
1	Chicken	Othmarschen	ND		0031	C(1)
2	Chicken	Essen	ND		0031	C(2)
11	Chichen	Enteritidis	ST-11	ST-4 Complex	0032	H(3)/G(3)/A(3)/J(1)/C(1)
7	Chichen	Enteritidis	ST-11	ST-4 Complex	0033	K(5)/F(2)
2	Chicken	Enteritidis	ST-11	ST-4 Complex	0034	G(2)
1	Chichen	Enteritidis	ND		0035	K(1)
1	Chichen	Entertidis	ST-11	ST-4 CorEnteritidis	0036	G(1)
8	Chicken	Derby	ST-40	ST-57 Complex	0037	C(8)
1	Duck	Albany	ST-45	ST-292 Complex	0038	L(1)

Fig 3. Dendrogram of PFGE profiles

PFGE patterns and the corresponding dendrogram for 100 isolates obtained in the present study are depicted. The 4PFGE clusters were marked on the node as I to IV. The different clusters observed are designated on the left side of the figure. Displayed on the right hand side are number, serotype, PFGE-Pattern, sequence types (STs), ST complex and source (number)

Şekil 3. PFGR profillerinin dendogramı

Çalışmadaki PFGE görüntüleri ve ilgili 100 izolatın dendogramı. 4PFGE kümeleri I'den IV'e kadar işaretlenmiştir. Elde edilen farklı kümeler şeklin sol tarafında belirtilmiştir. Sağ tarafta numara, serotip, PFGR-görüntüsü, sekans tipleri (STs), ST kompleks ve kaynakları (numara)

Each isolates showed antimicrobial resistance to at least one antimicrobial agent. The rate of resistance of *Salmonella* spp. to antimicrobial agents was higher than that previously observed [21,22], but sulfisoxazole, doxycycline and tetracycline were showed the higher resistance in 13 antimicrobial agents. The most of the isolates displayed a high level of susceptibility to most of the antimicrobial agents tested in this study, the antimicrobial susceptibility must will present a challenge for veterinary medicine and farm animal husbandry, and could also pose a threat to public health. *Fig. 2* shows *Salmonella* spp. resistance pattern of ofloxacin (NOR) at 10% is a reflection that it is least resistant to the organism, whereas the fluoroquinolones generally have been previously known to have a good sensitivity to *Salmonella* spp.. 100% resistance of *Salmonella* spp. from poultry sources to amoxicillin potassium clavulanat is in line with previous works [23], even though amoxicillin potassium clavulanat has been known to be of variable resistance. Tetracycline have repeatedly shown high levels of resistance of 60%-65% to which the work was lowed [24].

Some previous studies showed that the PFGE patterns of *S.* Enteritidis in China are similar even when the isolates have different phage types. The present investigation also confirmed that 18 *S. Enteritidis* isolates with different MLST pattern displayed different PFGE patterns in agreement with previous reports [25,26]. The PFGE results showed that different places had the same isolates, these strains maybe come from the same farms, or the slaughterhouse circulation caused by cross contamination.

This study shows that the domestic serotypes are *S. Enteritidis* and *S. Indian* in chicken and duck slaughterhouses in Shandong province, and provides detailed information about *Salmonella* spp. isolates from poultry. In addition, the appearance of resistant *Salmonella* spp. isolates from poultry suggests the need for a more prudent use of antibiotics and the importance of controlling this pathogen in poultry products. As human salmonellosis has been repeatedly related to the consumption of products worldwide, continuous research must be conducted to minimize the *Salmonella* spp. contamination in poultry slaughterhouse.

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REFERENCES

1. Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, O'Brien SJ, Jones TF, Fazil A, Hoekstra RM: The global burden of nontyphoidal *Salmonella* gastroenteritis. *Clin Infect Dis*, 50, 882-889, 2010. DOI: 10.1086/650733

2. Rostagno MH, Callaway TR: Pre-harvest risk factors for *Salmonella* Enterica in pork production. *Food Res Int*, 45, 634-640, 2012. DOI: 10.1016/j.foodres.2011.04.041

3. Brooks JT, Matyas BT, Fontana J, DeGroot MA, Beuchat LR, Hoekstra M, Friedman CR: An outbreak of *Salmonella* serotype Typhimurium infections with an unusually long incubation period. *Foodborne Pathog Dis*, 9, 245-248, 2012. DOI: 10.1089/fpd.2011.0992

4. Janmohamed K, Zenner D, Little C, Lane C, Wain J, Charlett A, Adak B, Morgan D: National outbreak of *Salmonella* Enteritidis phage type 14b in England, September to December 2009: Case-control study. *Euro Surveill,* 16, Pii: 19840, 2011.

5. Gast RK: Understanding *Salmonella* Enteritidis in laying chickens: the contributions of experimental infections. *Int J Food Microbiol*, 21, 107-116, 1994. DOI: 10.1016/0168-1605(94)90204-6

6. Kim A, Lee YJ, Kang MS, Kwag SI, Cho JK: Dissemination and tracking of *Salmonella* spp. in integrated broiler operation. *J Vet Sci*, 8, 155-161, 2007. DOI: 10.4142/jvs.2007.8.2.155

7. International Organization for Standardization (ISO): Microbiology of Food and Animal Feeding stuffs-Horizontal Method for the Detection of *Salmonella* spp. International Organization for Standardization: Geneva, Switzerland, 2007.

8. Kim JS, Lee GG, Park JS, Jung YH, Kwak HS, Kim SB, Nam YS, Kwon ST: A novel multiplex PCR assay for rapid and simultaneous detection of five pathogenic bacteria: *Escherichia coli* O157: H7, *Salmonella*, *Staphylococcus aureus, Listeria monocytogenes,* and *Vibrio parahaemolyticus*. *J Food Prot*, 70, 1656-1662, 2007.

9. Grimont PAD, Weill FX: Antigenic formulae of the *Salmonella*Serovars. Paris: World Health Organization, Institut Pasteur, 166p, 2007.

10. Wu W, Wang H, Lu J, Wu J, Chen M, Xu Y, Lu Y: Genetic diversity of *Salmonella*entericaserovarTyphi and Paratyphi in Shenzhen, China from 2002 through 2007. *BMC Microbiol*, 10, 32, 2010. DOI: 10.1186/1471-2180-10-32

11. Römling U, Tümmler B: Achieving 100% typeability of *Pseudomonas aeruginosa* by pulsed-field gel electrophoresis. *J Clin Microbiol*, 38, 464-465, 2000.

12. CLSI: Performance Standards for Antimicrobial Susceptibility Testing, Twenty-Second Informational ament Wayne Pennsylvania: Clinical and Laboratory Standards Institute; 2012.

13. Gai W, Wang J, Wang J, Cui Z, Qu Z, Cui J, Du X, Huang X, Zhao J: Molecular classification and drug resistance analysis of *Escherichia coli* isolated from poultry in China. *Int J Clin Exp Med*, 8, 836-844, 2015.

14. Song YK, Shin DH, Byun JW, Jeon KY, Jung BY: Seroprevalence of *Salmonella* Pullorum and Gallinarum in grandparent poultry stock farms during the 2006-2007. *Kor J Vet Publ Hlth*, 33, 131-136, 2009.

15. Bae DH, Dessie HK, Baek HJ, Kim SG, Lee HS, Lee YJ: Prevalence and characteristics of *Salmonella* spp solated from poultry slaughterhouses in Korea. *Vet Med Sci*, 75, 1193-1200, 2013.

16. Arsenault J, Letellier A, Quessy S, Boulianne M: Prevalence and risk factors for *Salmonella* and *Campylobacter* spp. carcass contamination in broiler chickens slaughtered in Quebec, Canada. *J Food Prot*, 70, 1820-1828, 2007.

17. Capita R, Alonso-Calleja C, Prieto M: Prevalence of *Salmonell a*enterica serovars and genovars from chicken carcasses in slaughterhouses in Spain. *J Appl Microbiol*, 103, 1366-1375, 2007.

18. Duffy G, Cloak OM, O'Sullivan MG, Guillet A, Sheridan JJ, Blair IS, McDowell DA: The incidence and antibiotic resistance profiles of *Salmonella* spp. on Irish retail meat products. *Food Microbiol*, 16, 623-631, 1999. DOI: 10.1006/fmic.1999.0278

19. Jørgensen F, Bailey R, Williams S, Henderson P, Wareing DR, Bolton FJ, Frost JA, Ward L, Humphrey TJ: Prevalence and numbers of *Salmonella* and *Campylobacter* spp. on raw, whole chickens in relation to sampling methods. *Int J Food Microbiol*, 76, 151-164, 2002. DOI: 10.1016/S0168-1605(02)00027-2

20. Cheong HJ, Lee YJ, Hwang IS, Kee SY, Cheong HW, Song JY, Kim JM, Park YH, Jung JH, Kim WJ: Characteristics of non-typhoidal *Salmonella* isolates from human and broiler-chickens in southwestern Seoul, Korea. *J Korean Med Sci*, 22, 773-778, 2007.

21. Kuang X, Hao H, Dai M, Wang Y, Ahmad I, Liu Z, Zonghui Y: Serotypes and antimicrobial susceptibility of *Salmonella* spp. isolated

from farm animals in China. *Front Microbiol*, 6, 602, 2015. DOI: 10.3389/fmicb.2015.00602

22. Morar A, Sala C, Imre K: Occurrence and antimicrobial susceptibility of *Salmonella* isolates recovered from the pig slaughter process in Romania. *J Infect Dev Ctries*, 9, 99-104, 2015. DOI: 10.3855/jidc.5236

23. Ezekiel CN, Olarinmoye AO, Oyinloye JMA, Olaoye OB, Edun AO: Distribution, antibiogram and multidrug resistance in *Enterobacteriaceae* from commercial poultry feeds in Nigeria. *Afr J Microbiol Res*, 5, 294-301, 2011.

24. Sakaridis I, Soultos N, Iossifidou E, Koidis P, Ambrosiadis I: Prevalence and antimicrobial resistance of *Salmonella* serovars from chicken carcasses in northern Greece. *J Food Saf*, 2011. DOI: 10.1111/j.1745-4565.2010.00286.x

25. Bai L, Lan R, Zhang X, Cui S, Xu J, Guo Y, Li F, Zhang D: Prevalence of *Salmonella* isolates from chicken and pig slaughterhouses and emergence of ciprofloxacin and cefotaxime co-resistant *S.* enterica serovar Indiana in Henan, China. *PLoS One*, 10, e0144532, 2015. DOI: 10.1371/journal.pone.0144532

26. Wang Y, Liu C, Zhang Z, Hu Y, Cao C, Wang X, Xi M, Xia X, Yang B, Meng J: Distribution and molecular characterization of *Salmonella* enterica hypermutators in retail food in China. *J Food Prot*, 78, 1481-1487, 2015. DOI: 10.4315/0362-028X.JFP-14-462

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Short Communication

Effect of Tween 80 on Conjugated Linoleic Acid Production by *Lactobacillus* Strains in Reconstituted Skim Milk Powder [1]

Emrah TORLAK [1] Suzan YALÇIN [2] Fatih ERCİ [3]

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1 Department of Molecular Biology and Genetics, Faculty of Science, Necmettin Erbakan University, TR-42090 Konya - TURKEY
2 Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Selcuk University, TR-42030 Konya - TURKEY
3 Department of Biotechnology, Faculty of Science, Necmettin Erbakan University, TR-42090 Konya - TURKEY

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Abstract

In this study, three conjugated linoleic acid (CLA)-producing strains of *Lactobacillus* were cultured up to 36 in reconstituted skim milk powder (10%) supplemented with 2000 μg/ml of free linoleic acid (LA) and various concentrations of Tween 80. During the incubation, total CLA levels in the culture supernatants were determined by UV-spectrophotometry. The CLA levels significantly (P<0.05) increased with the addition of 5 and 20 mg/ml Tween 80. However, increasing Tween 80 concentration from 20 to 40 mg/ml did not appear to enhance CLA levels. Similar increase patterns were observed in the growth rate and CLA production of *Lactobacillus* strains during the incubation.

Keywords: *Conjugated linoleic acid, Lactobacillus, Skim milk, Tween 80*

Lactobacillus Suşlarının Rekonstitüe Yağsız Süt Tozunda Konjuge Linoleik Asit Üretimine Tween 80'in Etkisi

Özet

Bu çalışmada üç adet konjuge linoleik asit (KLA) üreten *Lactobacillus* suşu 2000 μg/ml linoleik asit (LA) ve çeşitli konsantrasyonlarda Tween 80 ilave edilmiş rekonstitüe yağsız süt tozu (%10) içinde 36 saate kadar kültüre edilmiştir. İnkübasyon boyunca kültür süpernatantlarında toplam KLA düzeyleri UV-spektrofotometre ile tespit edilmiştir. KLA düzeyleri 5 ve 20 mg/ml Tween 80 ilavesi ile önemli (P<0.05) seviyede artmıştır. Bununla birlikte, Tween 80 konsantrasyonunun 20 mg/ml'den 40 mg/ml'ye arttırılması KLA düzeyinde bir artışa neden olmamıştır. İnkübasyon esnasında *Lactobacillus* suşlarının gelişme hızı ve KLA üretimlerinde benzer artış oranları gözlenmiştir.

Anahtar sözcükler: *Konjuge linoleik asit, Lactobacillus, Yağsız süt, Tween 80*

INTRODUCTION

Conjugated linoleic acid (CLA) is a collective name that refers to a wide variety of positional and geometric isomers of linoleic acid (LA) with conjugated double bonds at several positions from C7 to C14. In recent years, CLA, especially its isomers *cis*-9, *trans*-11 and *trans*-10, *cis*-12, has attracted attention among food and medical scientists due to its various beneficial biological effects. It has been reported in animal models to have anticarcinogenic, anti-atherogenic, anti-inflammatory and anti-diabetic activity and the ability to reduce the catabolic effects of immune stimulation [1].

Dairy products derived from ruminant animals are the major source of CLA in human diet. The major isomer of CLA in milk fat is *cis*-9, *trans*-11, and it represents 80% of the total CLA [2]. The CLA levels of various dairy products were reported in the range of 0.55-9.12 mg/g of fat [3]. These values are clearly lower compared to the recommended daily intake of CLA for appreciation of health benefits. Recommended therapeutic daily intake of CLA for an anticarcinogenic response in humans were reported in the range of 3.0-3.5 g/day based on diet and cancer risk studies and on the amount of CLA required for an anticarcinogenic response extrapolated from rats to humans [4,5]. Therefore, the development of strategies to increase CLA levels in dairy products is an ongoing subject of research.

Results of previous studies showed that many strains of lactic acid bacteria, especially from the genera *Lactobacillus*, *Bifidobacterium* and *Lactococcus* are able to efficiently

İletişim (Correspondence)
+90 506 1499841
ferci@konya.edu.tr

convert LA to CLA in either synthetic medium or milk [6]. Therefore, the incorporation of suitable strains of lactic acid bacteria and free LA into processed foods is a reasonable option to increase CLA concentration in the human diet [7]. Antibacterial effect of free LA is the main challenge in the use of lactic acid bacteria for the purpose of increasing the CLA levels in foods. The growth rate and CLA production of lactic acid bacteria may be negatively affected by the presence of higher concentrations of free LA [8].

It is well documented that Tween 80 (polyoxyethylene sorbitan monooleate), a nonionic surfactant is used widely as an additive in foods, pharmaceutical preparations, and as an emulsifier, dispersant or stabilizer [9]. Also, Tween 80 has ability to neutralize the antimicrobial effects of several substances including free fatty acids and positive effect on CLA production by lactic acid bacteria [8,10]. According to our knowledge, the effect of Tween 80 concentration on CLA-producing bacteria has not previously been reported. Therefore, in this study, the effect of various concentration of Tween 80 on growth rate and CLA production of selected *Lactobacillus* strains were evaluated in reconstituted skim milk powder supplemented with free LA.

MATERIAL and METHODS

Microorganisms and Growth Conditions

The *Lactobacillus* strains used were previously isolated from Tulum cheese, which is a traditional Turkish goat's milk cheese. The isolates were primarily evaluated based on colony and cell morphology, Gram reaction and catalase test. Presumptive *Lactobacillus* strains were identified by API 50 CHL biochemically-based identification system (bioMérieux, Marcyll'Etoile, France). After initial screening for CLA conversation in MRS broth (Lab M, Bury, UK), three strains from different *Lactobacillus* species (*L. rhamnosus*, *L. plantarum* and *L. brevis*) were assayed in this study.

Spray dried skim milk powder (1.1% fat) was kindly provided by Enka Milk Company (Konya, Turkey). Autoclaved reconstituted skim milk powder (10%) supplemented with 2000 µg/ml of LA (Sigma-Aldrich, Milwaukee, WI, USA) and 0, 5, 20 or 40 mg/ml of Tween 80 (Merck, Darmstadt, Germany) was designated as test medium. Stock cultures of microorganisms maintained in brain heart infusion broth (Liofilchem, Roseto Degli Abruzzi, Italy) supplemented with 20% glycerol at -18°C were activated in MRS broth. The activated cultures were inoculated into skim milk (10%) at a ratio of 1% (v/v) and incubated at 37°C. Thereafter, 0.1 ml of overnight cultures were transferred to 10 ml test media and incubated at 37°C up to 36 h. All incubations were done under anaerobic conditions. At the end of incubation times of 12, 24 and 36 h, total CLA levels and *Lactobacillus* counts in cultures were determined.

Enumeration of Lactobacillus

Lactobacillus counts were performed by pour plate technique using MRS agar (Lab M). Dilutions of culture were made by peptone salt diluent (Lab M). One milliliter of the dilutions was transferred in two sterile plates per dilution and 15 ml MRS agar was then added per plate. After incubation at 30°C for 72 h, colonies on plates were counted and *Lactobacillus* counts in cultures were calculated as log CFU/ml.

Total CLA Assay

Lipid extraction from cultures was carried out by a procedure described by Barrett et al.[6] with some minor modifications. After specified incubation period, 1 ml of cultures was centrifuged at 18600 *g* for 2 min (Hettich, Tuttlingen, Germany). Then, the supernatants were mixed with 2 ml of isopropanol by vortexing and allowed to stand for 3 min. The lipids were extracted by addition 3 ml of hexane and vortexing. Final solutions were centrifuged at 6000 *g* for 3 min and the resulting supernatants were used in CLA quantitation assay.

The UV-spectrophotometric method was used for quantification of total CLA in the supernatants of Lactobacillus cultures. According to the literature [10,11] the most widely used methods for analysis of CLA in milk and microbiological media are gas chromatography (GC) and silver-ion high-performance liquid chromatography (Ag+-HPLC). However, the chromatographic methods can be considered as laborious and time-consuming for total CLA quantification. Alternatively, conjugated double bonds of CLA isomers can be detected using a UV-spectrophotometer at a wavelength of 233 nm [11]. It was previously reported that UV-spectrophotometry is a simple, rapid, cheap and accurate measurement method for CLA analysis. However, it should be noted that UV-spectrophotometry is not able to distinguish between CLA isomers [10].

Spectrophotometric assay based on the procedure of Rodríguez-Alcalá et al.[11] was used to quantitate the total CLA concentration in the culture supernatants. Absorbances were obtained using a UV-spectrophotometer at a wavelength of 233 nm (Thermo Scientific, Waltham, MA, USA) from 2 ml of the lipid extracts in hexane placed into quartz cuvettes. The measurements were compared to a calibration curve constructed with solutions of *cis*-9, *trans*-11 CLA isomer (Sigma-Aldrich) in hexane. Culture supernatants containing CLA at a concentration exceeding linear concentration range were diluted with appropriate volumes of hexane.

The calibration curve constructed using the *cis*-9, *trans*-11 CLA isomer, demonstrated that an increase in the CLA concentration up to 50 µg/ml coincided with a linear increase (R^2>0.99) in the absorbance up to 2.2. The UV-spectrophotometric method was extremely sensitive

and could detect and quantitate CLA concentration in the supernatant (2.3 μg/ml) obtained from sterile skim milk medium. The precision of method was evaluated by the multiple analyses of supernatants with different CLA contents. The relative standard deviations of repeatability were in range of 6.2-11.1%, respectively.

Statistical Analysis

Results of three independent trials were analyzed by one-way analysis of variance (ANOVA) using statistical software (SPSS Inc., Chicago, USA). Mean values were compared using the Duncan grouping test at P<0.05.

RESULTS

The changes in total CLA level in the supernatants of *Lactobacillus* cultures grown in test medium supplemented with free LA (2000 μg/ml) and Tween 80 (0, 5, 20 or 40 mg/ml) during 36 h incubation are shown in *Fig. 1*. The level of CLA in supernatant of sterile skim milk was determined as 2.3 μg/ml. CLA concentrations in supernatants obtained from test medium without addition of Tween 80 significantly (P<0.05) increased from 2.3 to 37-64 μg/ml after 24 h of incubation. However, CLA levels in Tween 80-free test medium remained relatively constant during 36 h of incubation. CLA concentrations of the culture supernatants obtained after 36 h incubation of *Lactobacillus* strains in test medium with 5 mg/ml of Tween 80 ranged from 97 to 189 μg/ml. The levels of CLA in culture supernatants of test medium supplemented with 20 mg/ml of Tween 80 reached up to 198-327 and 233-384 μg/ml at the end of 24 and 36 h incubations, respectively. The CLA levels observed in culture supernatants obtained from test medium with 40 mg/ml of Tween 80 after 36 h of incubation were in the range of 244 to 357 μg/ml. At the end of 36 h incubation, the highest CLA levels were observed in test media inoculated with *L. brevis*. Our results showed that CLA conversion by selected Lactobacillus strains in skim milk with free LA was significantly (P<0.05) enhanced with the addition of 5 μg/ml of Tween 80, and increasing concentration of Tween 80 to 20 μg/ml resulted in a significant (P<0.05) increase in CLA conversions. However, CLA levels did not change significantly (P>0.05) at the two higher Tween 80 concentrations (20 and 40 mg/ml).

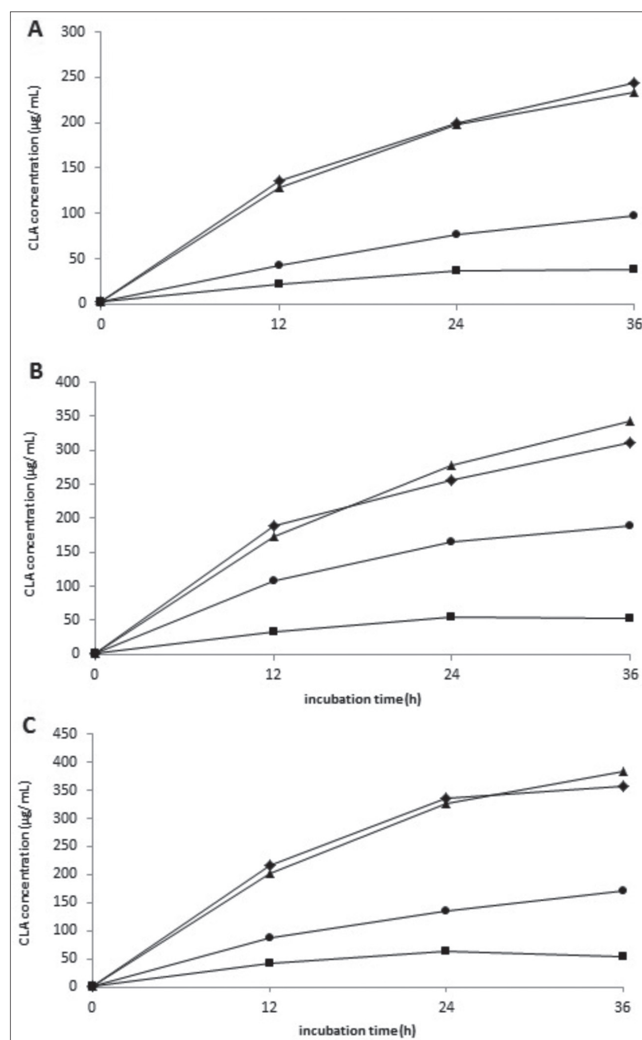

Fig 1. Total CLA concentration (μg/ml) of the culture supernatants obtained after incubation of the selected *Lactobacillus* strains ((A) *L. rhamnosus*; (B) *L. plantarum*; (C) *L. brevis*) in skim milk with free LA (2000 μg/ml) and Tween 80 ((▲) 0 mg/ml; (●) 5 mg/ml; (♦) 20 mg/ml; (■) 40 mg/ml) for 12, 24 and 36 h

Şekil 1. Serbest LA (2000 μg/ml) ve Tween 80 ((▲) 0 mg/ml; (●) 5 mg/ml; (♦) 20 mg/ml; (■) 40 mg/ml) içeren yağsız sütten *Lactobacillus* suşlarının ((A) *L. rhamnosus*; (B) *L. plantarum*; (C) *L. brevis*) 12, 24 ve 36 saat inkübasyonu sonunda elde edilen kültür süpernatantlarındaki toplam KLA konsantrasyonu (μg/ml)

The spectrophotometric results obtained revealed that CLA production by *Lactobacillus* strains in Tween 80-added test media were biphasic, indicating an initial rapid increase in the CLA levels during the 12 h of incubation, followed by gradually slower production rates. CLA is an intermediate in the bio-hydrogenation of polyunsaturated fatty acids [12]. Therefore, in addition to changes in the available amount of free LA, decrease in the CLA production rates during the incubation period can be attributed to the further transformation of CLA to saturated fatty acids.

Microbial growth curves of *Lactobacillus* strains in test media for up to 36 h of incubation are given in *Fig. 2*. The growth patterns of selected strains observed in test media were similar to those of CLA conversions. The numbers of *Lactobacillus* strains in test medium without the addition of Tween 80 increased from 4.1-4.7 to 5.3-6.2 log CFU/ml during incubation. *Lactobacillus* counts in test medium with 5 mg/ml of Tween 80 after 36 h of incubation were in the range of 7.6 to 8.1 log CFU/ml. A final *Lactobacillus* counts in 20 and 40 mg/ml Tween 80-added test medium were determined as 8.7-8.8 and 8.5-8.8 log CFU/ml, respectively. Plate counts showed that growth of *Lactobacillus* strains in the presence of 2000 μg/ml of free LA was increased significantly (P<0.05) through the addition of Tween 80. However, counts of *Lactobacillus*

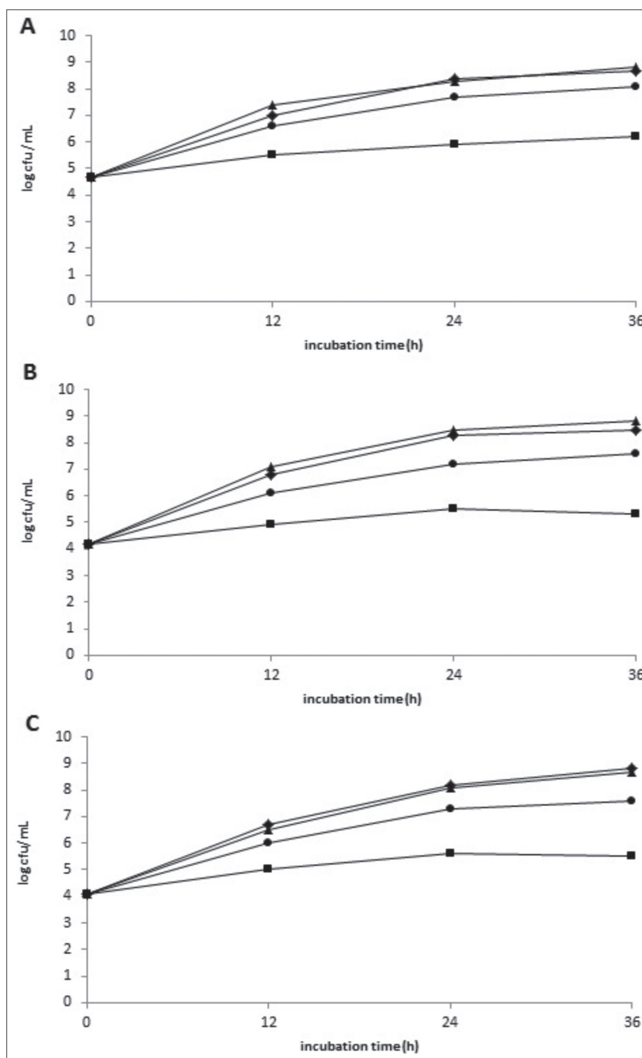

Fig 2. Plate counts of the selected *Lactobacillus* strains ((A) *L. rhamnosus*; (B) *L. plantarum*; (C) *L. brevis*) after 12, 24 and 36 h incubation in skim milk with free LA (2000 µg/ml) and Tween 80 ((▲) 0 mg/ml; (●) 5 mg/ml; (♦) 20 mg/ml; (■) 40 mg/ml)

Şekil 2. Serbest LA (2000 µg/ml) ve Tween 80 ((▲) 0 mg/ml; (●) 5 mg/ml; (♦) 20 mg/ml; (■) 40 mg/ml) içeren yağsız sütte *Lactobacillus* suşlarının ((A) *L. rhamnosus*; (B) *L. plantarum*; (C) *L. brevis*) 12, 24 ve 36 saat inkübasyonu sonunda sayısı

strains did not change significantly (P>0.05) when Tween 80 concentration increased from 20 to 40 mg/ml.

DISCUSSION

The antimicrobial effect of free LA against Gram positive bacteria, including Lactobacillus strains, has been previously documented in several studies [13]. Lin et al.[14] reported that growth of six lactic acid bacteria in skim milk was negatively affected by addition of free LA (1000 and 5000 µg/ml). In study of Jiang et al.[15], a negative correlation was found between the growth of CLA-producing strains of Propionibacterium and the LA concentration in MRS broth. Similarly, Boyaval et al.[16] reported that 100 µg/ml of LA had a strong bacteriostatic effect on the growth and metabolism of *Propionibacterium freudenreichii* ssp. shermanii in a lactate-yeast extract based medium. In study of Talon et al.[17], plate counts of the six lactic acid bacteria in the culture media with 500 µg/ml of LA remained at their initial levels of inoculation or decreased during the incubation.

The possible antibacterial mechanism for free fatty acids was postulated to proceed via disruption of the bacterial cell membrane resulting in a change in membrane permeability. Cell membranes have long been regarded as a primary target for the antimicrobial effect of free fatty acids. Exposure to free fatty acids causes the detrimental effects on bacterial cells such as formation transient or permanent pores, leakage of intracellular materials, inhibition of enzyme activity, impairment of nutrient uptake and the generation of toxic per-oxidation [13]. Generally, Gram-positive bacteria are more sensitive to long chain un-saturated free fatty acids than Gram-negatives [18].

In accordance with results of present study, neutralization of the antimicrobial properties of free fatty acids by Tween 80 was previously evidenced by several authors [16]. Li et al.[19] reported that Tween 80 can act as protective agent against leakage of intracellular materials. However, the precise mechanism of the Tween 80 in neutralizing the antibacterial activity of free fatty acids is still unclear. In study of Rainio et al.[8], negative effects of free LA (1000 µg/ml) on growth rate and CLA production of *P. freudenreichii* ssp. shermanii could be tolerated by the addition of 15 mg/ml Tween 80. Van Nieuwenhove et al.[7] reported that CLA production by lactic acid bacteria was positively affected by neutralization of negative effects of LA on bacterial metabolism. Wang et al.[10] suggested that Tween 80 in the formulation of MRS broth (1 mg/ml) reduces the antimicrobial effect of fatty acids against CLA-producing bacteria and thus stimulates their growth and CLA conversation.

In conclusion, the results obtained from this study suggest that addition of Tween 80 to milk together with free LA is a very feasible strategy to increase CLA conversion by lactic acid bacteria. Thus, the CLA content of fermented dairy products can be enhanced considerable by the use of CLA-producing lactic acid bacteria. Alternatively, a concentrated form of CLA obtained from synthetic media or milk can be used as a food additive. Further studies are still required to determine the commercialization potential of this strategy in terms of applicability and acceptable daily intake of Tween 80.

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REFERENCES

1. Gonçalves DC, Lira FS, Carnevali LC, Rosa JC, Pimentel GD, Seelaender M: Conjugated linoleic acid: Good or bad nutrient. *Diabetol Metab Syndr*, 2, 62, 2010. DOI: 10.1186/1758-5996-2-62

2. Abd El-Salam MH, El-Shafei K, Sharaf OM, Effat BA, Asem FM, El-Aasar M: Screening of some potentially probiotic lactic acid bacteria for their ability to synthesis conjugated linoleic acid. *Int J Dairy Technol*, 63, 62-69, 2010. DOI: 10.1111/j.1471-0307.2009.00541.x

3. Lin TY, Lee F: Conjugated linoleic acid as affected by food source and processing. *Sci Agric*, 45, 284-295, 1997.

4. Ha Y, Grimm N, Pariza M: Newly recognized anticarcinogenic fatty acids: Identification and quantification in natural and processed cheeses. *J Agric Food Chem*, 37, 75-81, 1989. DOI: 10.1021/jf00085a018

5. Ip C, Singh M, Thompson HJ, Scimeca JA: Conjugated linoleic-acid suppresses mammary carcinogenesis and proliferative activity of the mammary-gland in the rat. *Cancer Res*, 54, 1212–1215, 1994,

6. Barrett E, Ross RP, Fitzgerald GF, Stanton C: Rapid screening method for analyzing the conjugated linoleic acid production capabilities of bacterial cultures. *Appl Environ Microbiol*, 73, 2333-2337, 2007. DOI: 10.1128/AEM.01855-06

7. Van Nieuwenhove CP, Oliszewski R, González SN, Pérez Chaia AB: Conjugated linoleic acid conversion by dairy bacteria cultured in MRS broth and buffalo milk. *Lett Appl Microbiol*, 44, 467-474, 2007. DOI: 10.1111/j.1472-765X.2007.02135.x

8. Rainio A, Vahvaselkä M, Suomalainen T, Laakso S: Reduction of linoleic acid inhibition in production of conjugated linoleic acid by *Propionibacterium freudenreichii ssp. shermanii*. *Can J Microbiol*, 47, 735-740, 2001. DOI: 10.1139/w01-073

9. Ema M, Hara H, Matsumoto M, Hirata-Koizumi M, Hirose A, Kamata E: Evaluation of developmental neurotoxicity of polysorbate 80 in rats. *Reprod Toxicol*, 25, 89-99, 2008. DOI: 10.1016/j.reprotox.2007.08.003

10. Wang LM, Lv JP, Chu ZQ, Cui YY, Ren XH: Production of conjugated linoleic acid by *Propionibacterium freudenreichii*. *Food Chem*, 103, 313-318, 2007. DOI: 10.1016/j.foodchem.2006.07.065

11. Rodríguez-Alcalá LM, Braga T, Xavier Malcata F, Gomes A, Fontecha J: Quantitative and qualitative determination of CLA produced by *Bifidobacterium* and lactic acid bacteria by combining spectrophotometric and Ag+-HPLC techniques. *Food Chem*, 125, 1373-1378, 2011. DOI: 10.1016/j.foodchem.2010.10.008

12. Ando A, Ogawa J, Kishino S, Shimizu S: Conjugated linoleic acid production from castor oil by *Lactobacillus plantarum* JCM 1551. *Enzyme Microb Technol*, 35, 40-45, 2004. DOI: 10.1016/j.enzmictec.2004.03.013

13. Desbois AP, Smith VJ: Antibacterial free fatty acids: Activities, mechanisms of action and biotechnological potential. *Appl Microbiol Biotechnol*, 85, 1629-1642, 2010. DOI: 10.1007/s00253-009-2355-3

14. Lin TY, Lin CW, Lee CH: Conjugated linoleic acid concentration as affected by lactic cultures and added linoleic acid. *Food Chem*, 67, 1-5, 1999. DOI: 10.1016/S0308-8146(99)00077-1

15. Jiang J, Björck L, Fondén R: Production of conjugated linoleic acid by dairy starter cultures. *J Appl Microbiol*, 85, 95-102, 1998. DOI: 10.1046/j.1365-2672.1998.00481.x

16. Boyaval P, Corre C, Dupuis C, Roussel E: Effects of free fatty acids on propionic acid bacteria. *Lait*, 75, 17-29, 1995. DOI: 10.1051/lait:199512

17. Talon R, Walter D, Montel MC: Growth and effect of staphylococci and lactic acid bacteria on unsaturated free fatty acids. *Meat Sci*, 54, 41-47, 2000. DOI: 10.1016/S0309-1740(99)00068-6

18. Galbraith H, Miller TB, Paton AM, Thompson JK: Antibacterial activity of long chain fatty acids and the reversal with calcium, magnesium, ergocalciferol and cholesterol. *J Appl Bacteriol*, 34, 803-813, 1971. DOI: 10.1111/j.1365-2672.1971.tb01019.x

19. Li JY, Zhang LW, Du M, Han X, Yi HX, Guo CF, Zhang YC, Luo X, Zhang YH, Shan YJ, Hou AJ: Effect of Tween series on growth and cis-9, trans-11 conjugated linoleic acid production of *Lactobacillus acidophilus* F0221 in the presence of bile salts. *Int J Mol Sci*, 12, 9138-9154, 2011. DOI: 10.3390/ijms12129138

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Short Communication

The Effect of Different Storage Temperatures and Times on the Viability of *Helicobacter pullorum* [1]

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[1] Department of Microbiology, Faculty of Veterinary Medicine, Istanbul University, TR-34320 Avcılar, Istanbul - TURKEY

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Abstract

In the experimental study, four chicks were inoculated orally with 1x10^9 cfu/ml of *H. pullorum*, ATCC 51801 strain. Stool samples from each animal were collected and a pool of faecal content has been achieved. Totally 77 swabs taken from the sample pool were stored at -18°C, +4°C, + 25°C, respectively and were examined by culture every hour within first 24 h. In conclusion, the survival time of *H. pullorum* was determined as 11 hours at +25°C, 14 hours at +4°C and 16 hours at -18°C.

Keywords: *H. pullorum, Viability, Chicks, Feces*

Helicobacter pullorum'un Değişik Isılarda ve Sürelerde Dayanıklılığının Belirlenmesi

Özet

Bu deneysel çalışmada, 4 adet civcive 1x10^9 cfu/ml *H. pullorum*, ATCC 51801 suşu oral yolla inokule edildi. Dışkı örnekleri toplandı ve dışkı havuzu oluşturuldu. Toplamda dışkı havuzundan alınan 77 svab -18°C, +4°C ve + 20-25°C'de saklandı ve 24 saat içinde her saat başı kültürel metot ile incelendi. Sonuç olarak, *H. pullorum*'un dayanıklılığı +25°C'de 11 saat, +4°C'de 14 saat ve -18°C'de 16 saat olarak belirlendi.

Anahtar sözcükler: *H. pullorum, Dayanıklılık, Civciv, Dışkı*

INTRODUCTION

Helicobacter pullorum, first described by Stanley *et al.*[1], has been associated with hepatobiliary and gastrointestinal diseases in chickens and in human beings. This putative enterohepatic pathogen, or its DNA, has been detected in the intestinal contents of chickens [2-4], guinea fowl [5], turkeys [6] and a psittacine bird [7]. In humans, *H. pullorum* has been isolated from feces [8,9]. Although it has not yet been clearly proven that *H. pullorum* has zoonotic potential. As poultry carcasses can be contaminated by *H. pullorum* [2] during slaughtering, the potential role of these bacteria as an emerging foodborne human pathogen needs to be considered [4].

Like other *Helicobacter* species, *H. pullorum* is fastidious bacterial pathogen difficult to isolate [10,11]. Although it has been mentioned by many researchers that samples for isolation procedures should be send to the laboratories within minimum time period and with cold chain [3,4], there is no reported study about transportation procedures or limitations of the samples. Therefore, examining the viability of *H. pullorum* in different temperatures and time periods was decided. Several authors reported that different atmospheric environments, incubation period and incubation temperatures had been examined for the isolation of *H. pullorum* [1,3,4,10]. Culture is time consuming and difficult, and fresh samples are needed for this purpose, therefore PCR is also recommended to use along with culture [1,12].

The aim of this study is to examine the viability of *Helicobacter pullorum* in different temperatures and time periods.

İletişim (Correspondence)
+90 212 4737070/17360
beren@istanbul.edu.tr

MATERIAL and METHODS

This study was approved by the Animal Care Committee of Istanbul University, Faculty of Veterinary Medicine, Approval no: 2012/49.

In the experimental study, four 19-day-old chicks, which were *determined H. pullorum* negative by cultural methods and PCR, were *used*. The chicks were inoculated orally with 1x10^9 cfu/ml of *Helicobacter pullorum*, ATCC 51801 strain and clinically examined on an hourly basis. After 24 h, stool samples from each animal were collected and examined by culture and PCR analyses for confirmation of the experimental infection. It was confirmed with detection of *H. pullorum* by culture described by Ceelen *et al.*[3] and Zanoni *et al.*[4] and by PCR according to Stanley *et al.*[1] and Ceelen *et al.*[3].

After confirmation of the experimental infection, cecal and colon contents of each animal were collected followed after the necropsy of the animals and a pool of faecal content have been achieved [13].

Totally 77 swab samples were collected with Stuart agar gel medium transport swabs (Copan Diagnostics) from the sample pool. This phase regarded as hour "0". Two of the swab samples were used for isolation and confirmation of *H. pullorum* occurrence by PCR and the remaining swabs were divided into three groups consisting of 25 swabs. Sample groups were stored at -18°C, +4°C, +25°C, respectively and those samples were examined by culture every hour within first 24 h and the last inoculation onto medium was performed at the 48th h.

The swabs were inoculated into Brain Heart Infusion Broth (HiMedia) supplemented with inactivated horse serum and glucose (Sigma Chemical Co). Dilutions were inoculated onto Blood Agar Base No 2 (Oxoid) with 5% sheep blood using the modified filter technique of Steele and McDermott [14]. 100 µl of dilutions was spread onto 47 mm large in diameter and 0.65 µm pore size sterile filter (Sartorius) that has been previously placed on the Blood Agar Base No 2 surface. The plate was incubated at 37°C for 1 h in a microaerobic atmosphere. After incubation, the filter was removed and the agar surface streaked with a sterilized loop. Then plates were incubated under microaerophilic condition using gas generating kit (CampyGen, Oxoid) at 37°C for 7 days and examined daily for microbial growth. Very small, greyish-white, alpha hemolytic colonies were selected and purified on a Blood Agar Base No 2 plate. The biochemical characterization of the isolates was performed using the following tests: catalase, cytochrome oxidase, urease, hippurate and indoxyl acetate hydrolysis, nitrate reduction, hydrogen sulphide production in triple sugar iron agar, growth in the presence of 3.5% (w/v) NaCl, 1% (w/v) glycine and 1% (w/v) bile, growth at 25°C, 42°C and on MacConkey Agar ((BBL, Becton, Dickinson and Company), susceptibility to nalidixic acid and cephalotin (BBL, Becton, Dickinson and Company) [3,4].

RESULTS

Growth and biochemical characteristics of *H. pullorum* were shown in *Table 1*.

H. pullorum was isolated from the swab samples taken at hour "0" and occurrence of the agent confirmed by PCR from the sample taken at the same hour. The survival duration of the microorganism were 11 h, 14 h and 16 h at 25°C, +4°C and -18°C, respectively.

DISCUSSION

In this study, isolation medium, atmospheric conditions, incubation time, and incubation temperature provided in optimal conditions for recovery of *H. pullorum* from the experimentally infected chicks' cecal and colon contents. The effects of different storage temperatures and times on the viability of *H. pullorum* which were stored in their natural environment such as feces were determined. The survival time of *H. pullorum* was determined as 11 h at +25°C, 14 h at +4°C and 16 h at -18°C.

Although it has been considered that detection of the number of bacteria in the each inoculated medium would

Table 1. *Growth and biochemical characteristics of H. pullorum*
Tablo 1. *H. pullorum'un üreme ve biyokimyasal özellikleri*

Growth and Biochemical Characteristics	*H. pullorum*
Gram-negative	+
Oxidase	+
Catalase	+
Urease	-
Hippurate hydrolysis	+
Indoxyl acetate hydrolysis	-
Nitrate reduction	+
Trace H_2S in TSI	+
Growth on Media Containing	
1% (w/v) glycine	-
1% (w/v) bile	+
3.5% (w/v) NaCl	-
Growth at	
42°C (mO^2)	+
25°C (mO^2)	-
Antimicrobial Susceptibility	
Nalidixic acid (30 µg)	S
Cephalotin (30 µg)	R

***TSI** = Triple sugar iron, **NaCl** = Sodium chloride, **mO²** = microaerobic atmosphere; **S** = Susceptible, **R** = Resistant*

contribute the results of the study, due to the difficulties of isolation procedures this step has not been performed. However as a result of this study, it is revealed that the samples should be processed without any storage time, and in a case if they need to be stored, -18°C would be better instead of higher temperatures like +4°C or +25°C.

Different ratios were determined in the studies on the presence and prevalence of *H. pullorum* even few studies have been conducted worldwide [2-4,8,12,15,16]. The authors suggested that it is difficult to compare those results due to the different diagnostic methods and different kinds of samples, such as frozen versus fresh samples that have been used in each study [12]. Ceelen *et al.*[3] examined the occurrence of *H. pullorum* in broilers from samples stored at -20°C and -70°C, by using both polymerase chain reaction (PCR) and isolation, respectively. The researchers suggested that their low isolation rate of *H. pullorum* from cecal samples might have been the result of examining frozen, as opposed to fresh, samples. Manfreda *et al.*[12] reported that the relatively high isolation rate (78.47%) in their study compared with other studies [2] might have been due to the usage of fresh materials instead of frozen samples.

H. pullorum were not isolated from any of the samples while *H. pullorum* DNA was detected in 55.21% (53/96) by PCR in a study in which the presence of *H. pullorum* in caecum and colon of 96 broiler chickens from different commercial slaughtering facilities were examined [15]. The authors indicated that, some of the samples could be collected only by authorized veterinarians instead of themselves, therefore the delivery time of the samples to the veterinarians were not clear, and those samples were transferred to the laboratory within 6 hours on 4°C. In addition to this situation the processing of the sample has taken approximately one hour in the laboratory. The researchers suggest that the reason of lack of isolation in the study might have been due to the time period passed between sampling and culture.

When the survival time of the bacteria in feces is considered, it can be suggested that the time period for the transmission of the agent via contaminated poultry meat is relatively limited. It has been revealed that, studies such as detailed examination of occurrence of *H. pullorum* on the retail poultry products, determination of viability period of the agent on poultry carcasses would be helpful for understanding the transmission of the bacteria through human and designating the bacteria as a food-borne pathogen. The role of *H. pullorum* among the food-borne infections will be better understood by further epidemiological studies based on the current study.

REFERENCES

1. Stanley J, Linton D, Burnens AP, Dewhirst FE, On SLW, Porter A: *Helicobacter pullorum* sp. nov.-genotype and phenotype of a new species isolated from poultry and from human patients with gastroenteritis. *Microbiology,* 140, 3441-3449, 1994. DOI: 10.1099/13500872-140-12-3441

2. Atabay HI, Corry JEL, On SLW: Identification of unusual Campylobacter-like isolates from poultry products as *Helicobacter pullorum*. *J Appl Microbiol*, 84, 1017-1024, 1998. DOI: 10.1046/j.1365-2672.1998.00438.x

3. Ceelen LM, Decostere A, Van den Bulck K, Stephen LW, Margo B, Ducatelle R, Haesebrouck F: *Helicobacter pullorum* in chickens, Belgium. *Emerg Infect Dis,* 12, 263-267, 2006.

4. Zanoni RG, Rossi M, Giacomucci D, Sanguinetti V, Manfreda G: Occurence and antibiotic susceptibility of *Helicobacter pullorum* from broiler chickens and commercial laying hens in Italy. *Int J Food Microbiol*, 116, 168-173, 2007. DOI: 10.1016/j.ijfoodmicro.2006.12.007

5. Nebbia P, Tramuta C, Ortoffi M, Bert E, Cerruti Sola S, Robino P: Identification of enteric *Helicobacter* in avian species. *Schweizer Archiv für Tierheilkunde*, 149, 403-407, 2007. DOI: 10.1024/0036-7281.149.9.403

6. Zanoni RG, Piva S, Rossi M, Pasquali F, Lucchi A, De Cesare A, Manfreda G: Occurrence of *Helicobacter pullorum* in turkeys. *Vet Microbiol*, 149, 492-496, 2011. DOI: 10.1016/j.vetmic.2010.11.013

7. Ceelen LM, Decostere A, Martel A, Pasmans F, Haesebrouck F: First report of *Helicobacter pullorum* in the faece of a diarrhoeic psittacine bird *(Psephotus haematogaster)*. *Vet Rec*, 159, 389-390, 2006. DOI: 10.1136/vr.159.12.389

8. Burnens AP, Stanley J, Nicolet J: Possible association of *Helicobacter pullorum* with lesions of vibrionic hepatitis in poultry. **In,** Campylobacters, Helicobacters and Related Organism. 291-293, Springer US, 1996.

9. Steinbrueckner BG, Hearter K, Pelz S, Weiner JA, Rump W, Deissler S, Bereswill M: Isolation of *Helicobacter pullorum* from patients with enteritis. *Scand J Infect Dis*, 29, 315-318, 1997. DOI: 10.3109/00365549709019053

10. Andersen LP: New *Helicobacter* species in humans. *Dig Dis,* 19, 112-115, 2001. DOI: 10.1159/000050664

11. Taneera J, Moran AP, Hynes SO, Nilsson HO, Al-Soud W, Wadstrom T: Influence of activated charcoal, porcine gastric mucin and beta-cyclodextrin on the morphology and growth of intestinal and gastric *Helicobacter* spp.. *Microbiology,* 148, 677-684, 2002. DOI: 10.1099/00221287-148-3-677

12. Manfreda G, Rossi M, Sanguinetti V, Gavioli R, Lozito P, De Cesare A, Zanoni RG: Prevalence of *Helicobacter pullorum* in broiler chickens reared in intensive and extensive farms. *World's Poultry Sci J,* 62, 551-552, 2006.

13. Eleroğlu H, Yıldırım A, Şekeroğlu A, Duman M: Comparison of the growth performance and carcass characteristics of two slow-growing broiler genotypes fed diets supplemented with dry oregano (*Origanum vulgare* L.) or lemon balm (*Melissa officinalis* L.) leaves under the organic system. *Kafkas Univ Vet Fak Derg*, *20*, 49-58, 2014. DOI: 10.9775/kvfd.2013.9444

14. Steele TW, McDermott SN: The use of membrane filters applied directly to the surface of agar plates for the isolation of *Campylobacter jejuni* from feces. *Pathology*, 16, 263-265, 1984.

15. Basaran-Kahraman B, Ak S: Investigation of *Helicobacter pullorum* occurence in chicken in Marmara region of Turkey. *J Fac Vet Med Istanbul Univ*, 39, 63-66, 2012.

16. Borges V, Santos A, Correia CB, Saraiva M, Ménard A, Vieira L, Sampaio DA, Pinheiro M, Gomes JP, Oleastro M: *Helicobacter pullorum* isolated from fresh chicken meat: Antibiotic resistance and genomic traits of an emerging foodborne pathogen. *Appl Environ Microbiol*, 81, 8155-8163, 2015. DOI: 10.1128/AEM.02394-15

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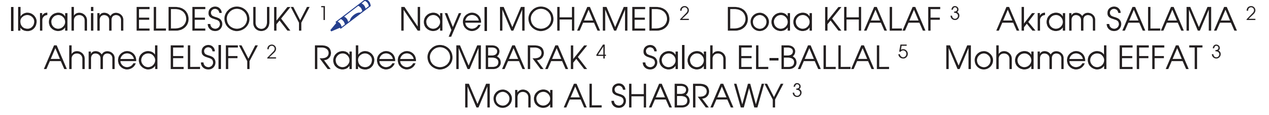
Short Communication

Candida Mastitis in Dairy Cattle with Molecular Detection *of Candida albicans*


Ibrahim ELDESOUKY [1] Nayel MOHAMED [2] Doaa KHALAF [3] Akram SALAMA [2]
Ahmed ELSIFY [2] Rabee OMBARAK [4] Salah EL-BALLAL [5] Mohamed EFFAT [3]
Mona AL SHABRAWY [3]

[1] Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Kafrelsheikh University 33516, EGYPT
[2] Department of Animal Medicine and Infectious Diseases, Faculty of Veterinary Medicine, University of Sadat City 32897, EGYPT
[3] Department of Microbiology and Immunology, National Research Centre, Dokki, Giza 12611, EGYPT
[4] Food hygiene and control department, Faculty of veterinary medicine, University of Sadat City32897, EGYPT
[5] Department of Pathology, Faculty of Veterinary Medicine, University of Sadat City 32897, EGYPT

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Abstract

This study focused on determining the prevalence of *Candida* species involved in dairy cattle mastitis with molecular detection of *Candida albicans*. A total of 150 milk samples were collected from dairy cattle showing clinical mastitis. Isolation and identification of organisms through phenotypical and physiological criteria on different media were performed as well as molecular identification of *C. albicans*. Fourty one isolates of *Candida* species were recovered with a prevalence of 27.3%. *C. albicans* was the dominating species (29.3%). Out of 12 strains phenotypically identified as *C. albicans*, 8 were confirmed by PCR using species specific primer for the 26S rRNA gene of *C. albicans*. A specific virulence determinant Phospholipase B1 gene was detected in all molecularly identified *C. albicans* isolates. This study has clearly shown the prevalence of *Candida* mastitis and providing a new attractive diagnostic molecular tool for mycotic mastitis caused by *C. albicans*.

Keywords: *Candida, Cattle, Mastitis*

Candida albicans'ın Moleküler Tespiti İle Sütçü İneklerde *Candida* Mastitisi

Özet

Bu çalışmada *Candida albicans*'ın moleküler yöntemle belirlenmesi suretiyle sütçü inek mastitlerinde *Candida* türlerinin prevalansının belirlenmesi amaçlanmıştır. Klinik olarak mastitis semptomları gösteren toplam 150 adet sütçü ineğe ait süt örnekleri toplandı. *C. albicans*'ın moleküler yöntemle belirlenmesinin yanında organizmaların fenotipik ve değişik kültürlerdeki fizyolojik kriterleri baz alınarak izolasyon ve identifikasyonu yapıldı. *Candida* türlerinin 41 izolatı %27.3 prevalans oranı ile elde edildi. *C. albicans* en çok belirlenen tür olarak kaydedildi (%29.3). Fenotipik olarak *C. albicans* olduğu belirlenen 12 adetten 8'i 26S rRNA geni için spesifik primer çifti kullanılarak yapılan PCR ile onaylandı. Moleküler yöntemle *C. albicans* olduğu belirlenen tüm izolatlarda spesifik virulans olarak Phospholipase B1 geni tespit edildi. Bu çalışma ile Candida mastitislerinin prevalansı belirlenmiş ve *C. albicans* kaynaklı mikotik mastitislerde diagnostik moleküler yöntemin kullanılabilirliği gösterilmiştir.

Anahtar sözcükler: *Candida, Sığır, Mastitis*

INTRODUCTION

Cattle mastitis is regarded as the most prevalent and economically important disease on all continents, with annual great losses in the dairy industry world-wide [1]. A wide variety of microorganisms have been found as etiological agents of mastitis in cattle. In addition to bacterial agents, several other groups of microorganisms such as fungi and algae from *Prototheca* genus capable of inducing an inflammatory process in

İletişim (Correspondence)
+81 85 2306487; Fax: +81 58 2306489
ibrahim543@yahoo.com

the udder [2]. Yeasts are groups of unicellular opportunistic organisms, ever present in the natural surroundings of dairy cattle and are normal inhabitants of the skin of the udder and teats, in which they exist in low numbers [3].

Fungal mastitis has been described as related to treatment directed against other pathogens using contaminated syringes, cannulas, or contaminated antibiotic preparations [4]. In recent years, predominance of the genus *Candida* was reported in various studies of cattle mastitis [5,6]. Several species of *Candida* have been implicated in subclinical and clinical mastitis [6]. Identification of *Candida albicans* by conventional methods based on morphological features and reproductive structures may take days to weeks to develop in culture, and evaluation of these characteristics requires expertise in mycology [7]. PCR-based detection of fungal DNA sequences can be rapid, sensitive and specific [8]. Phospholipase B1 (PLB1) considers one of the main virulence factor secreted by *C. albicans*. This enzyme digests the phospholipid constituents of host cell membrane leading to cell lysis with alterations of surface characteristics that facilitate adherence and subsequent infection [9]. Few reports exist on the prevalence of fungal mastitis in Egyptian cattle specially those caused by *Candida* species. Therefore, the aim of the present study was to determine the prevalence of *Candida* species in dairy cattle suffering from mastitis, with molecular based identification of *C. albicans* isolates.

MATERIAL and METHODS

Sampling, Isolation And Identification Procedures

A total of 150 milk samples were collected from dairy cattle showing clinical mastitis from 3 different dairy cattle farms at Minufiya Governorate, Egypt. The study was complied with the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, University of Sadat city. Milk samples (100 µl) were inoculated in Sabouraud Dextrose Broth and then incubated at 37°C for 24 h. Thereafter, 50 µl of each broth cultures was plated on Sabouraud Dextrose Agar (SDA) with Chloramphenicol (0.05 mg/ml) and chromogenic agar (Pronadisa Laboratorios Conda, S.A.).

The plates were incubated at 37°C for 72 hs. Yeast identification was performed based on the observed morphological characteristics, like formation of Chlamydoconidia, pseudohyphae and germ tube development. Additional characteristics taken into account include; growth at 45°C, growth in the presence of 0.1% cyclohexamide (Sigma TM), urea hydrolysis, acidic pH tolerance, and carbohydrates assimilation and/or fermentation (glucose, galactose, lactose, maltose, xylose, and sucrose), accordingly to the methodology described by Barnett et al.[10].

Molecular Identification of C. albicans

Total chromosomal DNA was isolated from *C. albicans* (Analytik jena kit, according to the manufacturer's instructions) and subjected to PCR amplification. The primer set was previously designed as species universal primer specific for the amplification of a fragment of 175 bp specific to the 26S rRNA of *C. albicans* [11]. DNA samples were amplified in a total of 25 µl of the following reaction mixture: 12.5 µl DreamTaq TM Green Master Mix (2X) (Thermo Fisher Scientific, Inc), 1 µl of each primer (10 µM) (Biobasic inc. company, Canada), 5 µl template DNA (1 µg) and 5.5 µl nuclease-free water. Amplification was carried out in the thermal cycler according to the following protocol: initial denaturation at 95°C for 5 min, followed by 35 cycles of 94°C for 1 min, 52°C for 1 min, and 72°C for 1 min, with a final extension at 72°C for 5 min. The amplified PCR product was electrophoresed on a 1.5% agarose gel in Tris-Acetate-EDTA buffer. A 100-bp DNA ladder (Invitrogen, Carlsbad, CA) was used as a molecular weight marker.

Detection of the Phospholipase B1 in C. albicans Isolates

Total chromosomal DNA isolated from *C. albicans* culture was subjected to PCR with oligonucleotide primers previously designed by Mukherjee et al.[12]. PCR amplification reaction mixtures was performed in a final volume of 25 µl consisted of 12.5 µl DreamTaq TM Green Master Mix (2X) (Thermo Fisher Scientific, Inc), 1 µl of each primer (10 µM) (Biobasic inc. company, Canada), 5.5 µl nuclease free water and 5 µl template DNA (1 µg). The PCR cycling conditions consisted of initial denaturation at 94°C for 5 min, followed by 35 cycles each of denaturation at 94°C for 1 min, annealing at 47°C for 1 min, and extension at 72°C for 1 min, followed by final extension at 72°C for 5 min. The amplified PCR product was electrophoresed on a 1.5% agarose gel in Tris-Acetate-EDTA buffer.

RESULTS

Prevalence of Candida Species in Dairy Cattle with Mastitis

Out of 150 milk samples analyzed from cattle with mastitis, 41 *Candida* isolates were recovered (27.3%). Based on cultural and morphological and biochemical characteristics, six different species of *Candida* were identified. *C. albicans* was the predominant one (29.3%), followed by *C. tropicalis, C. guilliermondii (*19.5%, each), *C. glabrata* (14.6%), *C. krusei* (12.2%) and *C. kefyr* (4.9%) *(Table 1)*.

Molecular Identification of C. albicans

In this study, PCR based on species specific universal oligonucleotides primers was used to confirm phenotypic identification of *C. albicans*. Out of 12 isolates presumptively

Table 1. *Candida species isolated from cattle with mastitis*
Tablo 1. *Mastitisli ineklerden izole edilen Candida türleri*

Candida Isolates	**No**	**%**
C. albicans	12	29.3
C. tropicalis	8	19.5
C. guilliermondii	8	19.5
C. glabrata	6	14.6
C. krusei	5	12.2
C. kefyr	2	4.9
Total	41	100

high incidence of mycotic mastitis especially that caused by *Candida* species has been noticed recently [4]. In the present study, the percentage of *Candida* isolation was 27.3%. Similar results were obtained by Zaragosa et al.[13] in Mexico (25.7%) and Geraldo et al.[14] in Brazil (29.35%). However, many studies conducted in other countries revealed higher frequency of *Candida* mastitis (79.4% in China and 71.9% in Algeria) respectively [5,6]. A higher percentage of isolation of *Candida* species from clinical cases reveal that the incidence of yeast mastitis is increasing which may be due to unhygienic conditions. Moreover, the development of antibiotic resistance in the bacteria, which prolongs the course of treatment favoring chances for the fungal species such as *Candida* to infect as secondary invader. The present study showed a clear overall predominance of *C. albicans* among the *Candida* species. This finding confirms a tendency seen in another study conducted in India [15], but contrary the other report that described low prevalence of *C. albicans* in mastitic cattle [5]. The present study revealed that *C. tropicalis* and *C. guilliermondii* were the second most frequent species isolated while *C. krusei*, and *C. kefyr* were less frequently isolated, in contrast to some reports [5,6].

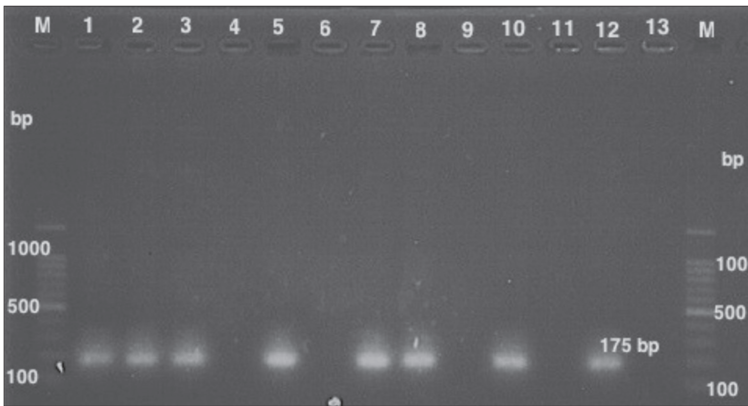

Fig 1. PCR test for detection of *C. albicans*. Agarose gel electrophoresis of amplified PCR products (175bp) by using species specific primer of *C. albicans*. Lane M: 100 bp ladders molecular size marker, lanes 1-3, 5, 7-8, 10 and 12 represent *C. albicans*, lanes 4, 6, 9 and 11 represent non *C. albicans* isolates, lane 13 control negative

Şekil 1. PCR ile *C. albicans*'ın belirlenmesi. *C. albicans* için tür spesifik primerler kullanarak PCR ürünlerinin (175bp) amplifikasyonu ve agaroz jel elektroforeziste gösterilmesi. M: 100 bp moleküler uzunluk markırı, 1-3, 5, 7-8, 10 ve 12: *C. albicans* pozitif örnekler, 4, 6, 9 ve 11: *C. albicans* negatif örnekler, 13: negatif kontrol

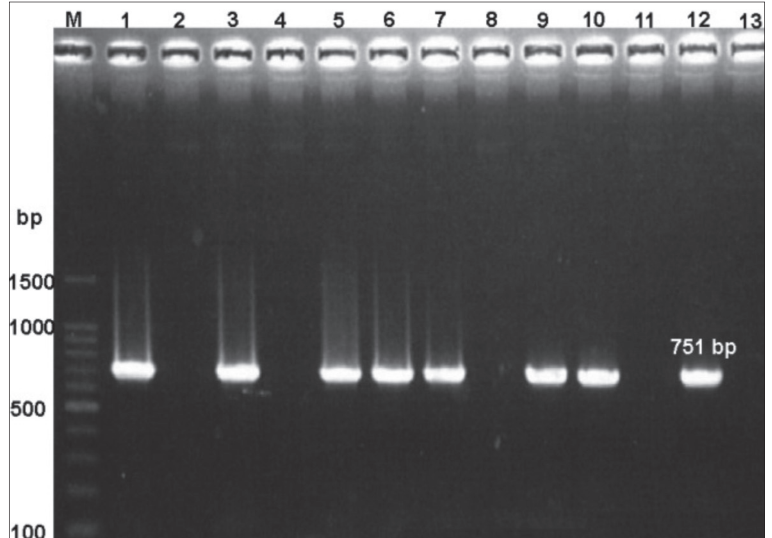

Fig 2. PCR test for detection of Phospholipase B1 in *C. albicans* isolates. Agarose gel electrophoresis of amplified PCR products (751bp) by using specific primer of Phospholipase B1 gene. Lane M: 100 bp ladders molecular size marker, lanes 1, 3, 5-7, 9-10 and 12 represent *C. albicans* isolates positive for Phospholipase B1. Lanes 2, 4, 8 and 11 represent non *C. albicans*. Lane 13 control negative

Şekil 2. PCR ile *C. albicans* izolatlarında Phospholipase B1'in belirlenmesi. Phospholipase B1 genine spesifik primerler kullanarak PCR ürünlerinin (751bp) amplifikasyonu ve agaroz jel elektroforeziste gösterilmesi. M: 100 bp moleküler uzunluk markırı, 1, 3, 5-7, 9-10 ve 12: Phospholipase B1 pozitif *C. albicans* izolatları, 2, 4, 8 ve 11: *C. albicans* negatif, 13: negatif kontrol

identified by phenotypic methods as *C. albicans,* 8 isolates yield the predicted 175 bp DNA fragments, while the other four isolates were negative *(Fig. 1)*. Specific PCR was carried out on molecularly identified *C. albicans* species to detect Phospholipase B1 gene as a virulence determinant. The PCR produced a DNA fragment of predicted size (751 bp) in all molecularly identified *C. albicans* isolates *(Fig. 2)*.

DISCUSSION

The prevalence of mastitis related to yeasts is usually low as compared with other agents of mastitis. However,

It is known that the phenotypic characterization of yeasts can lead to errors due to the fact that several species present similarities in their morphologies and biochemical/physiological characteristics [16]. Recently, molecular biology-based techniques have been adapted to the identification of *C. albicans* which include DNA-DNA tests using analyses with restriction endonucleases, methods based in pulsed field gel electrophoresis, DNA tests using probes. In particular, PCR has increasingly been used for *Candida* diagnosis, as it is quick, simple, specific, sensitive and reliable [11]. In the present study, 12 isolates identified morphologically as *C. albicans* were tested using species universal primer amplifying a fragment of the rRNA gene (175 bp) that could distinguish individual *C. albicans* from other *Candida* species. Out of 12 *C. albicans* isolates, 8 isolates yield DNA fragments of the predicted size. The PCR negative strain could be *C. dubliniensis* as the phenotypic methods for the identification of *Candida* species are often unable to discriminate *C. albicans* and *C. dubliniensis* [17]. *C. albicans* Phospholipase B1 gene is considered important virulence determinant, and could potentially facilitates increased penetration of fungal hyphal elements by directly damaging host cell membranes [9]. PCR was used by many authors for detection of phospholipase activity in pathogenic fungi [18]. In the current study, all isolates of genetically identified *C. albicans* were tested and identified with specific primers identical to the 751-bp region of the Phospholipase B1 gene.

The results of the present study reveal the relatively higher incidence of *Candida* mastitis especially *C. albicans* which seems to be of interest as a probable cause of mycotic mastitis in dairy cattle. It is evident that reliance on the variable expression of phenotypic characteristics of isolated yeasts can lead to inconsistent results. Phospholipase B1 provides new attractive and diagnostic targets for mycotic mastitis caused by *C. albicans.*

REFERENCES

1. Bradley A: Bovine mastitis: An evolving disease. *Vet J*, 164 , 116-128, 2002. DOI: 10.1053/tvjl.2002.0724

2. Krukowski H, Lisowski A, Rozanski P, Skorka A: Yeasts and algae isolated from cows with mastitis in the south-eastern part of Poland. *Pol J Vet Sci,* 9, 181-184, 2006.

3. de Casia dos Santos R, Marin JM: Isolation of *Candida* spp. from mastitic bovine milk in Brazil. *Mycopathologia,* 159, 251-253, 2005. DOI: 10.1007/s11046-004-2229-2

4. Dworecka-Kaszak B, Krutkiewicz A, Szopa D, Kleczkowski M, Bieganska M: High prevalence of *Candida* yeast in milk samples from cows suffering from mastitis in poland. *Sci World J,* 2012, Article ID: 196347, 2012. DOI: 10.1100/2012/196347

5. Zhou Y, Ren Y, Fan C, Shao H, Zhang Z, Mao W, Wei C, Ni H, Zhu Z, Hou X, Piao F, Cui Y: Survey of mycotic mastitis in dairy cows from Heilongjiang Province, China. *Trop Anim Health Prod,* 45, 1709-1714, 2013. DOI: 10.1007/s11250-013-0419-y

6. Ksouri S, Djebir S, Hadef Y, Benakhla A: Survey of bovine mycotic mastitis in different mammary gland statuses in two north-eastern regions of Algeria. *Mycopathologia*, 179, 327-331, 2015. DOI: 10.1007/s11046-014-9845-2

7. Pfaller M, Wenzel R: Impact of the changing epidemiology of fungal infections in the 1990s. *Eur J Clin Microbiol Infect Dis,* 11, 287-291, 1992. DOI: 10.1007/BF01962067

8. Makimura K, Murayama SY, Yamaguchi H: Detection of a wide range of medically important fungi by the polymerase chain reaction. *J Med Microbiol*, 40, 358-364, 1994. DOI: 10.1099/00222615-40-5-358

9. Ibrahim AS, Mirbod F, Filler SG, Banno Y, Cole GT, Kitajima Y, Edwards Jr JE, Nozawa Y, Ghannoum MA: Evidence implicating phospholipase as a virulence factor of *Candida albicans*. *Infect Immun,* 63, 1993-1998, 1995.

10. Barnett JA, Payne, RW, Yarrow D: Yeast, Characteristics and Identification. Third ed., Cambridge University Press, Cambridge, UK, 2000.

11. Mannarelli BM, Kurtzman CP: Rapid identification of *Candida albicans* and other human pathogenic yeasts by using short oligonucleotides in a PCR. *J Clin Microbiol*, 36, 1634-1641, 1998.

12. Mukherjee PK, Seshan KR, Leidich SD, Chandra J, Cole GT, Ghannoum MA: Reintroduction of the PLB1 gene into *Candida albicans* restores virulence *in vivo*. *Microbiol*, 147, 2585-2597, 2001. DOI: 10.1099/00221287-147-9-2585

13. Zaragoza CS, Olivares RA, Watty AE, Moctezuma Ade L, Tanaca LV: Yeasts isolation from bovine mammary glands under different mastitis status in the Mexican High Plateu. *Rev Iberoam Micol,* 28, 79-82, 2011. DOI: 10.1016/j.riam.2011.01.002

14. Costa GM, Pereira UP, Souza-Dias MA, Silva N: Yeast mastitis outbreak in a Brazilian dairy herd. *Braz J Vet Res Anim Sci,* 49, 239-243, 2012.

15. Pachauri S, Varshney P, Dash SK, Gupta MK: Involvement of fungal species in bovine mastitis in and around Mathura, India. *Vet World,* 6, 393-395, 2013. DOI: 10.5455/vetworld.2013.393-395

16. Spanamberg A, Ramos JP, Leoncini O, Alves SH, Valente P: High frequency of potentially pathogenic yeast species in goat's raw milk and creamed cheese in Southern Brazil. *Acta Sci Vet*, 37, 133-141, 2009.

17. Marinho SA, Teixeira AB, Santos OS, Cazanova RF, Ferreira CA, Cherubini K, de Oliveira SD: Identification of *Candida* spp. by phenotypic tests and PCR. *Braz J Microbiol*, 41, 286-294, 2010. DOI: 10.1590/S1517-83822010000200004

18. Ghannoum MA: Extracellular phospholipases as universal virulence factor in pathogenic fungi. *Med Mycol J*, 39, 55-59, 1998.

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Case Report

The Effect of Deslorelin Acetate in the Treatment of Persistent Urinary Incontinence after Operation of Ectopic Ureter in a Golden Retriever Bitch

Çağrı GÜLTEKİN [1] Eser ÖZGENCİL [1] Deniz SEYREK-İNTAŞ [2]

[1] Department of Surgery, Faculty of VeterinaryMedicine, University ofNearEast, Near East Boulevard, 99138, Nicosia, TURKISH REPUBLIC of NORTHERN CYPRUS
[2] Department of Surgery, Faculty of VeterinaryMedicine, University of Uludağ, TR-16059 Bursa - TURKEY

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Abstract

In this report, deslorelin acetate (suprelorin) implant, a GnRH depot analogue, was used for the first time in postoperatively observed urinary incontinence treatment of one year old female Golden Retriever puppy which came to our hospital with urinary incontinence complaint, diagnosed with left unilateral extramural ectopic ureter, treated for her ectopic ureter with ureteroneocystostomy operation and undergone ovariohysterectomy operation at the same time. In the examination at the end of eight months of postoperative period, it was observed that urinary incontinence which was thought to be due to urinary sphincter deficiency following the ectopic ureter surgery has been completely disappeared. The positive effect of deslorelin acetate on the treatment of urinary incontinence after ovariohysterectomy, which was reported in the literature in a limited number, was also found to be effective in the treatment of urinary sphincter deficiency, which was frequently observed following the ectopic ureter surgery.

Keywords: *Ureteral ectopi, Dog, Deslorelin acetate, Urinary incontinence, Urethral sphincter mechanism incompetence*

Bir Dişi Golden Retrieverda Ektopik Üreter Operasyonu Sonrası Kalıcı Üriner İnkontinensin Tedavisinde Deslorelin Asetatın Etkisi

Özet

Bu raporda üriner inkontinens şikayeti ile hastanemize getirilen ve sol unilateral ekstramural ektopik üreter tanısı konarak üreteroneo-sistostomi operasyonu ile ektopik üreteri onarılan ve aynı anda ovariohisterektomi operasyonu da geçiren 1 yaşlı, dişi Golden Retriever yavrusunda gözlenen postoperatif üriner inkontinensin tedavisinde ilk kez kullanılan GnRH depo analoğu deslorelin asetat(suprelorin) implantının etkinliği ele alındı. Postoperatif sekizinci ayda yapılan muayenelerde ektopik üreter operasyonunu takiben üriner sfinkter yetmezliği kaynaklı olduğu düşünülen üriner inkontinensin tamamen ortadan kalktığı gözlendi. Ovariohisterektomi sonrası üriner inkontinens tedavisindeki etkinliği önceden bilinen deslorelin asetatın, ektopik üreter operasyonunu takiben sıklıkla gözlenen üriner sfinkter yetmezliğinde de etkili olduğu düşünüldü.

Anahtar sözcükler: *Ektopik üreter, Köpek, Deslorelin asetat, Üriner inkontinens,Üretral sfinkter yetmezliği*

INTRODUCTION

Ectopic ureter (EU) is defined as a ureteral opening that enters the urinary tract in any location other than the trigone of the bladder and is the most common cause urinary incontinence (UI) in young dog. It has been reported that Golden Retriever, Labrador Retriever, Siberian Husky, Newfoundland, and English Bulldog breeds were to be at risk include the EUs [1]. EUs are classified as extramural (eEU) or intramural (iEU) depending on their anatomic course in dogs [2,3]. EU may be associated with other urogenital abnormalities such as hydroureter (HU), hydronephrosis (HN), urethral sphincter mechanism incompetence (USMI), vestibulovaginal malformations, hypoplastic bladder or kidney [2-4], urinary tract infections (UTI), renal dysplasia, short urethras, persistent paramesonephric remnants and vaginal septum or dual vaginas [5].

Ectopic ureters can be diagnosed with contrast radio-graphy, excretory urography, abdominal ultrasonography, cystoscopy, helical computed tomography, or a combination of these diagnostic procedures [3,6]. Contrast radiographic

İletişim (Correspondence)
+90 533 8473930
cagri.gultekin@neu.edu.tr

studies, including excretory intravenous urography (IVU) and retrograde contrast vaginourethrocystography may reveal EUs, but identification of both ureteral openings is often not possible [7,8].

Surgical procedures, which are aimed at repositioning the ureteral orifice(s) within the bladder lumen and treating primary sphincter incompetence, are necessary to successfully manage small animal patients with ectopic ureters [9]. If the ureter is extraluminal, ureteroneocystostomy must be performed by resecting the ureter as distal as possible and reimplanting in the bladder lumen [1,3].

Generally postoperative persistent UI can be managed in many cases pharmacologically with the alfa adrenergic stimulant phenylpropanolamine or estrogen compounds that increase sensitivity of alfa-adrenergic receptors in the urethra [1]. The daily supplementation of oestrogen only results in 61-65% of incontinent bitches becoming continent [10]. Unfortunately phenylpropanolamine is not completely effective in the treatment USMI [11,12]. Recently gonadotropin-releasing hormone (GnRH) analogues were shown to improve continence in ovariectomized female dogs [1,13,14].

With this report, in a Golden Retriever female dog treated with submucosal tunel technique and diagnosed left unilateral eEU by the help of plain and contrast radiography was evaluated ultrasonographic and subjectively regressing of postoperative UI with the using GnRH depot analogue (deslorelin acetate 9.4 mg; Suprelorin, Virbac, France) implante previously unused for this purpose.

CASE HISTORY

A 1-year-old female intact Golden Retriever bitch was referred to Animal Teaching Hospital, Faculty of Veterinary Medicine, Near East University-Turkish Republic of Northern Cyprus with a history of UI. The owner complained that the bitch had been incontinent since purchasing her as a 1-month-old puppy. The clinical examination was normal except for serious perivulvar dermatitis and odor. Blood, serum and urine samples were collected for hematologic, biochemical and urine analysis. Hematologic and biochemical results from serum sample showed no any abnormality. In urine analysis, it was detected that the urine was yellow and blurry, spesific gravity was 1020, pH was 8.0. There was (++) positive erytrocytes and (+++) positive leucocytes and (+) positive nitrate in the urine test strip. In microscopic evaluation of urine sample, it was noted that there were 50-100 leucocytes and 5-20 eritrocytes in microscopic field (40x magnification). UTI was diagnosed according to laboratory parameters and under controlled by the antibiotherapy with Ciprofloxacine (Cipro 500 mg, Biofarma) 11 to 15 mg/kg per day and 11 to 20 mg of Amoxicillin and 2.75 to 5 mg of Clavulanic acid (Synulox 500 mg, Zoetis) per kg of body weight every eight to twelve hours orally.

To evaluate cause of UI with plain and contrast radiography the dog was sedated. In the latero lateral (L/L) and ventro dorsal (V/D) plain radiographic evaluation; bilateral HU and mild HN were also present *(Fig. 1)*. Dilatated eEU was clearly demonstrable with L/L pelvic negative and double contrast cystography *(Fig. 2)*. The dog under the general anaesthesia was positioned in dorsal recumbency for a ventral midline laparatomy and during the abdominal explanation, it was observed that right ureter has been enters the dorsolateral caudal surface of the bladder and empties into the trigone normally but had dilatated, left ureter has been bypasses the bladder to enter urethral lumen extramurally and dilatated much more than right ureter *(Fig. 3)*. Left eEU was corrected ureteroneocystostomy operation using submucosal tunel technique *(Fig. 4)*. In the same time ovariohysterectomy operation was performed in order to prevent overpopulation and at the request of the owner.

At the end of the examination and interviews with the owner after 20 days postoperatively, UI are determined decreased by 50% compared to preoperative period. In the UI treatment is thought to be originated of USMI, GnRH depot analog deslorelin acetate (suprelorin) implant was used instead of the classical medicaments using treatmet of urinary incontinence. An implant containing 9.4 mg deslorelin (Suprelorin, Virbac, France) was administered subcutaneously in the interscapular region by using a single use applicator. The dog was evaluated radiographically,

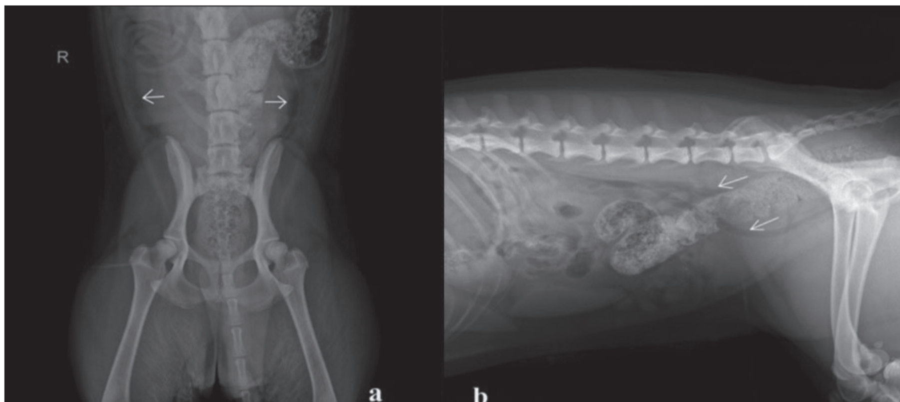


Fig 1. Ventro-dorsal *(left)* and lateral *(right)* negative contrast radiographs demonstrating both ureters as dilated, radiolucent tubular structures *(arrows)* extending from the level of the kidneys caudally with the left ureter passing the bladder ventral to the descending colon into the pelvis

Şekil 1. Ventro-dorsal *(solda)* ve laterolateral *(sağda)* negatif kontrast radyografide her iki üreter, dilate, radyolusent, tübüler yapılar *(oklar)* olarak böbreklerin seviyesinden kaudale doğru uzanmakta olup sol üreterin idrar kesesini pas geçip colon descendens'in ventralinden pelvisin içine doğru seyrettiği görülmektedir

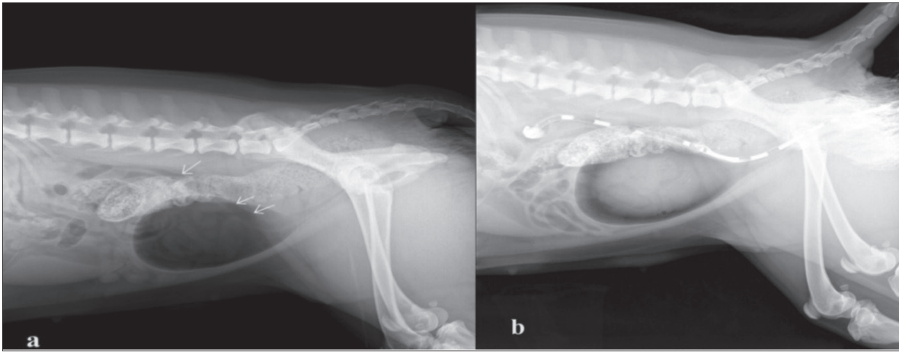


Fig 2. Dilatated eEU was clearly demonstrable with L/L pelvic negative and double contrast cystography *(arrows)*

Şekil 2. Latero lateral (L/L) pelvik negative ve çift contrast sistografide dilate eEU görüntüsü *(oklar)*

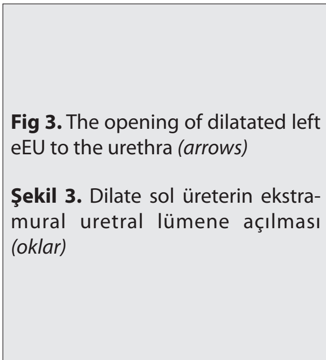
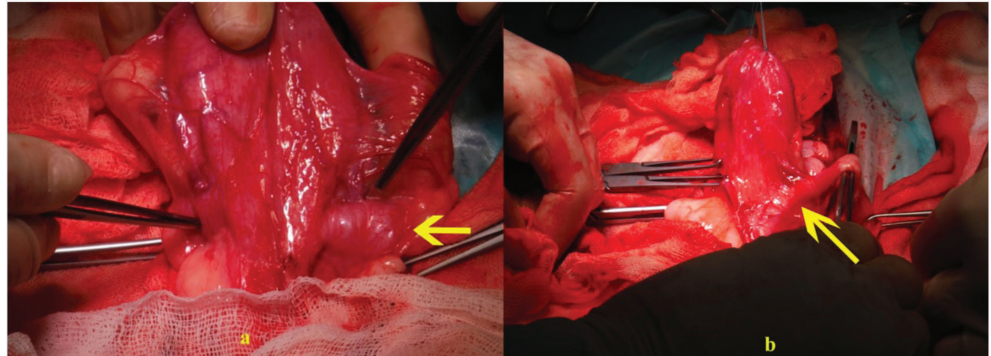


Fig 3. The opening of dilatated left eEU to the urethra *(arrows)*

Şekil 3. Dilate sol üreterin ekstramural uretral lümene açılması *(oklar)*



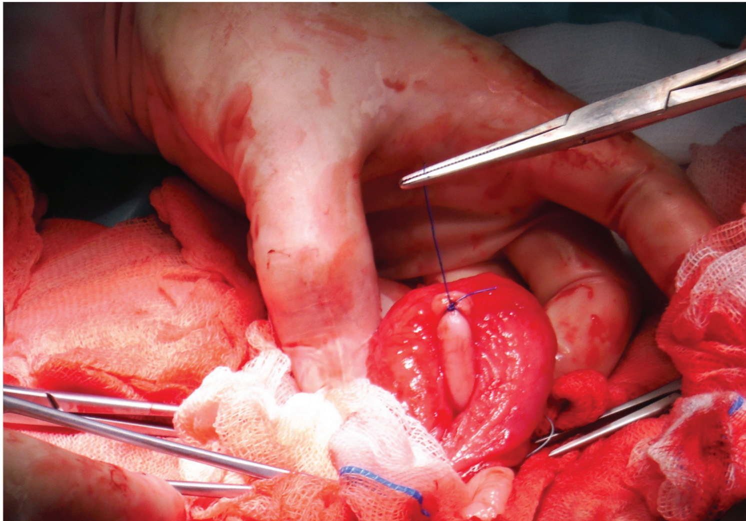


Fig 4. Left eEU was corrected ureteroneocystostomy operation using submucosal tunel technique

Şekil 4. Submukozal tünel tekniği kullanılarak sol eEU'nun üretroneosistostomi operasyonuyla düzeltilmesi

ultrasonographically and subjectivelly each month. In the first month after the implant was placed, it was observed that narrowing of the diameter of the left ureter and regressing of HN. At the end of interviews with owner, UI are determined regressing by 90% compared to before period in the second month. In the examination after 8 months, it was observed that UI completely disappeared. The positive effect of deslorelin acetate on the observed UI after OHE was also effective in the improvement of UI observed following the ectopic ureter operation.

DISCUSSION

It has known that the EUs resulting in inappropriate ureteral tube termination and malposition of the ureteral orifice [5] were heritable condition in the Golden Retriever females and males [1,7,15,16]. In the study of Reicher et al.[2] related with EU cases, they indicated early start of UI, observation of a higher incidence of left unilateral EU and that urethra shaping of urethral opening is usually more common in females than in males; and the data were consistent with the data of our case.

Suspected bladder and urethral functional anomalies, such as USMI, have been reported in 75 to 89 percent of female dogs [5] and the most common clinical sign associated with EU was UTI [2]. Dilatation of the renal pelvis and hydroureter may be associated with maldevelopment of structures or acquired infection [16]. It has been

reported that the affected female Golden puppies did not grown as rapidly nor were as active as other litter members, as they were very prone to bacterial infections [7]. Postoperative complications have been included persistent incontinence, hydronephrosis, and the risks associated with open abdominal surgery and urinary incontinence is the most common clinical sign in dogs with EU [1,3,16]. In our case, UI, HU, HN and UTI were determined in addition to EU. UTI and UI taken under control with the repair of EU and antibiotherapy were observed within the significant findings. In one-year-old phenomenon, UTI antibiotherapy determined as a result of laboratory and urine tests was taken under control with EU operative treatment and it was verified with laboratory and urine analysis that treatment of EU and incontinence has a big contribution on not repeating in postoperative period.

The first step in the treatment of incontinence patients is alpha-adrenergic agonists (which are commonly used to stimulate the alpha-adrenergic receptors expressed in the internal urethral sphincter, leading to an increase in the urethral closure pressure). The success rate varies from 86% to 97% for phenylpropanolamine and 74-93% for ephedrine. But the use of alpha-adrenergic agonists is contraindicated in diseases where an increase in blood pressure should be avoided, as in most kidney diseases, heart problems or glaucoma [13,14]. The subcutaneous application of GnRH depot analogues (Deslorelinacetate 4.7 mg/dog) increases bladder compliance and is successful as a single therapy in approximately 50% of bitches with UI. GnRH analogues are especially suitable for patients showing serious side effects after therapy with alpha adrenergic agonists or if alpha-adrenergic agonists are contraindicated. So far, there have been no reports on side effects after the use of GnRH analogues in ovariectomized bitches [13]. In the literature, GnRH depot analogue was reported to have restored incontinence only in castrated male dog [17] and cat [18]. In our case, it was observed that urinary incontinence thought to be caused from OHE or USMI which continues after OHE done with ectopic ureter correction was removed with deslorelin acetate implant, reported as GnRH agonist in a limited number in the literature, in a 7-month period. Unlike the side effects of other drugs, no adverse effects of deslorelin acetate were determined and it was examined that deslorelin acetate was successful in a case of the treatment of USMI-induced urinary incontinence and intending to support further research will be appropriate.

REFERENCES

1. Davidson AP, Westrop JL: Diagnosis and management of urinary ectopia. *Vet Clin North Am: Small Anim Pract*, 44, 343-353, 2014. DOI: 10.1016/j.cvsm.2013.11.007

2. Reichler IM, Specker EC, Hubler M, Boos A, Haessig M, Arnold S: Ectopic ureters in dogs: Clinical features, surgical techniques and outcome. *Vet Surg*, 41, 515-522, 2012. DOI: 10.1111/j.1532-950X.2012.00952.x

3. Fossum TW: Surgery of the kidney and ureter - Ectopic ureter. **In,** Fossum TW (Ed): Textbook of Small Animal Surgery. 3rd ed., 635-663, St. Louis, MO, Mosby, 2007.

4. Holt PE, Moore AH: Canine ureteral ectopia: An analysis of 175 cases and comparison of surgical treatments. *Vet Rec*,136, 345-349, 1995. DOI: 10.1136/vr.136.14.345

5. Weisse C, Berent A: Correcting ectopic ureters in juvenile dogs: Cystoscopic-guided laser ablation is a minimally invasive alternative to surgery. http://veterinarynews. dvm360.com/correcting-ectopic-ureters-juvenile-dogs. *Accessed:* 01.04.2015.

6. Lamb CR, Gregory SP: Ultrasonographic findings in 14 dogs with ectopic ureter. *Vet Radiol Ultrasound,* 39, 218-223,1998. DOI: 10.1111/j.1740-8261.1998.tb00343.x

7. Hedburg K: Ectopic ureter in Golden Retrievers. http://www.australiangoldenretrieverbreeders.com/assets/ectopic_ureter_in_golden_retrievers_-_karen_hedburg.pdf, *Accessed:* 30.04.2015.

8. Özgencil E, Beşaltı Ö: Ureteral ectopia in two Dalmatian bitches. *Turk J Vet Anim Sci,* 21, 503-506, 1997.

9. McLoughlin MA, Chew DJ: Diagnosis and surgical management of ectopic ureters. *Clin Tech Small AnimPract*, 15, 17-24, 2000. DOI: 10.1053/svms.2000.7302

10. Arnold S, Arnold P, Hubler M, Casal M, Rusch P: Incontinentiaurinaebei der kastriertenHu¨ndin:Ha¨ufigkeitund Rassedisposition. *Schweiz Arch Tierheilkd,* 131, 259-263,1989.

11. Arnold S, Hubler M, Reichler I: Urinary incontinence in Spayed bitches: New insights into the pathophysiology and options for medical treatment. *Reprod Dom Anim,* 44 (Suppl. 2): 190-192, 2009. DOI: 10.1111/j.1439-0531.2009.01407.x

12. Scott L, Leddy M, Berney F, Davot JL: Evaluation of pheny-propanolamine in the treatment of urethral sphincter mechanism incompetence in the bitch. *J Small Anim Pract,* 43, 493-496, 2002. DOI: 10.1111/j.1748-5827.2002.tb00020.x

13. Reichler IM, Hubler M: Incontinence in the bitch: An update. *Reprod Dom Ani m*,49 (Suppl. 2): 75-80, 2014. DOI: 10.1111/rda.12298

14. Ponglowhapan S, Khalid M, Church D: Canine urinary incontinence post-neutering: A review of associated factors, pathophysiology and treatment options. *Thai J Vet Med,* 42 (3): 259-265, 2012.

15. Hayes HM: Breed associations of canine ectopic ureter: A study of 217 female cases. *J Small Anim Pract,* 25, 501-504, 1984. DOI: 10.1111/j.1748-5827.1984.tb03422.x

16. Cannizzo KL, McLoughlin MA, Mattoon JS, Samii VF, Chew DJ, DiBartola SP: Evaluation of transurethral cystoscopy and excretory urography for diagnosis of ectopic ureters in female dogs: 25 cases (1992-2000). *Am Vet Med Assoc*, 223, 475-481, 2003.

17. Greer M: Deslorelin implant for urinary incontinence treatment in a neutered male dog, a case study. *7thInternational Symposiumon Canineand Feline Reproduction.* July 26 to 29, 2012. Whistler, Canada. http://www.ivis.org/proceedings/iscfr/2012/62.pdf?LA=1, *Accessed:* 22.09.2015.

18. Pisu MC, Veronesi MC: Effectiveness of deslorelin acetate subcutaneous implantation in a domestic queen with after-spaying urinary incontinence. *J Feline Med Surg*, 16, 366-368, 2013. DOI: 10.1177/1098612X13498250

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Case Report

Dermoid Cyst Penetrating the Abdominal Cavity in a Persian Cat [1]

Volkan İPEK [1] Ersin CANPOLAT [2]

[1] *This case report was presented at VI. National Veterinary Pathology Congress (with international participation), 19-23 September, Kuşadası/Aydın, 2012*

[1] Department of Pathology, Faculty of Veterinary Medicine, Uludağ University, TR-16059 Bursa - TURKEY
[2] Armilla Veterinary Health Center, TR-16080, Bursa - TURKEY

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Abstract

This report describes a case of dermoid cyst localized subcutaneously in the abdominal region, 2 cm to the right of linea alba in a six-year-old, neutered male Persian cat admitted with complaints of painful defecation and abdominal licking. A laparatomy was performed and the mass was seen to have penetrated the periton and extended into the abdominal space with a stalk located among the abdominal muscles and aponeuroses. Macroscopically, the oval shaped mass was approximately 3 cm in diameter, was capsulated with a grayish white tissue, had a smooth surface, and soft consistency. The cut surface of the mass contained abundant hair. Histopathologically, the mass was encapsulated with fibrous tissue. Beneath the fibrous capsule, dermal structures such as mature hair follicles, sweat glands, and sebaceous glands were observed. In the center of the mass, stratified squamous epithelium was observed lining the luminal surface of the cyst. The cyst cavity was filled with flacks of keratin and hair shafts.

Keywords: *Dermoid cyst, Persian cat, Abdominal region, Pain*

Bir İran Kedisinde Abdominal Boşlukla İlişkili Dermoid Kist

Özet

Bu raporda kliniğe ağrılı dışkılama ve abdominal yalama şikayeti ile getirilen, 6 yaşlı, kısırlaştırılmış, erkek bir İran kedisinde abdominal bölgede, linea albanın 2 cm sağında gözlenen, deri altı yerleşimli bir dermoid kist olgusu tanımlanmıştır. Laporatomide kitlenin peritona penetre olduğu ve abdominal kaslar ile aponevrozlar arasında bulunan bir sap ile abdominal boşluğa uzandığı görüldü. Makroskobik incelemede, oval şekilli kitle yaklaşık 3 cm çapında, kapsüllü, grimsi beyaz renkte, düzgün yüzeye sahip ve yumuşak kıvamlıydı. Kitlenin kesit yüzünde bol miktarda kıl mevcuttu. Histopatolojik incelemede, kitle fibröz kapsül ile sarılıydı ve kapsül altında olgun kıl follikülleri, ter bezleri ve sebasöz bezler mevcuttu. Lüminal yüzeyi çok katlı yassı epitelle döşeli olan kist boşluğunun, keratin parçaları ve kıl gövdeleri ile dolu olduğu görüldü.

Anahtar sözcükler: *Dermoid kist, İran kedisi, Abdominal bölge, Ağrı*

INTRODUCTION

Dermoid cyst is a rare congenital or acquired tumor like developmental anomaly and has been reported in dogs and cats [1-5]. The term dermoid cyst generically includes three different formations, namely epidermoid cyst, "true" dermoid cyst, and teratoid cyst [6]. Both epidermoid and dermoid cysts are surrounded by stratified squamous epithelium, however dermoid cysts contain hair follicles, sweat glands, and sebaceous glands as additional distinctive features [6,7]. Teratoid cyst, on the other hand, is a cystic form of teratoma and contains ectodermal, mesodermal, and endodermal structures [6]. Dermoid cyst usually forms as a result of embryonal fissures' closure defects [2,8], but a traumatic origin has also been suggested [2]. There are limited numbers of reports about dermoid cysts in cats [2,3-5,9]. In this case report, we evaluated a periton-penetrating subcutaneous dermoid cyst occurring in the abdominal region of a Persian cat.

CASE HISTORY

A six-year-old, neutered male Persian cat was admitted to a private veterinary health center with complaints of painful defecation and abdominal licking. A subcutaneous mass was palpated in the abdominal region, approximately

İletişim (Correspondence)
+90 224 2940863 Fax: +90 224 2941202
volkanipek@uludag.edu.tr

2 cm to the right of linea alba, and radiography was performed. Radiologic evaluation revealed an approximately three cm sized mass in connection with the abdominal muscles and abdominal cavity. A laparatomy was performed. The mass was seen to have penetrated the periton and extended into the abdominal space with a stalk located among the musculus rectus abdominis and aponeuroses of other abdominal muscles. The mass was well-capsulated and the intestinal serosal vessels in contact with the mass were congested. Following the excision, the mass was submitted for pathological examination.

Macroscopically, the oval shaped mass was approximately 3 cm in diameter (long axis), was capsulated with a grayish white tissue, had a smooth surface and soft consistency. The cut surface of the mass contained abundant hair *(Fig. 1)*. Tissue samples were taken into formalin and they were sectioned at 5 micrometer thickness after following routine tissue processing procedures. Histopathologically, the mass was encapsulated with fibrous tissue. Beneath the fibrous capsule, dermal structures such as mature hair follicles, sweat glands, and sebaceous glands were observed *(Fig. 2)*. In the center of the mass, stratified squamous epithelium *(Fig. 3)* was observed lining the luminal surface of the cyst. The cyst cavity was filled with flacks of keratin and hair shafts. No inflammation was observed.

DISCUSSION

In veterinary medicine, dermoid cyst is most frequently seen in dog breeds including Rhodesian Ridgeback, Siberian Husky, Shih Tzu, Boxer, and Kerry Blue [2,4,8]. Rhodesian Ridgeback has been reported to have a genetic tendency to dermoid cyst formation [1,2,4,10]. Dermoid cyst is a rare formation in cats and according to the authors' knowledge there are only a few reports about dermoid cysts and dermoid sinuses. In these reports, dermoid cysts and sinuses were seen in various locations such as dorsal

Fig 1. Cut surface of the mass contained abundant hair

Şekil 1. Kitlenin kesit yüzünde bol miktarda kıl varlığı

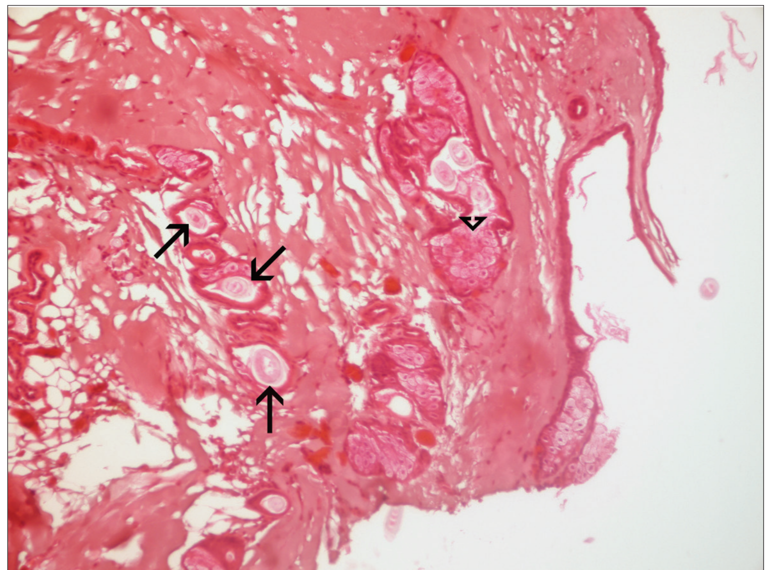

Fig 2. Mature hair follicles *(arrows)* and sebaceous glands *(arrowhead)*, Hematoxylin-eosin, x200

Şekil 2. Olgun kıl follikülleri *(oklar)* ve sebasöz bezler *(ok başı)*, Hematoksilen-eozin, 200x

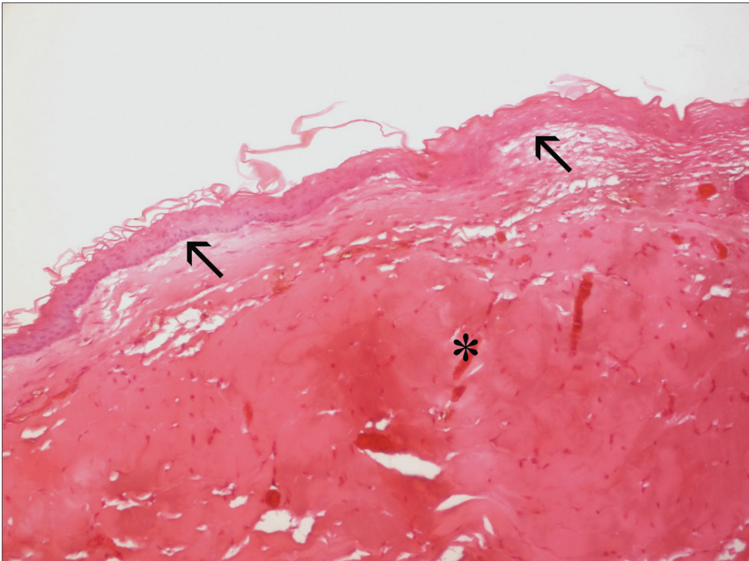

Fig 3. Keratinized stratified squamous epithelium lining the cyst wall *(arrow)* and dermis *(asterisk)*, Hematoxylin-eosin, x200

Şekil 3. Keratinize çok katlı yassı epitel ile örtülü kist duvarı *(ok)* ve dermis *(asterisk)*, Hematoksilen-eozin, 200x

midline, sublumbar area, flank area, in the thyroid glands, and intracranially [2,3-5,9,11]. While there is a significant predisposition for certain dog breeds, the number of reported cases in cats is too limited to suggest any such correlation.

Dermoid cysts occur as solitary or multiple [4,8], and are most commonly found on the dorsal midline of the head or along the vertebral column [2,12]. They are lined with stratified epithelium and generally contain hair, keratin, and sebum; there are hair follicles, sebaceous and apocrine glands related to the cyst wall [2,6,8]. In our case, there was a solitary cyst and the animal owner noticed the mass approximately one month prior to referral. The cyst was lined with keratinized stratified epithelium; there were hair follicles, sebaceous and sweat glands under the epithelium; and cyst cavity was filled with hair and keratin.

Clinically dermoid cysts resemble follicular cysts [2] and the differentiation is done upon the observation of fully formed hair shafts on cut surface in dermoid cysts [12]. Another similar structure is the dermoid sinus, which has a connection with skin surface as a distinctive feature [1,13], but the two terms have also been used synonymously [6,9]. In our case a diagnosis of dermoid cyst was made as no connection to the skin surface was observed and the cyst cavity contained hair.

Etiology of dermoid cyst is controversial. While some authors believe that dermoid cyst is a developmental anomaly forming as a result of a defect in neural tube split from the skin during embryogenesis [14] or epidermal closure defect along embryonic fissures that isolate an island of ectoderm in the dermis or subcutis [2], others classify it as a type of teratoma [15] or an acquired structure [6]. In our case, there was no clear evidence of a developmental or hereditary origin; the cat was middle-aged with no known history of the lesion at birth and he did not have a traumatic history either. However it was known that the cat had been treated for chronic, generalized *Microsporum canis* infection, affecting also the abdominal region, 2.5 years ago. There is no information about any relationship between cyst formation and the mentioned infection.

Clinical symptoms vary depending on the site of lesion and serious problems may develop in some cases [3,9]. We believe that painful defecation might have occurred as a result of the compression of the cyst to the intestines in our case as evidenced by the congested veins of the intestine in contact with the cyst. Painful defecation was also completely resolved following the operation. Although risk of postoperative herniation has been reported following the removal of dermoid cysts in abdominal region of cats [4], we did not encounter such a problem in this case.

Penetration of dermoid cyst into the abdominal cavity is an unreported finding. In a previous report, dermoid cysts were observed located within the abdominal muscles in the sublumbar and left flank area in two cats, but there was no indication of penetration into the abdominal cavity [4]. A stalk located within the abdominal muscles and aponeuroses established the connection between the subcutis and the mass in the abdominal space in our case.

In conclusion, to the best of the authors' knowledge, this is the first case of a dermoid cyst reported in a Persian cat. Intrusion of the abdominal cavity with painful defecation is an unexpected, previously unreported finding. We recommend the veterinarians who encounter cats with complaints of painful defecation and/or mass present in the abdominal region to consider dermoid cyst in the differential diagnosis.

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REFERENCES

1. Tshamala M, Moens Y: True dermoid cyst in a Rhodesian Ridgeback. *J Small Anim Pract*, 41 (8): 352-353, 2000.

2. Akhtardanesh B, Kheirandish R, Azari O: Dermoid cyst in a domestic shorthair cat. *Asian Pac J Trop Biomed*, 2, 247-249, 2012. DOI: 10.1016/S2221-1691(12)60051-3

3. Henderson JP, Pearson GR, Smerdon TN: Dermoid cyst of the spinal cord associated with ataxia in a cat. *J Small Anim Pract*, 34, 402-404, 1993. DOI: 10.1111/j.1748-5827.1993.tb02734.x

4. Rochat MC, Campbell GA, Panciera RJ: Dermoid cysts in cats: Two cases and a review of the literature. *J Vet Diagn Invest*, 8 (4): 505-507, 1996.

5. Chenier S, Quesnel A, Girard C: Intracranial teratoma and dermoid cyst in a kitten. *J Vet Diagn Invest*, 10, 381-384, 1998. DOI: 10.1177/104063879801000417

6. Oryan A, Hashemnia M, Mohammadalipour A: Dermoid cyst in camel: A case report and brief literature review. *Comp Clin Path*, 21, 555-558, 2012. DOI: 10.1007/s00580-010-1128-9

7. Hansmann F, Herder V, Ernst H, Baumgartner W: Spinal epidermoid cyst in a SJL mouse: Case report and literature review. *J Comp Pathol*, 145, 373-377, 2011. DOI: 10.1016/j.jcpa.2011.03.002

8. Alam MM, Rahman MM: A three years retrospective study on the nature and cause of ocular dermoids in cross-bred calves. *Open Vet J*, 2 (1): 10-14, 2012.

9. Tong T, Simpson DJ: Spinal dermoid sinus in a Burmese cat with paraparesis. *Aust Vet J*, 87 (11): 450-454, 2009. DOI: 10.1111/j.1751-0813.2009.00487.x

10. Mann GE, Stration J: Dermoid sinus in the Rhodesian ridgeback. *J Small Anim Pract*, 7, 631-642, 1966. DOI: 10.1111/j.1748-5827.1966.tb04388.x

11. Kiviranta AM, Lappalainen AK, Hagner K, Jokinen T: Dermoid sinus and spina bifida in three dogs and a cat. *J Small Anim Pract*, 52, 319-324, 2011. DOI: 10.1111/j.1748-5827.2011.01062.x

12. Villalobos A: Tumors of the Skin and Soft Tissues. **In,** Kahn CM (Ed): The Merck Veterinary Manual. 10th ed., 863-864, Whitehouse Station, New Jersey, Merck & Co. Inc., 2010.

13. Hillyer LL, Jackson AP, Quinn GC, Day MJ: Epidermal (infundibular) and dermoid cysts in the dorsal midline of a three-year-old thoroughbred-cross gelding. *Vet Dermatol*, 14, 205-209, 2003. DOI: 10.1046/j.1365-3164.2003.00345.x

14. Howard-Martin M, Bowles MH: Intracranial dermoid cyst in a dog. *J Am Vet Med Assoc*, 192 (2): 215-216, 1988.

15. Kountakis SE, Minotti AM, Maillard A, Stiernberg CM: Teratomas of the head and neck. *Am J Otolaryngol*, 15 (4): 292-296, 1994.

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Case Report

A Case of Tuberculosis in a Free-living Long-legged Buzzard *(Buteo rufinus)*

Hasan ÖZEN [1] Musa KARAMAN [2] Serpil DAĞ [1]
Emin KARAKURT [1] Yalçın AKBULUT [3]

[1] Kafkas University, College of Veterinary Medicine, Department of Pathology, TR-36100 Kars - TURKEY
[2] Balıkesir University, College of Veterinary Medicine, Department of Pathology, TR-10145 Balıkesir - TURKEY
[3] Kafkas University, College of Veterinary Medicine, Department of Anatomy, TR-36100 Kars - TURKEY

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Abstract

A male Long-legged buzzard with a gunshot wound on his left wing was presented for treatment. However, the bird died shortly after, and then the routine necropsy was performed. At necropsy, numerous white-to-yellow nodular lesions sizing several mm to 1 cm in diameter were noted in liver, spleen, gizzard and lung. Microscopic examination of the nodules in lung and gizzard revealed classical formation of tubercles characterized by a caseous core surrounded by epitheloid cells, multinucleated giant cells, heterophils, macrophages, and outer fibrous capsule. Fibrous capsule formation was vague in tubercles located in liver and spleen. Acid fast bacteria were shown by Ziehl-Neelsen staining. Based on the observations a diagnosis of avian mycobacteriosis was made. This report indicates that avian tuberculosis might be an important disease in free living animals in Turkey as in other places, and more attention might be needed to monitor the disease.

Keywords: *Avian tuberculosis, Long-legged buzzard*

Doğada Serbest Yaşayan Bir Uzun Bacaklı Şahinde *(Buteo rufinus)* Tuberküloz Olgusu

Özet

Sol kanadında ateşli silah yarası bulunan bir erkek Uzun bacaklı şahin tedavi amaçlı getirilmiş, ancak kısa sürede ölmüş ve rutin nekropsisi yapılmıştır. Nekropside; karaciğer, dalak, ventrikulus ve akciğerlerde çok sayıda, yarıçapı birkaç mm ile 1 cm arasında değişen büyüklüklerde beyaz-sarı renkli nodüler lezyonlar dikkati çekti. Mikroskobik bakıda akciğer ve ventrikulusta kazeifiye merkez etrafında epiteloid hücreler, çok çekirdekli dev hücreleri, heterofil lökositler, makrofajlar ve en dışta fibröz bir kapsül ile karakterize klasik tüberkel yapısı gözlemlendi. Fibröz kapsül yapısı karaciğer ve dalaktaki tüberkellerde belirgin değildi. Ziehl-Neelsen boyama ile aside dirençli bakterilerin varlığı belirlendi. Yapılan gözlemler sonucunda kanatlı tüberkülozu tanısı konuldu. Bu olgu sunumu; diğer pek çok yerde olduğu gibi ülkemizde de kanatlı tüberkülozunun doğada serbest yaşayan hayvanların önemli bir sorunu olabileceğini göstermiş ve bu alanda daha fazla ilgiye ihtiyaç duyulduğunu ortaya koymuştur.

Anahtar sözcükler: *Kanatlı tüberkülozu, Uzun bacaklı şahin*

INTRODUCTION

Tuberculosis is a commonly seen disease affecting both human and animals [1]. Avian tuberculosis, also called avian mycobacteriosis, is occasionally reported both in various domestic and wild birds as well. The causative agents of the disease in birds are mostly *Mycobacterium avium* subsp. *avium* and less frequently *M. genavense* [2]. However, there are studies reporting that *M. genevanse* is the predominant mycobacterial species in pet birds [3,4]. Several other species of mycobacteria such as *M. tuberculosis, M. bovis, M. terrae, M. fortuitum*, and *M. scrofulaceum* was rarely recorded in the etiology of avian mycobacteriosis [2,5-7].

Susceptibility of different species of birds to the disease varies greatly. Although birds were broadly classified into four groups, namely highly susceptible, less susceptible, moderately resistant and highly resistant, based on their susceptibility to the disease [8], the reason why they differ is exactly not known. Birds living under the same housing

İletişim (Correspondence)
+90 474 2426836, Fax: +90 474 2426853
hasanozen@hotmail.com

conditions were reported to differ in contracting the disease, and hence genetic and environmental conditions were suggested [9,10]. Even feather color in doves was shown to be a factor probably in relation to chromosomal association of immune response [11]. Countless stressors such as overcrowding, malnutrition and concurrent infections that impair immune system are known to be some important predisposing factors [6,12].

Reports of mycobacteriosis in wild and captive birds are very common worldwide where affective monitoring and protection studies performed regularly [3,6,7,13,14]. However, there are only limited numbers of reports describing avian tuberculosis in Turkey. Besides, most of these cases are on pigeons [15-18]. There are also reports in a hen [19], chickens [20] geese [21], a peafowl and pheasants [10]. Avian mycobacteriosis in raptors was only described once in Turkey before, however the subject of that case, which was also a Long-legged buzzard, was a captive bird held in a park under a protection program [12]. Therefore, a case of avian tuberculosis in a raptor living freely in Turkey was found to be worth of reporting.

CASE HISTORY

An adult male Long-legged buzzard *(Buteo rufinus)* was referred to Kafkas University Wildlife Safety, Rescue, Rehabilitation, and Research Center in Kars-Turkey for gunshot treatment. Clinically the bird was depressed and emaciated, however it died shortly after without the chance of treatment. Thereafter, routine necropsy was performed. In gross examination, body condition of the bird was poor with atrophied pectoral muscles, and there was an apparently old open wound at the level of left ulna, which was detected to be broken. Intestinal content revealed presence of diarrhea. No other gross lesions or abnormalities were observed in the head and the skin. Upon inspection of the internal organs, multiple white-to-yellow firm nodular lesions sizing several mm to 1 cm in diameter were seen in liver, spleen, gizzard and lung *(Fig. 1)*, which caused the speculation of tuberculosis.

Tissue samples from all organs were collected and routinely processed for 10% formalin fixation and paraffin embedding. Tissue sections were then cut and stained with hematoxylin and eosin (HE) for evaluation of histopathological changes and with Ziehl-Neelsen for demonstration of acid-fast bacteria.

In microscopic examination of lungs *(Fig. 2)* and gizzard, typical tuberculoid granulomas characterized by non-mineralized or mineralized central necrosis, surrounded by heterophils, macrophages, lymphocytes, epitheloid cells, multinucleated giant cells and outer fibrous capsule were

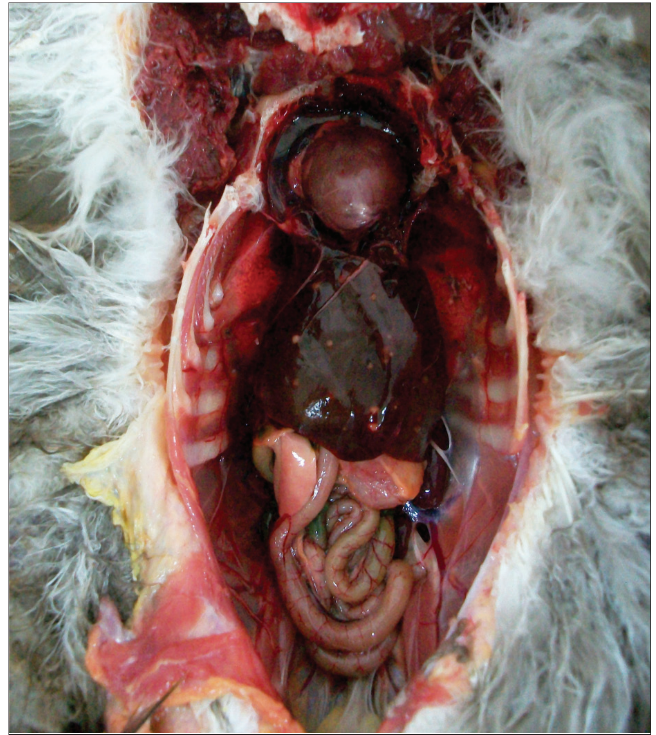


Fig 1. White-to yellow nodules located on liver and lungs

Şekil 1. Karaciğer ve akciğerlerde beyaz sarı renkli nodüller

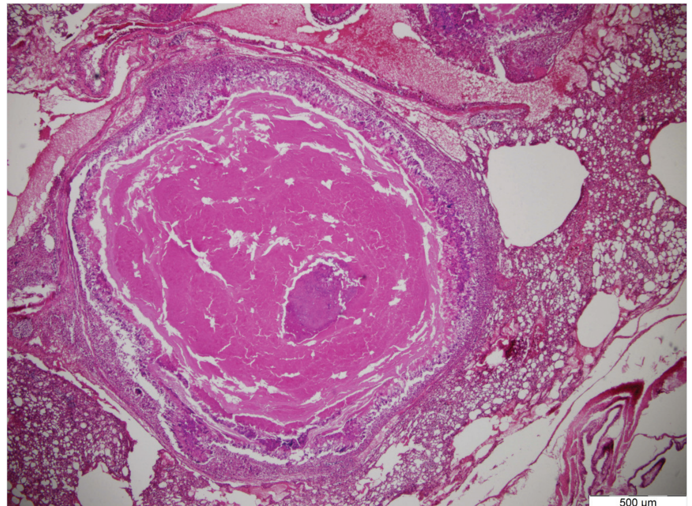


Fig 2. Microscopic view of a granuloma characterized by a mineralized caseous core surrounded by epitheloid cells, multinucleated giant cells, heterophils and macrophages with outer fibrous capsule formation in lung

Şekil 2. Akciğerde mineralize kazeoz merkez etrafında epiteloid hücreler, çok çekirdekli dev hücreleri, heterofil ve makrofajlar ile karakterize çevresinde fibröz kapsül şekillenmiş bir granulomun mikroskobik görüntüsü

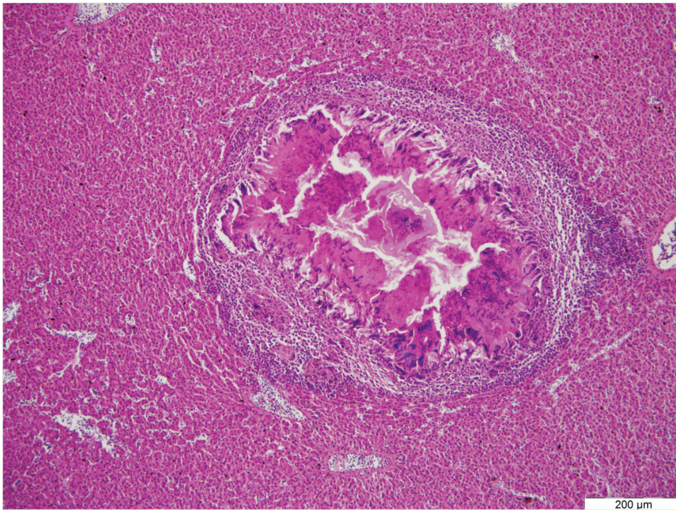


Fig 3. Microscopic view of a granuloma characterized by a caseous core surrounded by epitheloid cells, multinucleated giant cells, heterophils and macrophages in liver

Şekil 3. Karaciğerde kazeoz merkez etrafında epiteloid hücreler, çok çekirdekli dev hücreleri, heterofil ve makrofajlar ile karakterize bir granulomun mikroskobik görüntüsü

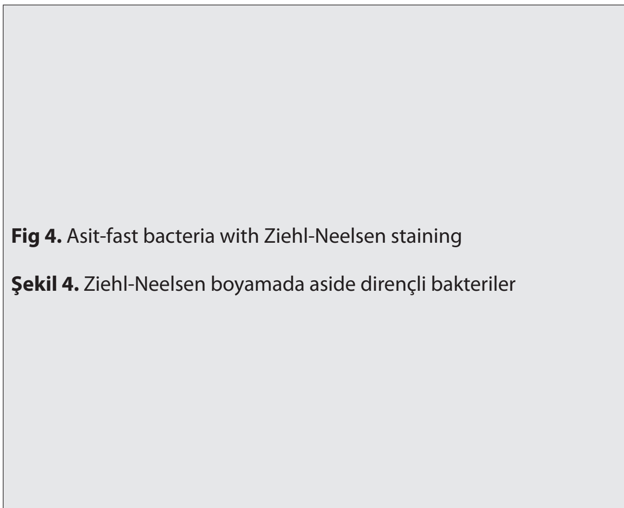
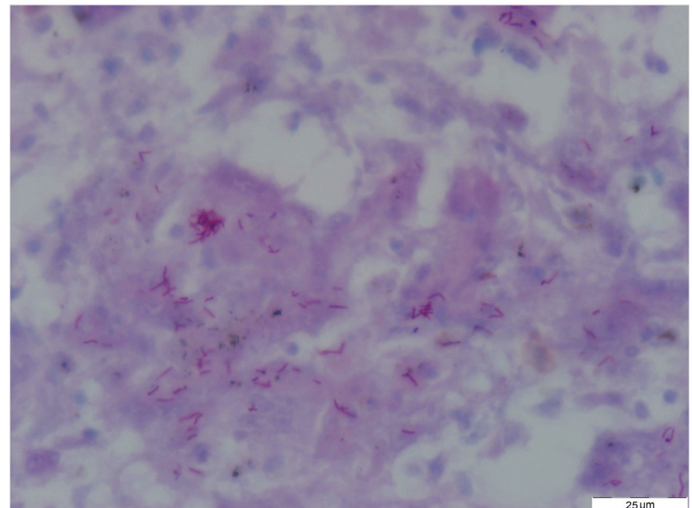


Fig 4. Asit-fast bacteria with Ziehl-Neelsen staining

Şekil 4. Ziehl-Neelsen boyamada aside dirençli bakteriler



observed. Fibrous capsule formation around the tubercles was vague in liver *(Fig. 3)* and spleen. Where tubercles not formed yet, sheets of inflammatory cells mostly composed of macrophages and lymphocytes were noted. Ziehl-Neelsen staining revealed the presence of acid-fast bacteria located both intracellular within the giant cells and as free *(Fig. 4)*. Based on the typical tuberculoid lesions and view of acid-fast bacteria, a diagnosis of avian mycobacteriosis was made. Microscopy of kidney revealed severe glomerular and tubular degeneration with infiltration of inflammatory cells and congestion.

DISCUSSION

Avian tuberculosis is a ubiquitous disease worldwide. It is common in gallinaceous poultry and wild animals living in captivity [6]. Although occasional case reports or investigations on poultry, captive and wild birds are present elsewhere, there is only few in Turkey [10,15-18,20]. Avian tuberculosis in a raptor was only reported once before in Turkey, and it was a captive Long-legged buzzard [12]. In the current case report, avian mycobacteriosis was described also in a Long-legged buzzard, which was differently from the previous case, living free in nature.

Mycobacterial agents are highly resistant to environmental conditions and can survive several years in soil. Contaminated soil, water and infected prey in wild and housing equipment in captivity can help spreading of the infection [13]. Continuous shedding of microorganisms with droppings and aerosol are the main sources for transmission to susceptible birds. Proper sanitation measures taken after if the disease is diagnosed in a flock of poultry animals or captive birds may easily help preventing the spread or the reoccurrence [22]. However, since such measures cannot be applied in nature it is almost impossible to control the disease in free-living animals.

Lesions in avian tuberculosis are mostly seen in liver, spleen, and gastrointestinal system organs [11,23]. Lung and facial lesions can also be observed occasionally [10,14]. In the present case, white to yellow tubercles were also noted in liver, spleen, gizzard and lung. Microscopic view of the lesions revealed classical formation of tubercles with macrophages, epitheloid cells, giant cells, lymphocytes

and an outer fibrous capsule with a central caseous core as reported previously [24]. However, fibrous capsule formation was not clear in liver and spleen. The reason of vague fibrous capsule formation in liver and spleen in opposite to lung and gizzard might be explained by extensive connective tissue presence in the later organs. In addition, we did not detect any lesions in intestines, where tuberculoid lesions are often reported. In birds, alimentary tract is the main route of transmission of the infection, since most lesions are generally localized in this system. However, presence of lung lesions in addition to liver, spleen and gizzard, might suggest that aerosol transmission were involved in the current case. On the other hand possibility of alimentary transmission cannot be omitted based on the size of tubercle in gizzard. In the previously reported case in a Long-legged buzzard in Turkey, lesions were detected in wing, spleen, intestines and lungs but no tubercles were reported in liver. In that case report, because of the close contact with other birds such as sparrows, starlings and crows, the disease was speculated to be contracted from such animals [12]. In the present case, it is not even possible to speculate the source of infection since the animal was a free living animal and, besides no reports was before present from the region where the animal was located.

Avian tuberculosis is a chronic disease, and hence is seen mostly in adult animals. However, considerable amount of young companion birds with ages less than a year were previously reported to have tuberculosis [13]. In the current case the bird was also a mature animal. In wild birds with tuberculosis, mostly subclinical disease state is observed. Emaciation, stagnation, drowsiness, and diarrhea were often the recorded clinical signs in birds [2,12]. However, probable affects of gunshot and following unknown state of the bird for a while did not let the observation of any signs except pectoral muscle atrophy.

In conclusion, the aim of this case presentation was to provide awareness to the wild-life diseases besides describing the pathological changes in a tuberculoid bird detected coincidentally. Insufficient wild life facilities as well as absence of monitoring programs in Turkey probably limit the observation of diseases occurring in wild animals in general. Therefore, it is clear from the lack of enough disease reports in wild animals that more attention is undoubtedly needed for wild-life animals and the diseases. Control measures can only then be applied. In addition, it must be mentioned that since the infected wild birds can be a serious source for captive birds as well as poultry in avian tuberculosis, regular monitoring of the disease in wild can help estimating the risk for poultry.

REFERENCES

1. Ghodbane R, Drancourt M: Non-human sources of *Mycobacterium tuberculosis. Tuberculosis,* 93, 589-595, 2013. DOI: 10.1016/j.tube.2013.09.005

2. Dhama K, Mahendran M, Tiwari R, Singh SD, Kumar D, Singh S, Sawant PM: Tuberculosis in birds: Insights into the Mycobacterium infections. *Vet Med Int*, Article ID: 712369, 2011. DOI: 10.4061/2011/712369

3. Hoop RK, Böttger EC, Pfyffer GE: Etiological agents of mycobacterioses in pet birds between 1986 and 1995. *J Clin Microbiol*, 34 (4): 991-992, 1996.

4. Holsboer Buogo C, Bacciarini L, Robert N, Bodmer T, Nocolet J: Vorkommen von *Mycobacterium genavense* bei Vögeln. *Schweizer Arch Tierheilkd,* 139 (9): 397-402, 1997.

5. Smit T, Eger A, Haagsma J, Bakhuizen T: Avian tuberculosis in wild birds in the Netherlands. *J Wildlife Dis*, 23, 485-487, 1987. DOI: 10.7589/0090-3558-23.3.485

6. Tell LA, Woods L, Cromie RL: Mycobacteriosis in birds. *Res Sci Tech Off Int Epiz*, 20 (1): 180-203, 2001.

7. Schmidt V, Schneider S, Schlömer J, Krautwald-Junghanns ME, Richter E: Transmission of tuberculosis between men and pet birds: a case report. *Avian Pathol*, 37, 589-592, 2008. DOI: 10.1080/03079450802428901

8. Hejlicek K, Treml F: Comparison of the pathogenesis and epizootiologic importance of avian mycobacteriosis in various types of domestic and free-living syntropic birds. *Vet Med-Czech,* 40, 187-194, 1995.

9. Cromie RL, Brown MJ, Price DJ, Stanford JL: Susceptibility of captive wildfowl to avian tuberculosis: the importance of genetic and environmental factors. *Tubercle*, 72, 105-109, 1991. DOI: 10.1016/0041-3879(91)90036-R

10. Kul O, Tunca R, Hazıroğlu R, Diker KS, Karahan S: An outbreak of avian tuberculosis in peafowl *(Pavo cristatus)* and pheasants *(Phasianus colchicus)* in a zoological aviary in Turkey. *Vet Med-Czech*, 50, 446-450, 2005.

11. Saggese MD, Tizard I, Phalen DN: Mycobacteriosis in naturally infected ring-neck doves *(Streptopelia risoria):* Investigation of the associaition between feather colour and susceptibility to infection, disease and lesion type. *Avian Pathol*, 37, 443-450, 2008. DOI: 10.1080/03079450802210655

12. Atasever A, Beyaz L, Kibar M, Gümüssoy KS: A case of tuberculosis and aspergillosis in a Long-Legged Buzzard *(Buteo rufinus). Revue Med Vet*, 157 (1): 26-29, 2006.

13. Manarolla G, Liandris E, Pisoni G, Sassera D, Grilli G, Gallazzi D, Sironi G, Moroni P, Piccinini R, Rampin T: Avian mycobacteriosis in companion birds: 20-year survey. *Vet Microbiol*, 133, 323-327, 2009. DOI: 10.1016/j.vetmic.2008.07.017

14. Mayahi M, Mosavari N, Esmaeilzadeh S, Parvandar Asadollahi K: Avian tuberculosis in naturally infected lofts of domestic pigeons, isolation, molecular identification and study of necropsy findings. *Intern J Appl Res Vet Med*, 11 (3): 194-201, 2013.

15. Sezen İY, Erer H, Erganis O: Bir güvercinde tüberküloz olgusu. *Selçuk* Üniv *Vet Fak Derg*, 2 (1): 163-166, 1986.

16. Kutsal O, Sağlam M: Güvercinlerde tüberkuloz olgularının değerlendirilmesi. *Ankara* Üniv *Vet Fak Derg*, 35 (2.3): 545-552, 1988.

17. Gürel A, Arun SS, Yesildere T: Üç farklı evcil güvercin sürüsünde spontan tüberkülozis olguları. *Istanbul Univ Vet Fak Derg*, 23 (1): 131-139, 1997.

18. Terim Kapakin KA, Alçığır G: Bir güvercinde tüberküloz olgusu. *Kakfas Unıv Vet Fak Derg*, 15, 477-479, 2009. DOI: 10.9775/kvfd.2009.061-G

19. Beytut E, Atabay Hİ, Akça A: Tuberculosis and Sarcosporidiosis in the periorbital location in a hen. *Kafkas Univ Vet Fak Derg*, 7 (2): 213-217, 2001.

20. Terim Kapakin KA, Sağlam YS, Altun S: Histopathological examinations of tuberculosis cases detected in chickens grown by a family enterprice. *Atatürk Univ Vet Bil Derg*, 5 (3): 141-146, 2010.

21. Özcan K, Beytut E, Aydın F, Tuzcu M: Tuberculosis in geese *(Anser anser)* in Turkey. *Avian Dis*, 45, 755-759, 2001. DOI: 10.2307/1592924

22. Gill IJ, Blandy ML: Control of avian tuberculosis in a commercial poultry flock. *Aust Vet J*, 63, 422-423, 1986. DOI: 10.1111/j.1751-0813.1986.tb15889.x

23. Millán J, Negre N, Castellanos E, de Juan L, Mateos A, Parpal L, Aranaz A: Avian mycobacteriosis in free-living raptors in Majorca Island, Spain. *Avian Pathol*, 39, 1-6, 2010. DOI: 10.1080/03079450903389945

24. Kriz P, Makovcova J, Skoric M, Huml O, Pokorny J: Avian mycobacteriosis in an individual of the endangered Mauritian Pink pigeon *(Nesoenas mayeri)* species: A case report. *Vet Med-Czech*, 60, 101-104, 2015. DOI: 10.17221/7984-VETMED

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Letter to the Editor

Thromboelastographic Evaluation of Coagulation in a Dog with Anticoagulant Rodenticide Intoxication
(Antikoagülant Rodentisit Zehirlenmesi Olan Bir Köpekte Koagülasyonun Tromboelastografik Olarak Değerlendirilmesi)

Oya ERALP İNAN [1] Meriç KOCATÜRK [2] Zafer MECİTOĞLU [2] Zeki YILMAZ [2]

[1] Medical and Surgical Experimental Animal Practice and Research Center, Eskişehir Osmangazi University, TR-26480 Eskisehir - TURKEY
[2] Department of Internal Medicine, Faculty of Veterinary Medicine, Uludag University, TR-165059 Bursa - TURKEY

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Dear Editor,

As known, clinicians have a responsibility to make a true diagnosis and to provide effective treatment plan as soon as possible in the clinical setting. For this purpose, alternative diagnostic methods have been used to minimize time consuming effect during the diagnostic work-up in human and veterinary medicine. Thus, this case presentation provides a novel diagnostic approach by use of Thromboelastography (TEG) to evaluate coagulation cascade in a dog with anticoagulant rodenticid intoxication.

Anticoagulant rodenticids are leading to multiple clinical and laboratory disorders including primarily internal/external bleeding, anemia and thrombocytopenia [1]. Traditional methods to evaluate coagulation system include active coagulation time, prothrombin time (PT) and activated partial thromboplastin time (aPTT) [2]. These procedures need not only to comprehensive and high technology laboratory equipment, but also to have some disadvantages due to long measurement time and economic reason in veterinary medicine. To solve this problem, TEG provides valuable information of haemostasis by measuring graphically and numerous the clotting time, clot firmness and fibrinolysis of the patient. TEG is used in veterinary medicine [3], but there is no report yet of the TEG evaluation of anticoagulant rodenticid intoxication in dogs.

A pointer (18 kg, 9 months old and female) was presented to the Animal Hospital (Uludag University, Faculty of Veterinary Medicine, Bursa - Turkey) with the complaint of severe depression and inappetence. Anticoagulant rodenticid (brodifacoum) intoxication was considered as a possible cause according to the owner's history. In the physical examination, a muffled heart sound, anaemic mucous membranes and an echimosis at the medial right regio tarsi were the most noticeable findings. Pericardial effusion was suspected based on the electrocardiographic (electrical alternans and small complexes of QRS) and thoracic radiographic observations (globoid heart shape), and then it was confirmed by echocardiographic examination. Pericardiocynthesis did not performed due to bleeding risk. Laboratory analysis included a neutrophilic leukocytosis (WBC: 19.600 µL; N: 18.400 µL), normal hematocrit (Hct: 42.1%), mild thrombocytopenia (175.000 µL) and mild hypoproteinemia (TP: 4.3 g/dL). To evaluate the coagulation system, kaolin-activated TEG (Hameoscop, TEG 5000, USA) was performed with Na citrated whole blood [4]. On TEG parameters [4], reaction time (R) is primarily influenced by plasma clotting factors, and coagulation time (K) is influenced by coagulation factors, fibrinogen plasma concentration and platelet count. Alpha angle (α-angle) is reflecting the speed of fibrin cross-linking. Maximum amplitude (MA) is the measurement of the peak rigidity manifested by the clot. The G value is more indicative of small changes in clot rigidity or fibrinolysis. In this case, anti-coagulant rodenticide intoxication was suspected based on the clinical and laboratory test results as well as ancillary diagnostic procedures, but it was confirmed by TEG result showing prolonged R time (a flat line on the graphic, *Fig. 1*). Thrombocytopenia and hypoproteinemia in this case were probably due to internal (pericardial) and external (ecchymosis) bleedings.

Vitamin K1 (Konakion, 10 mg Amp, Roche) was suggested at a dosage of 2.5 mg/kg, SC, for two weeks [3]. At the end of this period, clinical and laboratory evaluations were resulted in full recovery (data not shown). TEG analysis revealed a nearly normal platelet map as a graphical format *(Fig. 2)*. Compared to reference ranges [4], all variables except R time (13.1 min, ref.:1.8-8.6 min) were in normal ranges. Prolonged R time means that fibrin polymerization time was prolonged by factor deficiency

İletişim (Correspondence)

+90 222 2393578

oya.eralp@gmail.com

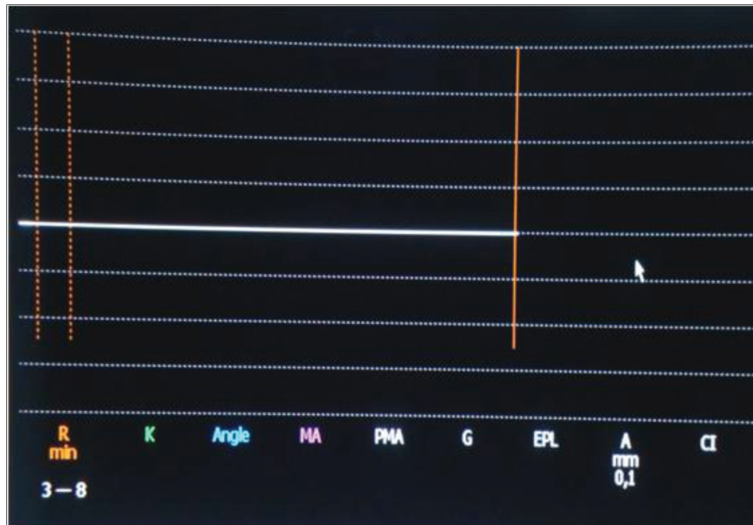

Fig 1. TEG analysis was truncated with a vertical brown colour line (after G value) due to very prolonged reaction time (R) in a dog with an anticoagulant rodenticit toxication

Şekil 1. TEG analizi G harfinden sonra dikey kahverengi bir çizgi ile antikoagülant rodentisit toksikasyonlu köpeğin çok uzamış olan reaksiyon süresi (R) sebebiyle sonlandırılmıştır

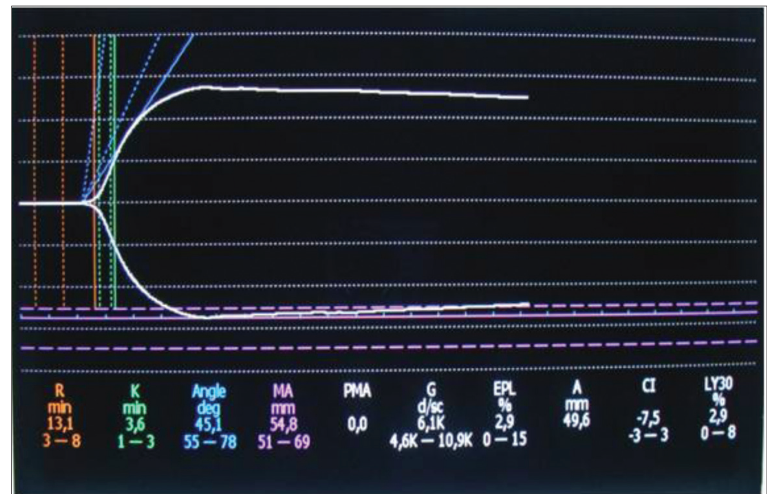

Fig 2. TEG analysis result seems to be a normal except minor prolongation of R time and indicated a normalisation of coagulation system after Vit. K1 treatment

Şekil 2. TEG analizi sonucu, R süresinin hafif bir uzaması haricinde, normal görülmektedir ve Vit.K1 tedavisi sonrasında koagülasyon sisteminin düzeldiğini göstermektedir

(FII, VII, IX and X). K (3.6 min, ref.: 1.3-5.7 min), α-angle (45.1 degree, ref.: 36.9-74.6 degree), MA (54.8 mm, ref.: 42.9-67.9 mm) and G values (6.1 Kdyn/cm^2, ref.: 3.2-9.6 Kdyn/cm^2) indicated a normal and stable clot formation for this case, as well [4].

In conclusion, TEG measurements are clinically relevant in diagnosis of dogs with bleeding and also useful for monitoring treatment. TEG analysis are easy to run and need a short time period to give information of haemostasis. Also, clinician should be kept in mind that TEG results have a higher negative or positive predictive value in identifying bleeding dogs than PT, aPTT and D-dimer, which are widely used in veterinary medicine [1,3].

REFERENCES

1. Valchev I, Binev R, Yordanova V, Nikolov Y: Anticoagulant rodenticide intoxication in animals - A review. *Turk J Vet Anim Sci,* 32 (4): 237-243, 2008.

2. Ok M, Er C, Yıldız R, Cöl R, Aydoğdu U, Sen I, Güzelbektas H: Evaluation of acute phase proteins, some cytokines and hemostatic parameters in dogs with sepsis. *Kafkas Univ Vet Fak Derg,* 21, 761-766, 2015. DOI: 10.9775/kvfd.2015.13418

3. Lubas G, Caldin M, Wiinber B, Kristensen AT: Laboratory Testing of Coagulation Disorders. **In,** Weiss DJ, Wardrop KJ (Eds): Schalm's Veterinary Hematology. 6th ed., 1082-1100, Wiley-Blackwell, Iowa, 2010.

4. Bauer N, Eralp O, Moritz A: Establishment of reference intervals for kaolin-activated thromboelastography in dogs including an assessment of the effects of sex and anticoagulant use. *J Vet Diag Invest,* 21, 641-648, 2009.

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3- Yazarlar yayınlamak istedikleri makale ile ilgili olarak gerekli olan etik kurulu onayı aldıkları kurumu ve onay numarasını Materyal ve Metot bölümünde belirtmelidirler. Yayın kurulu gerekli gördüğünde etik kurul onay belgesini ayrıca isteyebilir.

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Orijinal Araştırma Makaleleri, yeterli bilimsel inceleme, gözlem ve deneylere dayanarak bir sonuca ulaşan orijinal ve özgün çalışmalardır.

Türkçe yazılmış makaleler Türkçe başlık, Türkçe özet ve anahtar sözcükler, yabancı dilde başlık, yabancı dilde özet ve anahtar sözcükler, Giriş, Materyal ve Metot, Bulgular, Tartışma ve Sonuç ile Kaynaklar bölümlerinden oluşur ve toplam (metin, tablo, şekil vs dahil) 12 sayfayı geçemez. Yabancı dilde yazılmış makaleler yabancı dilde başlık, yabancı dilde özet ve anahtar sözcükler, Türkçe başlık, Türkçe özet ve anahtar sözcükler dışında Türkçe makale yazım kurallarında belirtilen diğer bölümlerden oluşur. Türkçe ve yabancı dilde özetlerin her biri yaklaşık 200±20 sözcükten oluşmalıdır.

Kısa Bildiri, konu ile ilgili yeni bilgi ve bulguların bildirildiği fakat orijinal araştırma olarak sunulamayacak kadar kısa olan yazılardır. Kısa bildiriler, orijinal araştırma makalesi formatında olmalı, fakat özetlerin her biri 100 sözcüğü aşmamalı, referans sayısı 15'in altında olmalı ve 6 sayfayı aşmamalıdır. Ayrıca, en fazla 4 şekil veya tablo içermelidir.

Ön Rapor, kısmen tamamlanmış, yorumlanabilecek aşamaya gelmiş orijinal bir araştırmanın kısa (en çok 4 sayfa) anlatımıdır. Bunlar orijinal araştırma makalesi formatında yazılmalıdır.

Gözlem (Olgu Sunumu), uygulama, klinik veya laboratuar alanlarında ender olarak rastlanılan olguların sunulduğu makalelerdir. Bu yazıların başlık ve özetleri orijinal makale formatında yazılmalı, bundan sonraki bölümleri Giriş, Olgunun Tanımı, Tartışma ve Sonuç ile Kaynaklar bölümlerinden oluşmalı ve 4 sayfayı geçmemelidir.

Editöre Mektup, bilimsel veya pratik yararı olan bir konunun veya ilginç bir olgunun resimli ve kısa sunumudur ve 2 sayfayı geçmemelidir.

Derleme, güncel ve önemli bir konuyu, yazarın kendi görüş ve araştırmalarından elde ettiği bulguların da değerlendirildiği özgün yazılardır. Bu yazıların başlık ve özet bölümleri orijinal araştırma makalesi formatında yazılmalı, bundan sonraki bölümleri Giriş, Metin, Sonuç ve Kaynaklar bölümlerinden oluşmalı ve 12 sayfayı geçmemelidir.

5- Makale ile ilgili gerek görülen açıklayıcı bilgiler (tez, proje, destekleyen kuruluş vs) makale başlığının sonuna üst simge olarak işaret konularak makale başlığı altında italik yazıyla belirtilmelidir.

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Kaynak dergi ise, yazarların soyadları ve ilk adlarının başharfleri, makale adı, dergi adı (orijinal kısa ad), cilt ve sayı numarası, sayfa numarası ve yıl sıralamasına göre olmalı ve aşağıdaki örnekte belirtilen karakterler dikkate alınarak yazılmalıdır.

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Örnek: **Mcllwraith CW:** Disease of joints, tendons, ligaments, and related structures. **In,** Stashak TS (Ed): Adam's Lameness in Horses. 4th ed., 339-447, Lea and Febiger, Philadelphia, 1988.

DOI numarası bulunan kaynaklarda bu bilgi ilgili kaynak künyesinin sonuna eklenmelidir.

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Case Reports describe rare significant findings encountered in the application, clinic and laboratory of related fields. The title and summary of these articles should be written in the format of full-length original articles and the remaining sections should follow Introduction, Case History, Discussion and References without exceeding the total of 4 pages.

Letters to the Editor are short and picture-documented presentations of subjects with scientific or practical benefits or interesting cases without exceeding 2 pages.

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Example: **Gokce E, Erdogan HM:** An epidemiological study on neonatal lamb health. *Kafkas Univ Vet Fak Derg,* 15 (2): 225-236, 2009.

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DOI number should be added to the end of the reference.

In the references can be reached online only, the web address and connection date should be added at the end of the reference information. The generally accepted scientific writing instructions must be complied with the other references.

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