ISSN 1300 - 6045
e-ISSN 1309 - 2251

KAFKAS ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Journal of the Faculty of Veterinary Medicine, Kafkas University

(Yılda altı sayı yayımlanır)

(Published Bi-monthly)

http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

Cilt / Volume : 19

Sayı / Number : 4 TEMMUZ-AĞUSTOS / JULY - AUGUST

Yıl / Year : 2013

ISSN: 1300-6045
e-ISSN: 1309-2251

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This journal is indexed and abstracted by Thomson Reuters Services beginning with Volume 13 (1) 2007 in the followings:

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Bu dergi Kafkas Üniversitesi Veteriner Fakültesi tarafından iki ayda bir yayımlanır
This journal is published bi-monthly, by the Faculty of Veterinary Medicine, University of Kafkas

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Kafkas Üniversitesi Veteriner Fakültesi Dergisi Editörlüğü
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Fax: +90 474 2426853
E-mail: vetdergi@kafkas.edu.tr

E-ISSN: 1309-2251

ELEKTRONİK BASKI (ELECTRONIC EDITION)
http://vetdergi.kafkas.edu.tr

ONLINE MAKALE GÖNDERME (ONLINE SUBMISSION)
http://vetdergikafkas.org

BASKI (PRINT)
ESER OFSET MATBAACILIK Tel: +90 442 2334667 ERZURUM

Bu Sayının Hakem Listesi *(alfabetik sıra)*
The Referees List of This Issue *(in alphabetical order)*

AÇIKGÖZ Zümrüt	Ege Üniversitesi Ziraat Fakültesi Zootekni Bölümü
AKBAŞ Yavuz	Ege Üniversitesi Ziraat Fakültesi Zootekni Bölümü
ASLANTAŞ Özkan	Mustafa Kemal Üniversitesi Veteriner Fakültesi Mikrobiyoloji Anabilim Dalı
AVCI Mehmet	Harran Üniversitesi Veteriner Fakültesi Hayvan Besleme ve Beslenme Hastalıkları Anabilim Dalı
AYAŞAN Tugay	Doğu Akdeniz Tarımsal Araştırma Enstitüsü Müdürlüğü
BELGE Ali	Adnan Menderes Üniversitesi Veteriner Fakültesi Cerrahi Anabilim Dalı
ÇAKIR Ahmet	Ankara Üniversitesi Veteriner Fakültesi Anatomi Anabilim Dalı
ÇANKAYA Soner	Ondokuz Mayıs Üniversitesi Zirrat Fakültesi Zootekni Bölümü
ÇELİK İlhami	Selçuk Üniversitesi Veteriner Fakültesi Histoloji ve Embriyoloj Anabilim Dalı
ÇETİNKAYA Nurcan	Ondokuz Mayıs Üniversitesi Veteriner Fakültesi Hayvan Besleme ve Beslenme Hastalıkları Anabilim Dalı
ÇOBAN Ömer	Atatürk Üniversitesi Veteriner Fakültesi Zootekni Anabilim Dalı
ÇOBAN SABUNCUOĞLU Nilüfer	Atatürk Veteriner Fakültesi Zootekni Anabilim Dalı
DAĞOĞLU Gürdal	Fırat Üniversitesi Veteriner Fakültesi Farmakoloji ve Toksikoloji Anabilim Dalı
DEĞER Yeter	Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Biyokimya Anabilim Dalı
DEMİREL Gülcan	İstanbul Üniversitesi Veteriner Fakültesi Hayvan Besleme ve Beslenme Hastalıklkarı Anabilim Dalı
DURMAZ Rıza	Kırıkkale Üniversitesi Tıp Fakültesi Tıbbi Mikrobiyoloji Anabilim Dalı
EFE Ercan	Kahramanmaraş Sütçü İmam Üniversitesi Ziraat Fakültesi Zootekni Bölümü
ERDOĞAN Zeynep	Mustafa Kemal Üniversitesi Veteriner Fakültesi Hayvan Besleme ve Beslenme Hastalıkları Anabilim Dalı
ERENER Güray	Ahi Evran Üniversitesi Ziraat Fakültesi
ERGÜN Emel	Kırıkkale Üniversitesi Veteriner Fakültesi Histoloji ve Embriyoloji Anabilim Dalı
ERÖKSÜZ Hatice	Fırat Üniversitesi Veteriner Fakültesi Patoloji Anabilim Dalı
GÖRGÜLÜ Murat	Çukurova Üniversitesi Ziraat Fakültesi Zootekni Bölümü
GÜL Mehmet	Atatürk Üniversitesi Veteriner Fakültesi Hayvan Besleme ve Beslenme Hastalıkları Anabilim Dalı
GÜLBAHAR M. Yavuz	Ondokuz Mayıs Üniversitesi Veteriner Fakültesi Patoloji Anabilim Dalı
GÜLER Leyla	Konya Veteriner Kontrol ve Araştırma Enstitüsü
GÜRBÜZ Ümit	Selçuk Üniversitesi Veteriner Fakültesi Besin Hijyeni ve Teknoloji Anabilim Dalı
HADİMLİ Hasan Hüseyin	Selçuk Üniversitesi Veteriner Fakültesi Mikrobiyoloji Anabilim Dalı
HAYIRLI Armağan	Atatürk Üniversitesi Veteriner Fakültesi Hayvan Besleme ve Beslenme Hastalıkları Anabilim Dalı
İMİK Halit	Atatürk Üniversitesi Veteriner Fakültesi Hayvan Besleme ve Beslenme Hastalıkları Anabilim Dalı
KABAK Murat	Ondokuz Mayıs Üniversitesi Veteriner Fakültesi Anatomi Anabilim Dalı
KALAYCIOĞLU Atilla Taner	Kafkas Üniversitesi Veteriner Fakültesi Mikrobiyoloji Anabilim Dalı
KAMİLOĞLU Nadide Nabil	Kafkas Üniversitesi Veteriner Fakültesi Fizyoloji Anabilim Dalı
KARACA Fikret	Mustafa Kemal Üniversitesi Veteriner Fakültesi Dölerme ve Suni Tohumlama Anabilim Dalı
KARACA Orhan	Adnan Menderes Üniversitesi Ziraat Fakültesi Zootekni Bölümü
KARAKURUM Çağrı	Mehmet Akif Ersoy Üniversitesi Veteriner Fakültesi İç Hastalıkları Anabilim Dalı
KAYAALP Tamer	Çukurova Üniversitesi Ziraat Fakültesi Zootekni Bölümü
KAYGISIZ Ali	Kahramanmaraş Sütçü İmam Üniversitesi Ziraat Fakültesi Zootekni Bölümü
KUL Oğuz	Kırıkkale Üniversitesi Vetreriner Fakültesi Patoloji Anabilim Dalı
MATUR Erdal	İstanbul Üniversitesi Veteriner Fakültesi Fizyoloji Anabilim Dalı
MUĞLALI Hakan	Ondokuz Mayıs Üniversitesi Veteriner Fakültesi Hayvan Besleme ve Beslenme Hastalıkları Anabilim Dalı
NİSBET Cevat	Ondokuz Mayıs Üniversitesi Veteriner Fakültesi Biyokimya Anabilim Dalı
OĞUZ Halis	Selçuk Üniversitesi Veteriner Fakültesi Farmakoloji ve Toksikoloji Anabilim Dalı
ORAL Hasan	Kafkas Üniversitesi Veteriner Fakültesi Doğum ve Jinekoloji Anabilim Dalı
ORTATATLI Mustafa	Selçuk Üniversitesi Veteriner Fakültesi Patoloji Anabilim Dalı
ÖZTÜRK Dilek	Mehmet Akif Ersoy Üniversitesi VeterinerFakültesi Mikrobiyoloji Anabilim Dalı
ÖZTÜRK Ergin	Ondokuz Mayıs Üniversitesi Ziraat Fakültesi Yemler ve Hayvan Besleme Anabilim Dalı
PAPUÇCUOĞLU Serhat	İstanbul Üniversitesi Veteriner Fakültesi Dölerme ve Suni Tohumlama Anabilim Dalı
PEKCAN Zeynep	Kırıkkale Üniversitesi Veteriner Fakültesi Cerrahi Anabilim Dalı
SEZEN Yavuz	Gebze İleri Teknoloji Enstitüsü
SÖZMEN Mahmut	Ondokuz Mayıs Üniversitesi Veteriner Fakültesi Patoloji Anabilim Dalı
ŞEKER İbrahim	Fırat Üniversitesi Veteriner Fakültesi Zootekni Anabilim Dalı
TIPIRDAMAZ Sadettin	Selçuk Üniversitesi Veteriner Fakültesi Anatomi Anabilim Dalı

Bu Sayının Hakem Listesi *(alfabetik sıra)*
The Referees List of This Issue *(in alphabetical order)*

TİMURKAN Sema	Fırat Üniversitesi Veteriner Fakültesi Histoloji ve Embriyoloji Anabilim Dalı
TÜRKER Hakan	Abant İzzet Baysal Üniversitesi Fen Edebiyat Fakültesi Biyoloji Bölümü
TÜZER Erkut	İstanbul Üniversitesi Veteriner Fakültesi Parazitoloji Anabilim Dalı
URAL Kerem	Adnan Menderes Üniversitesi Veteriner Fakültesi İç Hastalıkları Anabilim Dalı
UZLU Erdoğan	Kafkas Üniversitesi Veteriner Fakültesi İç Hastalıkları Anabilim Dalı
ÜNVER Ahmet	Çanakkale Onsekiz Mart Üniversitesi Tıp Fakültesi Tıbbi Mikrobiyoloji Anabilim Dalı
YALÇIN Ebru	Uludağ Üniversitesi Veteriner Fakültesi İç Hastalıkları Anabilim Dalı
YILDIRIM UÇAR Yeliz	Erciyes Üniversitesi Veteriner FakültesiBesin Hijyeni ve Teknolojisi Anabilim Dalı
YILDIZ Hüseyin	Uludağ Üniversitesi Veteriner Fakültesi Anatomi Anabilim Dalı
YÖRÜK Mecit	Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Histoloji ve Embriyoloji Anabilim Dalı

İÇİNDEKİLER
(CONTENTS)

ARAŞTIRMA MAKALELERİ
(Research Articles)

Kafkas Univ Vet Fak Derg
19 (4): 553-560, 2013
DOI: 10.9775/kvfd.2012.8207

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

Studies on Chewing Lice (Phthiraptera: Amblycera, Ischnocera) Species from Domestic and Wild Birds in Turkey [1] [2]

Bilal DİK [1] Elif YAMAÇ [2] Uğur USLU [1]

[1] *This study was supported by Selçuk University Research Fund (BAP Project number: 11401042)*
[2] *This study has been presented as poster at the 21th National Biology Congress, Ege University, Science Faculty, İzmir, 2-7 September 2012*

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Makale Kodu (Article Code): KVFD-2012-8207

Summary

This study was performed to detect chewing lice species occurring on domestic and wild birds in Turkey between October 2010 and May 2012. For this aim, the birds were brought to Veterinary Faculty of Selçuk University and Biology Department of Science Faculty of Anadolu University were examined for their lice. The louse specimens collected from the birds were preserved in eppendorf tubes contains ethyl alcohol 70%. They were cleared in Potassium Hydroxide (KOH) 10% for a day, washed within distilled water for a day and transferred to alcohol 70%, 80%, 90% and 99% at consecutive days. Then, they were mounted on the slides in Canada balsam and identified to species. Among studied 26 bird species in 24 genera and 17 families belonging to 9 orders, seventeen species (65.38%) were found to be infested with lice and no louse specimen was found in 9 bird species (34.62%). The forty-one specimens (48.23%) of examined 85 birds were found to be infested with the lice, and 32 lice species were detected. Besides, the genera *Falcolipeurus, Coloceras* and *Struthiolipeurus*, the species; *Brueelia apiastri* (Denny, 1842); *Brueelia munda* (Nitzsch, 1866); *Coloceras piageti* (Johnston ve Harrison, 1912); *Coloceras israelensis* (Tendeiro, 1974); *Degeeriella nisus* (Giebel, 1866); *Degeeriella phylctopygus* (Nitzsch, 1861); *Degeeriella rufa* (Burmeister, 1838); *Falcolipeurus quadripustulatus* (Burmeister, 1838); *Laemobothrion vulturis* (Fabricius, J.C., 1775); *Menacanthus orioli* (Blagovestchensky, 1951); *Philopterus fringillae* (Scopoli, 1772); *Struthiolipeurus struthionis* (Gervais, 1844) were recorded for the first time from the birds in Turkey. *Columbicola columbae* was found on the Long-eared Owl as a first record.

Keywords: *Ardeicola, Falcolipeurus, Brueelia, Ciconophilus, Coloceras, Colpocephalum, Laemobothrion, Piagetiella, Struthiolipeurus, Philopterus*

Türkiye'deki Evcil ve Yabani Kuşlarda Bulunan Çiğneyici Bit (Phthiraptera: Amblycera, Ischnocera) Türleri Üzerine Araştırmalar

Özet

Bu araştırma Türkiye'deki evcil ve yabani kuşlarda görülen bit türlerini belirlemek amacıyla Ekim 2010 -Mayıs 2012 tarihleri arasında yapılmıştır. Bu amaçla Selçuk Üniversitesi Veteriner Fakültesi Kliniklerine ve Anadolu Üniversitesi Fen Fakültesi Biyoloji Bölümü'ne getirilen hasta, yaralı veya ölü kuşlarla, karayollarında ölü olarak bulunan kuşlar laboratuvar ortamında bit yönünden incelenmiştir. Toplanan bitler, içinde %70 alkol bulunan eppendorf tüplerde saklanmıştır. Bitler %10'luk Potasyum Hidroksit (KOH) içinde bir gün süreyle saydamlaştırılmış, 24 saat süreyle distile suda yıkanmış ve birer gün süreyle %70, %80, %90 ve %99'luk alkol serilerinden geçirildikten sonra, Kanada balsamı ile lam üzerine yapıştırılmış ve tür seviyesinde teşhis edilmişlerdir. İncelenen 9 takım, 17 aile, 24 cinse ait 26 kuş türünden 17'si (%65.38) bitlerle enfeste bulunmuş, 9 kuş (%34.62) türünde ise herhangi bir bite tesadüf edilmemiştir. Muayene edilen 85 kuşun 41'i (%48.23) bitlerle enfeste bulunmuş ve 32 bit türü saptanmıştır. *Falcolipeurus, Coloceras* ve *Struthiolipeurus* cinsleri ile; *Brueelia apiastri* (Denny, 1842); *Brueelia munda* (Nitzsch, 1866); *Coloceras piageti* (Johnston ve Harrison, 1912); *Coloceras israelensis* (Tendeiro, 1974); *Degeeriella nisus* (Giebel, 1866); *Degeeriella phylctopygus* (Nitzsch, 1861); *Degeeriella rufa* (Burmeister, 1838); *Falcolipeurus quadripustulatus* (Burmeister, 1838); *Laemobothrion vulturis* (Fabricius, J.C., 1775); *Menacanthus orioli* (Blagovestchensky, 1951); *Philopterus fringillae* (Scopoli, 1772); *Struthiolipeurus struthionis* (Gervais, 1844) Türkiye'deki kuşlardan ilk kez bildirilmiştir. Ayrıca, Kulaklı Orman Baykuşunda *C. columbae*'ye ilk kez rastlanmıştır.

Anahtar sözcükler: *Ardeicola, Falcolipeurus, Brueelia, Ciconophilus, Coloceras, Colpocephalum, Laemobothrion, Piagetiella, Struthiolipeurus, Philopterus*

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INTRODUCTION

One of the most common bird diseases is parasitic ones which are mostly caused by ectoparasites. Lice and mites are known as common ectoparasites of the birds. Although parasitic lice species on birds were previously considered order or suborder Mallophaga, according to recent classification, bird lice are belonging to the suborders Ischnocera and Amblycera in the order Phthiraptera. More than 250 genera and 6.000 lice species were described until now; about 4.500 species are valid species [1].

Although, Turkey has 468 species of bird [2], there is limited number of investigations about the lice species found on the birds in Turkey [3-6]. The number of lice species reported from the birds in Turkey has been reached about 100 species [7-38]. Although, most of the studies about lice species have been focused on only chickens [3,7,9,11,39-41].

As a result of the published studies, some information has been obtained with regard to the lice fauna found on bird species in Turkey. On the other hand, existing information has included 20% of known bird species in Turkey. Thus, it can be argued that our knowledge is not sufficient and we need to perform much more studies on the lice fauna of birds in Turkey. This study was performed to detect chewing lice species occurring on domestic and wild birds in Turkey.

MATERIAL and METHODS

This study was carried out to detect the lice species on the birds in Turkey between October 2010 and May 2012. The chewing lice were collected from 85 domestic and wild bird specimens that were found to be injured or died in the field or obtained from hunters during legal bird hunting seasons. The feathers of each bird were carefully examined and lice were removed with forceps. After visual examination, Bolfo (1% (w/w) 2-Isopropoxyphenyl N-methyl-carbamate) or Biyo Avispray (0.09% (v/w) Tetramethrin and 0.45 (w/w) piperonyl butoxide) were applied to birds placed in a white basin and were kept at least 15 min. The collected lice specimens were fixed in 70% ethanol. After clearing in 10% KOH for 24 h, they were washed with distilled water for 24 h and passed in alcohol series 70%, 80%, 90% and 99% in four consecutive days. The lice specimens were mounted on the slides in Canada balsam by using stereo-zoom microscope, and they were kept for drying in an incubator 50ºC for a few weeks. The lice species were examined under light microscope and were identified to species according to the relevant literatures [42-47].

RESULTS

Only one bird individual in the orders Struthioniformes, Pelecaniformes, Ciconiiformes and Coraciiformes, two bird samples in the orders Columbiformes and Psittaciformes, three bird samples in the order Strigiformes and eight bird specimens in the orders Falconiformes and Passeriformes were examined. While, seventeen of twenty six bird species (65.38%) were found to be infested, no louse was found on studied nine bird species (34.62%). It was determined that forty one of a total of eighty five birds (48.23%) has one louse species on their body, at least. A total of 32 lice species were determined, which of 11 and 21 species belongs to the suborders Amblycera and Ischnocera, respectively. The comprehensive information about the lice species found on the birds was presented in *Table 1*.

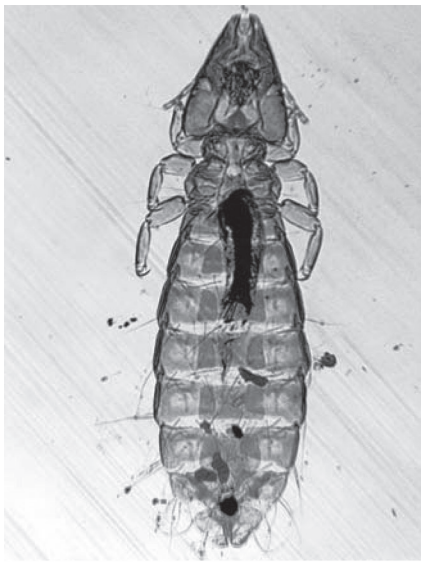

Fig 1. *Brueelia apiastri*, female, original
Şekil 1. *Brueelia apiastri,* dişi, orijinal

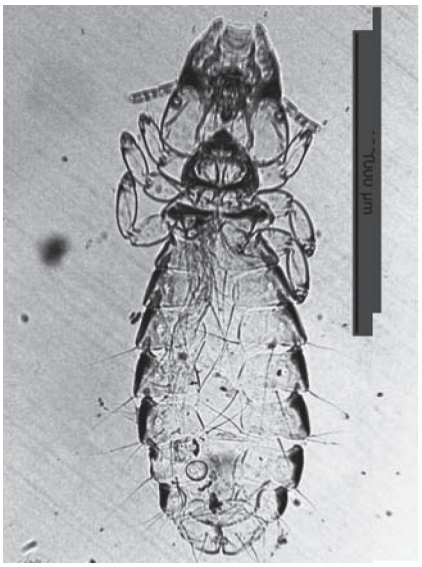

Fig 2. *Brueelia munda,* female, original
Şekil 2. *Brueelia munda,* dişi, orijinal

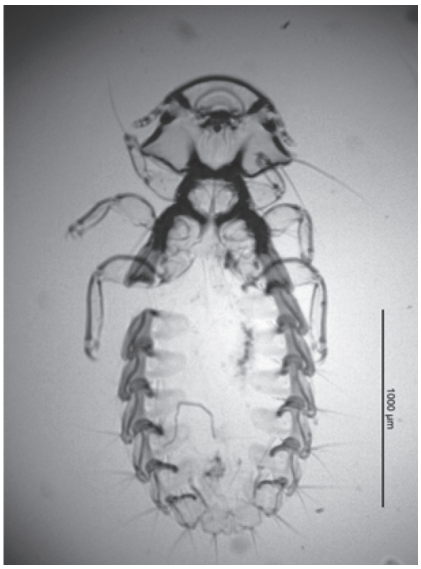

Fig 3. *Coloceras israelensis,* female, original
Şekil 3. *Coloceras israelensis,* dişi, orijinal

Table 1. *Studied bird species and detected lice species*
Tablo 1. *İncelenen kuş türleri ve tespit edilen bit türleri*

Order	Family	Genus	Species	Examined Bird Number	Infested Bird Number	Lice Species
Struthioniformes	Struthionidae	*Struthio*	*S. camelus* Ostrich	1	1	*Struthiolipeurus struthionis* *
Pelecaniformes	Pelecanidae	*Pelecanus*	*P. onocrotalus* Great White Pelican	2	2	*Colpocephalum eucarenum* *Pectinopygus forficulatus* *Piagetiella titan*
Ciconiiformes	Ciconiidae	*Ciconia*	*C. ciconia* White Stork	1	1	*Ardeicola ciconiae* *Ciconophilus quadripustulatus* *Colpocephalum zebra* *Neophilopterus incompletus*
Falconiformes	Accipitridae	*Aegypius*	*A. monachus* Eurasian Black Vulture	1	1	*Falcolipeurus quadripustulatus* * *Laemobothrion vulturis* * *Colpocephalum trachelioti*
		Circus	*C. aeruginosus* WesternMarsh-harrier	2	1	*Colpocephalum turbinatum* ** *Degeeriella fusca*
		Accipiter	*A. nisus* Eurasian Sparrowhawk	3	2	*Colpocephalum nanum* *Degeeriella nisus* *
			A. brevipes Levant Sparrowhawk	1	-	-
		Buteo	*B. buteo* Common Buzzard	2	2	*Degeeriella fulva* *Kurodaia fulvofasciata*
			B. rufinus Long-legged Buzzard	13	11	*Colpocephalum nanum* *Craspedorrhynchus platystomus* *Degeeriella fulva* *Laemobothrion maximum*
	Falconidae	*Pernis*	*P. apivorus* European Honey-buzzard	1	1	*Degeeriella phlyctopygus* *
		Falco	*F. tinnunculus* Common Kestrel	1	1	*Degeeriella rufa* *
Columbiformes	Columbidae	*Columba*	*C. livia* Rock Pigeon	4	4	*Columbicola columbae* *Coloceras israelensis* *
		Streptopelia	*S. decaocto* Eurasian Collared-dove	2	1	*Coloceras piageti* *
Psittaciformes	Psittacidae	*Melopsittacus*	*M. undulatus* Budgerigar	8	-	-
	Cacatuidae	*Nymphicus*	*N. hollandicus* Cockatiel	1	-	-
Strigiformes	Strigidae	*Athene*	*A. noctua* Little Owl	2	-	-
		Asio	*A. otus* Long-eared Owl	4	4	*Strigiphilus barbatus* *Columbicola columbae* **
Coraciiformes	Meropidae	*Merops*	*M. apiaster* European Bee-eater	1	1	*Brueelia apiastri* * *Meropoecus sp*
Passeriformes	Motacillidae	*Motacilla*	*M. alba* White Wagtail	1	-	-
	Sylviidae	*Sylvia*	*S. atricapilla* Blackcap	1	-	-
	Corvidae	*Pica*	*P. pica* Eurasian Magpie	1	-	-
	Oriolidae	*Oriolus*	*O. oriolus* Eurasian Golden Oriole	1	1	*Brueelia munda* * *Menacanthus orioli* *
	Sturnidae	*Sturnus*	*S. vulgaris* Common Starling	4	4	*Brueelia nebulosa* *Menacanthus eurysternus* **
	Passeridae	*Passer*	*P. domesticus* House Sparrow	22	3	*Menacanthus eurysternus* ** *Philopterus fringillae* *
	Fringillidae	*Fringilla*	*F. coelebs* Eurasian Chaffinch	1	-	-
		Carduelis	*C. cannabina* Eurasian Linnet	4	-	-
Total				85	41	

** First record in Turkey, ** First record from this host in Turkey*

Brueelia apiastri (Denny, 1842) *(Fig. 1); Brueelia munda* (Nitzsch, 1866) *(Fig. 2); Coloceras israelensis* (Tendeiro, 1974) *(Fig. 3); Coloceras piageti* (Johnston ve Harrison, 1912) *(Fig. 4); Degeeriella nisus* (Giebel, 1866) *(Fig. 5); Degeeriella phylctopygus* (Nitzsch, 1861) *(Fig. 6); Degeeriella rufa* (Burmeister, 1838) *(Fig. 7); Falcolipeurus quadripustulatus* (Burmeister, 1838) *(Fig. 8); Laemobothrion vulturis* (Fabricius, J.C., 1775) *(Fig. 9), Menacanthus orioli* (Blagovestchensky, 1951) *(Fig. 10); Philopterus fringillae* (Scopoli, 1772) *(Fig. 11); Struthiolipeurus struthionis* (Gervais, 1844) *(Fig. 12)* were reported for the first time in Turkey.

DISCUSSION

It was stated that, 3910 out of 5642 described lice species from birds had been approved as valid species about ten years ago. Among them, 2737 and 1173 species are belonging to the suborders Ischnocera and Amblycera, respectively [1]. In spite of increased number of investigations about lice fauna found on bird species; the numbers of investigated bird and reported lice species are still insufficient in Turkey. Approximately, 20% of total bird species have been examined for their lice species and no louse was found on some of them. The results of the studies carried out on chewing lice fauna in the birds in Turkey until now have showed that, the numbers of the lice species recorded have been reached about 100.

In this study, 85 bird individuals of 26 species in 24 genera, 17 families belonging to the 9 orders were examined. Therefore we can argue that the presented study is the most comprehensive investigation on the lice species on birds in Turkey. Forty-one bird individuals

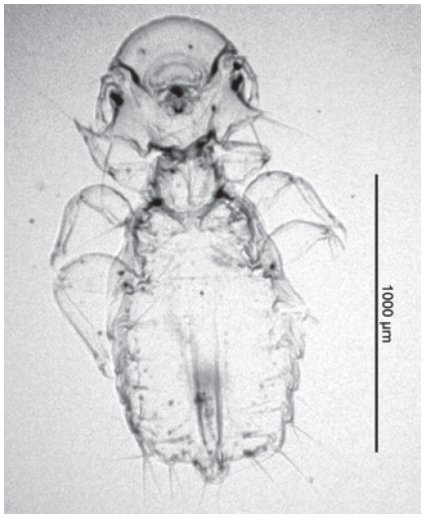

Fig 4. *Coloceras piageti*, male, original
Şekil 4. *Coloceras piageti*, erkek, orijinal

Fig 5. *Degeeriella nisus*, female, original
Şekil 5. *Degeeriella nisus,* dişi, orijinal

Fig 6. *Degeeriella phlyctopygus*, female, original
Şekil 6. *Degeeriella phlyctopygus*, dişi, orijinal

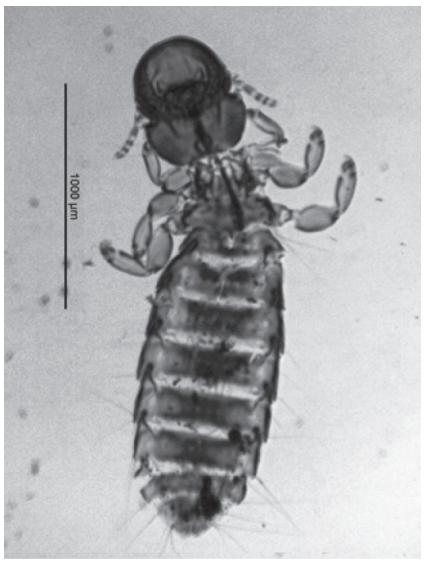

Fig 7. *Degeeriella rufa,* female, original
Şekil 7. *Degeeriella rufa,* dişi, orijinal

Fig 8. *Falcolipeurus quadripustulatus,* female, orig.
Şekil 8. *Falcolipeurus quadripustulatus,* dişi, orij.

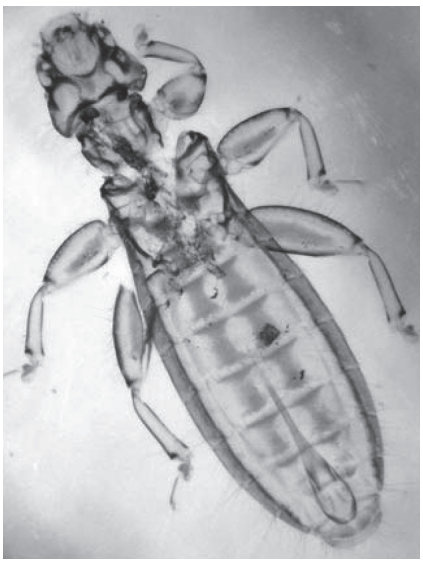

Fig 9. *Laemobothrion vulturis,* male, original
Şekil 9. *Laemobothrion vulturis*, erkek, orijinal

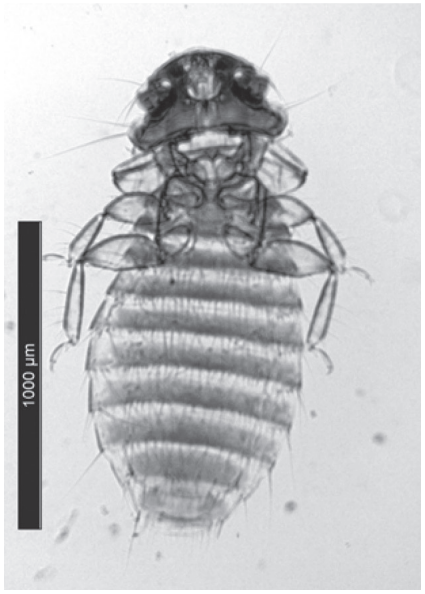

Fig 10. *Menacanthus orioli,* female, original
Şekil 10. *Menacanthus orioli,* dişi, orijinal

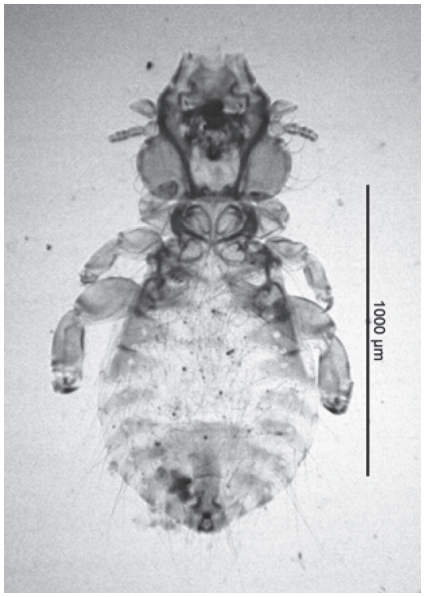

Fig 11. *Philopterus fringillae,* female, original
Şekil 11. *Philopterus fringillae,* dişi, orijinal

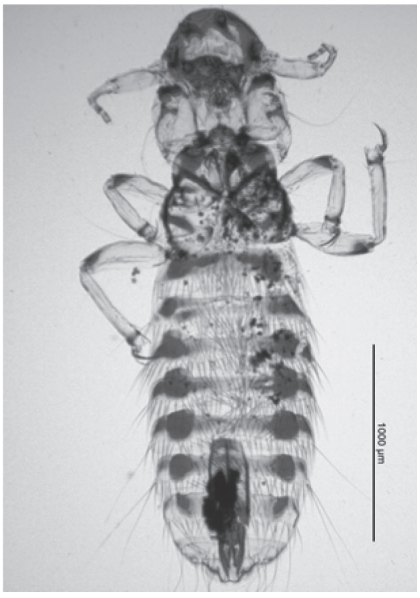

Fig 12. *Struthiolipeurus struthionis,* male, original
Şekil 12. *Struthiolipeurus struthionis,* erkek, orij.

belonging to seventeen species (65.38%) were found to be infested by the lice, while no louse was found on nine bird species (34.62%). Totally, 32 lice species, which of 11 species in six genera and 21 species in 13 genera are belonging to the suborders Amblycera and Ischnocera were detected, respectively. While the bird individuals and species numbers was not same for each order, infestation rates were 100% for Struthioniformes, Pelecaniformes, Ciconiiformes and Coraciiformes, 83.33% for Columbiformes, 79.17% for Falconiformes, 66.66% for Strigiformes and 22.86% for Passeriformes. But, it could not be detected any louse specimen on the order Psittaciformes.

The numbers of recorded lice species on the orders Falconiformes and Passeriformes were more than the others, due to the bird samples examined in these orders were much more from the others. Twenty-four individuals belonging to the eight bird species in the order Falconiformes were examined and 13 lice species were detected. For the order Passeriformes, total 35 individuals belonging to eight bird species were examined and five lice species were identified. Although, the order Passeriformes was the second order for lice species; the numbers of lice species detected on the birds in this order were less than the others, compared to the examined bird numbers.

Chewing lice usually parasitise on their specific host, but some of them are cosmopolitan and can found on different bird species. Twenty-eight lice species were found on their normal host; *C. columbae, C. nanum, D. fulva* and *M. eurysternus* were occurred on different bird species, as well as their normal hosts, in this study. *Colpocephalum nanum* was collected from Eurasian Sparrow Hawk and Long-legged Buzzard in this study. This louse species has also been reported on common Buzzard and Long-legged Buzzard in Turkey [16,21,27], previously. *Menacanthus eurysternus* has been recorded from several bird species throughout the world [1], but not in Turkey until now. Dik et al.[41] recorded that, *Brueelia nebulosa, Sturnidoecus sturni* and *Myrsidea cucullaris*, but no found *Menacanthus eurysternus* on Eurasian Starlings *(Sturnus vulgaris)* in a previous study. Three European Starlings and two House Sparrows *(Passer domesticus)* were found to be infested by *M. eurysternus*. Thus, *M. eurysternus* is recorded from these hosts for the first time and this species a new record for Turkey lice fauna. *Columbicola columbae* is found on rock pigeon *(Columba livia)*. However, this species was found on a Long-eared Owl *(Asio otus)* as well as Rock Pigeon in this study.

In a previous study, no louse species was found on 13 house sparrows [31]. Seventy-two individuals from over 10 different bird species belonging to the order Passeriformes had been examined near Aras River in Kars and infestation rate was determined as 12.50% [35]. In another report for louse species found on the birds belonging to the order Passeriformes in Kuyucuk Lake in Kars, 51 bird specimens representing 22 genera were examined and five lice species were detected from 11 bird individuals, and infestation rate was detected as 21.57% [37]. In present study, 35 bird specimens belonging to the eight species in the order Passeriformes were examined, infestation rate was found as 22.86% and five lice species; *B. munda, B. nebulosa, M. eurysternus, M. orioli* and *P. fringillae* were detected. Infestation rate was relatively low in this study as well as the other studies [31,35,37], because of only three of examined 22 House Sparrows had been infested for the louse specimens. This result related House Sparrow in this study is similar with the results of Dik [31] and its reasons

have been discussed in a previous paper [37]. As a result, *B. munda, M. orioli* and *P. fringillae* detected on Passeriformes birds were recorded for the first time in Turkey.

The genera *Colpocephalum, Kurodaia, Laemobothrion, Craspedorrhynchus* and *Degeeriella* were reported from the Falconiformes birds, previously. *Laemobothrion maximum, C. nanum, Colpocephalum* sp, *D. fulva* and *C. platystomus* have been reported from Long legged Buzzard *(Buteo rufinus)* [16,20,21,24,27,31,34]. We have also confirmed the presence of *L. maximum, C. nanum, C. platystomus* and *D. fulva* on Long-legged Buzzard in this study. Besides, it was found that prevalence of *C. platystomus* and *D. fulva* were higher and of *L. maxiumum* was lower than the other species. Dik and Yamaç [24] have reported that, *C. trachelioti* was found on Eurasian Black Vulture *(Aegypius monachus)* in Turkey. *Colpocephalum trachelioti, F. quadripustulatus* and *L. vulturis* were found on the Eurasian Black Vulture in this study. The genus *Falcolipeurus* and the species *L. vulturis* were firstly reported from Turkey. *Kurodaia fulvofasciata* was found on Common Buzzard *(Buteo buteo)* and reported as a first record for Turkey in a previous study [31]. İnci et al.[34] determined that all of lice species, except *K. fulvofasciata,* found on Common Buzzards also has been detected on the Long-legged Buzzards. Two Common Buzzard samples examined and they were infested with *D. fulva* and *K. fulvofasciata* in this study. Besides, İnci et al.[34] have also recorded that, *D. fulva* and *Colpocephalum* sp. from European Honey-Buzzards in Middle Anatolia. Additionally, *D. phlyctopygus* had been found on European Honey-Buzzard as a first record for Turkey in that study [34]. In previously studies; the Western Marsh-Harrier *(Circus aeruginosus)*, Northern Goshawk *(Accipiter gentilis)* and Common Kestrel *(Falco tinnunculus)* were reported as hosts of *D. fusca* [27], *Colpocephalum polonum* [36], and *Colpocephalum subzerafae* and *Laemobothrion tinnunculi* [38], respectively in Turkey. There was no detected any louse species on the Northern Goshawk in present study, while the Common Kestrel was infested by *D. rufa* and one of the Western Marsh-Harrier by *C. turbinatum, D. fusca*. Thus, *D. rufa* was reported for the first time in Turkey. *Colpocephalum turbinatum* occurs on Rock Pigeon [4] and some raptor species [1]. However, this species was recorded for the first time on Western Marsh-Harrier in Turkey. But, *C. subzerafae* and *L. tinnunculi* reported from Common kestrel by Ulutaş Esatgil et al.[38] could not found in this study.

Studies on the lice species occurring on the Owls (Strigiformes) are very limited in Turkey. Dik and Uslu [22] reported that, *Strigiphilus strigis* has been recorded from Eurasian Eagle-Owl *(Bubo bubo)* for the first time in Turkey. *Strigiphilus barbatus* has been firstly reported by Dik [31] and then by the other researchers [34,36] from Long-eared Owl *(Asio otus)* in Turkey. Two owl species were examined in this study and no louse species was found on the Little Owl *(Athene noctua),* while the four Long-eared Owl specimens were found to be infested with the lice. Three of them were infested by *S. barbatus* and the other was parasitized with *Columbicola columbae*, although this species is a specific for Rock Pigeon. As far as we know, this is the first record of this host-parasite association. This association can be explained by transferring of louse specimens from Rock Pigeon to Long-eared Owl while hunting. Louse individuals collected from the Owl was alive. Therefore, adaptation of *C. columbae* to owl as a new host could be another alternative explanation for this association.

Lice species in the genera *Bonomiella, Hohorstiella, Campanulates, Coloceras, Colpocephalum Columbicola* and *Physconelloides* occurs on the birds in the order Columbiformes [1]. *Hohorstiella lata, Colpocephalum turbinatum, Campanulates compar* and *C. columbae* have been recorded from Rock Pigeon in Turkey, previously [4,6,8,10,14,36,40]. The results of those studies showed that, the prevalence of *C. columbae* and *C. compar* were higher than the other species [4,6,8,10,14]. In a study, *Columbicola bacillus* was reported from Eurasian Collared Dove *(Streptopelia decaocto)* as a new record for this host in Turkey [32]. In this study, six bird specimens (4 Rock Pigeons and 2 Eurasian Collared Doves) in the order Columbiformes were examined and, all of the specimens with except of one Eurasian Collared Dove were infested by the lice species. As a result, *Columbicola columbae* and *Coloceras israelensis* on the Rock Pigeon and *Coloceras piageti* on the Eurasian Collared Dove were found. The last two species; *C. israelensis* and *C. piageti* were the first records for Turkey lice fauna. On the other hand, *C. compar, H. lata* and *C. bacillus* were not detected in the study.

Piagetiella titan, Pectinopygus forficulatus and *Colpocephalum eucarenum* have been recorded on Great White Pelican previously in Turkey [15,18,23]. According to results of those reports, *P. titan* and *P. forficulatus* were the most common lice species on Great White Pelican, but; *C. eucarenum* was found rarely on the pelicans. Two Great White Pelican specimens were examined in this study, and both of them were infested by *P. titan* and *P. forficulatus. Colpocephalum eucarenum* was detected only one pelican. While *P. titan* and *P. forficulatus* were obtained more numbers, only two male *C. eucarenum* specimens were found on the birds. These findings are similar to the results of the previous studies. Although, Dik [15] has reported that *P. titan* can cause erosive stomatitis in a white pelican, erosive stomatitis was not detected on the pelicans infested by *P. titan* in this study.

As far as we know, there is no study about the lice species on Struthioniformes in Turkey. Price et al.[1] reported that the genus *Struthiolipeurus* and the species *S. struthionis* in this genus has been occurred on Ostrich *(Struthio camelus).* In the present study, one Ostrich specimen was examined and a lot of lice specimens were collected from this bird, and they were identified as *S. struthionis,* as the first record for Turkey.

There is a little information about the lice species found on the birds belonging to the order Ciconiiformes in Turkey. Dik and Uslu [19] have reported that, presence of *Ardeicola ciconiae, Ciconophilus quadripustulatus, Colpocephalum zebra* and *Neophilopterus incompletus* from White Stork *(Ciconia ciconia)* in Konya. In addition to that, White Stork, Eurasian Bittern *(Botaurus stellaris)* and Great Egret *(Ardea alba)* samples have been examined for the louse, recently [31,34]. Although, there was no found any louse species on the Eurasian Bitter, *A. ciconiae, C. quadripustulatus, C. zebra* and *N. incompletus* were detected on the White Storks. Also, it was stated that, *Comatomenopon elongatum* was recorded as a first record on Great Egret for Turkey in that study [34]. In this study, all of the species reported from White Stork by Dik and Uslu [19] were detected from the White Stork specimen.

There is no any detailed study on the lice species of the order Psittaciformes in Turkey. Although, some of the bird samples belonging to this order were examined, but any louse specimen was found [31]. Dik [32] reported that *Afrimenopon waar* from budgerigar *(Melopsittacus undulatus)* as a new record for Turkey. In the current study, eight budgerigars and one cockatiel *(Nymphicus hollandicus)* were examined, but any louse specimen was obtained on all of them, probably they were pet animals and provided adequate living conditions by humans.

Among the bird species belonging to the order Coraciiformes; only European Bee-eater *(Merops apiaster)* has been examined for louse, previously in Turkey. *Meromenopon meropis* and *Meropoecus meropis* have been detected on the birds in those studies [35,36]. In this study, only one European Bee-eater was examined and *Meropoecus* sp. and *Brueelia apiastri* were detected on the bird while *Meromenopon meropis* was not found. *B. apiastri* was recorded for the first time from the Eurasian Bee-eater in Turkey in this study. *Meropoecus* specimens could not be identified to species; because all of them were nymph stages.

As a result, a total of 85 bird specimens belonging to the 26 species in the orders Struthioniformes, Pelecaniformes, Ciconiiformes, Coraciiformes, Columbiformes, Psittaciformes, Strigiformes, Falconiformes and Passeriformes were examined in this study. Seventeen (65.38%) of these species were infested with lice species and no louse specimen was found in nine bird species (34.62%). Forty-one of 85 bird specimens (48.23%) were found to be infested with lice specimens and a total of 32 lice species belonging to the suborders Amblycera (11 species) and Ischnocera (21 species) were recorded. The results of the study showed that, the genera Falcolipeurus, Colocerasand Struthiolipeurus, and the species; *Brueelia apiastri; Brueelia munda; Coloceras piageti; Coloceras israelensis; Degeeriella nisus; Degeeriella phylctopygus; Degeriella rufa; Falcolipeurus quadripustulatus; Laemobothrion vulturis; Menacanthus oriole; Philopterus fringillae; Struthiolipeurus struthionis* were reported for the first time in Turkey. Also, *C. columbae* was reported from Long-eared Owl, as a first record.

Ackkowledgements

We thank to Selçuk University Research Fund for supporting this study as titled of "Türkiye'deki evcil ve yabani kuşlarda bulunan çiğneyici bit (Phthiraptera: Amblycera, Ischnocera) türleri üzerine araştırmalar" (BAP Project number: 11401042)

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Kafkas Univ Vet Fak Derg
19 (4): 561-568, 2013
DOI: 10.9775/kvfd.2012.8235

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

The Effects of Seasonal Variation on the Microbial-N Flow to the Small Intestine and Prediction of Feed Intake in Grazing Karayaka Sheep [1]

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[1] *This study was supported by Ondokuz Mayis University Research Fund (Project number: PYO.VET.1901.10.03, 2010)*

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Makale Kodu (Article Code): KVFD-2012-8235

Summary

The objectives of the present study were to estimate the microbial-N flow to the small intestine and to predict the digestible organic matter intake (DOMI) in grazing Karayaka sheep based on urinary excretion of purine derivatives (xanthine, hypoxanthine, uric acid and allantoin) by the use of spot urine sampling under field conditions. In the trial, 10 Karayaka sheep from 2 to 3 years of age were used. The animals were grazed in a pasture for ten months and fed with concentrate and vetch plus oat hay for the other two months (January and February) indoors. Highly significant linear and cubic relationships (P<0.001) were found among months for purine derivatives index, purine derivatives excretion, purine derivatives absorption, microbial-N and DOMI. Through urine sampling and the determination of levels of excreted urinary PD and the Purine Derivatives:Creatinine ratio (PDC index), microbial-N values were estimated and they indicated that the protein nutrition of the sheep was insufficient. In conclusion, the prediction of protein nutrition of sheep under the field conditions may be possible with the use of spot urine sampling, urinary excreted PD and PDC index. The mean purine derivative levels in spot urine samples from sheep were highest in June, July and October. Protein nutrition of pastured sheep may be affected by weather changes, including rainfall. Spot urine sampling may useful in modeling the feed consumption of pasturing sheep. However, further studies are required under different field conditions with different breeds of sheep to develop spot urine sampling as a model.

Keywords: *Karayaka sheep, Spot sampling, Urinary purine derivatives, PDC index, microbial-N, Feed intake*

Merada Otlayan Karayaka Koyunlarında Mevsimsel Değişimin İnce Bağırsağa Geçen Mikrobiyal-N Miktarı ve Yem Tüketimine Etkisi

Özet

Merada otlayan Karayaka koyunlarında spot idrar örneklemesi ile idrarla atılan purin türevlerinden (ksantin, hipoksantin, ürik asit ve allantoin) ince bağırsağa geçen mikrobiyal-N miktarı ve sindirilebilir organik madde tüketiminin (SOMT) mevsimsel olarak belirlenmesi amaçlanmıştır. Araştırmada 2-3 yaşlarında 10 adet Karayaka koyunu kullanılmıştır. Hayvanlara yem materyali olarak 10 ay mera ve 2 ayda (Ocak, Şubat) kapalı ağıllarda fabrika yemi ve fiğ +yulaf otu ile beslenmiştir. Purin türevleri indeksi, idrarla atılan purin türevleri, absorbe edilen purin türevleri, mikrobiyel-N ve SOMT yönünden aylara göre bir değerlendirme yapıldığında gruplar arasında linear ve kübik (P<0.001) bir ilişki tespit edilmiştir. Mikrobiyal-N değerlerine bakıldığında koyunların protein yönünden yetersiz beslendikleri görülmüştür. Sonuç olarak spot idrar toplanarak denemenin yapıldığı benzer saha koşullarında otlatılan Karayaka koyunlarda protein beslenmesini tahmin etmekte idrarla atılan pürin türevleri ve Purin Türevleri: Kreatinin (PDC indeksi) kullanılabilir. Ortalama PT miktarı Karayaka koyunları için spot idrar toplamada en yüksek değerler Haziran, Temmuz ve Ekim aylarında belirlenmiştir. Bu aylardaki iklimsel değişikliklerden (yağış fazlalığı) dolayı meradaki koyunların protein beslenmesi etkilenmiştir. Ayrıca spot idrar toplama tekniği meradaki koyunların yem tüketimini belirlemede bir model olabilir. Bunun içinde farklı saha koşullarında farklı koyun ırklarıyla benzer çalışmaların yapılmasına ihtiyaç vardır.

Anahtar sözcükler: *Karayaka koyunu, Spot örnekleme, Idrar purin türevleri, PDC indeksi, Mikrobiyal-N, Yem tüketimi*

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INTRODUCTION

The Karayaka sheep is one of the indigenous breeds reared on the coastline of the Black Sea region of Turkey. Karayaka sheep are well adapted to the wet climate of the region and there is a total population of approximately one million [1].

The use of spot urine sampling has been proposed to predict protein nutrition and digestible organic matter intake in grazing sheep [2] and goats [3]. Microbial protein flow to the small intestine has been estimated total of purine derivatives excreted in the urine of ruminants [4,5]. Rumen microbes constitute the major source of protein supply to the ruminants. The purines from the rumen microbes are metabolized and excreted in the urine as their derivatives, hypoxanthine, xanthine, uric acid and allantoin. In sheep, hypoxanthine and xanthine are converted to uric acid by xanthine oxidase, and uric acid is further converted to allantoin by uricase. All four compounds are excreted in the urine of sheep. The synthesis of microbial protein is dependent on ruminal ammonia nitrogen supply [6] and digestible organic matter intake [7,8]. Reports that estimate microbial-N flow to the small intestine are mostly from European sheep breeds [2,8-11].

The objectives of the present study were to examine the microbial-N flow to the small intestine and to predict DOMI in grazing Karayaka sheep on the basis of urinary excretion of PD by the use of spot urine sampling under field conditions and to investigate the effects of seasonal variation on ruminal microbial synthesis.

MATERIAL and METHODS

The study was approved by the Local Ethics Committee on Animal Experiments of Ondokuz Mayis University (OMU, 30.12.2009, HADYEK/139).

Animal and Feed Materials

A total of 10 Karayaka sheep aged between 2 and 3 years, and with live weight ranging from 42.4 to 47.6 kg, were used in the this study that was undertaken in Akyazı village of Bafra town, Samsun province, Turkey. The animals were grazed in a pasture for ten months and fed a concentrate and vetch plus oat hay for the other two months (January and February) indoors.

Plant samples were taken by hand clipping to ground level at the beginning of the grazing experiment and repeated every month. They were collected from one square meter area in six different locations in a 0.5-1 ha area [12]. The important part of meadow and pasture was formed by *Agropyron cristatum, Lotus corniculatus L., Agropyron elongatum, Bromus inermis, Convolvulus sp., Trifolium pretense, Trifolium repense* and *Dactylis glomerata*. All plant samples were dried to a constant weight in a forced-air oven at 65°C for 48 h. Ash content was determined by heating in a muffle furnace at 550°C [13]. Organic matter (OM) was calculated as DM–ash. Nitrogen (N) content was analyzed with the Kjeldahl method, with the use of a semi-automated N analyzer. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined according to the methodology of VAN SOEST *et al.*[14].

The levels of purine derivatives were determined according to the methodology of CHEN *et al.*[15] using a spectrophotometer (Shimadzu UV-1700). Allantoin is first hydrolyzed under weak alkaline conditions and at 100°C to allantoic acid which is further hydrolysed to urea and glyoxylic acid in a weak acid solution. The glyoxylic acid is reacted with phenylhydrazine hydrochloride to produce a phenylhydrazone derivative of the acid. The product forms an unstable chromophore with potassium ferricyanide. The colour was read at 522 nm. Xanthine and hypoxanthine are converted to uric acid by treatment with xanthine oxidase and are thus determined as uric acid, the amount of which is determined by its absorbance at 293 nm, although other compounds may also absorb at this wavelength. When samples are treated with uricase, uric acid is converted to allantoin and other compounds that do not absorb UV at 293 nm. Therefore, the reduction in absorbance reading after treatment with uricase is correlated with the concentration of uric acid in the sample. After treatment, the absorbance of the standards should be zero, if the conversion is complete. Creatinine reacts with picrate ion formed in alkaline medium and a red-orange colour develops. The colour produced from the sample is then compared in a colorimeter at 505 nm with that produced by a known amount of creatinine under the same conditions [5].

Mean monthly rainfall and air temperature (°C) in Bafra in 2010 and 2011 were collated by the Samsun Meteorological Office and are presented in *Fig. 1*.

Urinary Sample Collection

Spot urinary samples were collected from each sheep between 10:00 am and 12:00 pm (2 h after grazing) on two consecutive days per month for a year. Urine samples were collected by closing the mouth and nose of the sheep by hand. Samples were usually collected over a 10 to 35 sec period. After collection, a 10 ml sub-sample was taken, acidified with 1 ml of 10% H_2SO_4 and diluted to 1:4 with distilled water. The samples were stored at -20°C until analysis for purine derivatives.

Estimation of Microbial-N Supply

The microbial-N supply was estimated using the following equation for sheep [5].

$$Y = 0.84X + (0.150\ W^{0.75}\ e^{-0.25X})$$

Where X (mmol/d) is absorption of purines (X mmol/d)

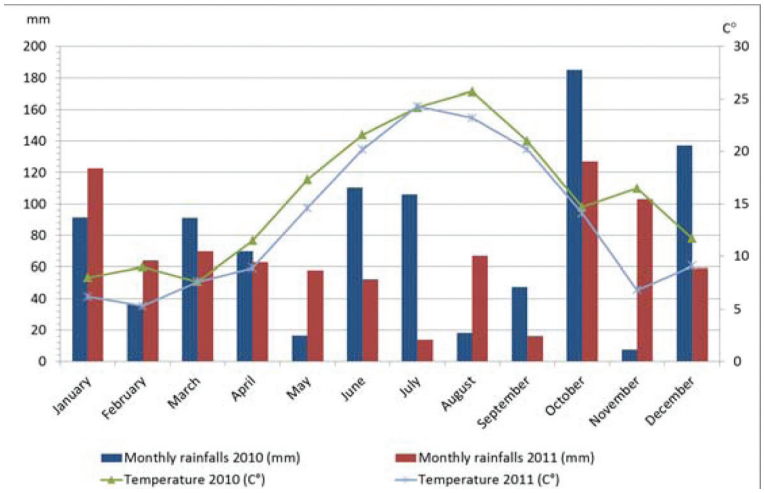

Fig 1. Mean monthly rainfall and air temperature (°C) in Bafra in 2010 and 2011

Şekil 1. Bafra'da 2010 ve 2011 yıllarında ortalama aylık yağış ve sıcaklık miktarları (°C)

and Y (mmol/d) is PD excretion in the urine. The calculation of X based on the above non-linear equation can be performed by means of the Newton Raphson iteration process, as shown below:

$X_{(n+1)} = X_n - [f(X_n) / f'(X_n)]$; where $f(X) = 0.84\ X + 0.150\ W^{0.75}\ e^{-0.25X} - Y$, and the derivative of f(X): $f'(X) = 0.84 - 0.038\ W^{0.75}\ e^{-0.25X}$

An initial value of $X_1 = Y \div 0.84$ is fed into the above equation to calculate X_2, and after the process is repeated for 4 iterations, X_5 should have reached a constant value.

The supply of microbial-N was estimated below from the relationship derived by CHEN and GOMES [11]. The assumptions used in the above equations are: digestibility of microbial purines is 0.83. the N content of purines is 70 mg N/mmol and the ratio of purine-N: total-N in mixed rumen microbes is 11.6:100 [5]. Therefore, the equation to determine microbial-N is: Microbial N (gN/d) = [X (mmol/d) x 70] / [0.116 x 0.83 x 1000]=0.727X

Microbial-N estimated from purine derivatives excretion corresponds to the quantity of microbial biomass reaching the duodenum, rather than that synthesized within the rumen. It is expressed as grams of microbial-N per kilogram of digestible organic matter apparently digested in the rumen. The model assumes that the digestible organic matter in the rumen is 65% of digestible organic matter intake [16].

Estimation of PDC Index

PDC index is determined by calculating the creatinine concentrations and total purine derivatives in the urine. The following equation is used to index the PDC:

$$PDC\ index = (PD/C) \times W^{0.75}$$

where W is the body weight (kg), and PD and C are purine derivatives and creatinine concentrations, respectively in mmol/L.

PD excretion (mmol/d) = PDC index × C where C is the daily creatinine excretion (mmol/kg $W^{0.75}$) for a specific breed of animals, which should have been previously measured from the complete urine collection. Average daily creatinine excretion was taken to be 503 µmol/kg $CA^{0.75}$ for sheep [5].

Estimation of Digestible Organic Matter İntake (DOMI)

The spot measurement of the PDC index can provide an estimate of feed intake. Digestible organic matter intake was calculated with the following equation [5].

DOMI (g /d) = 59.7xPDC-678

Statistical Analysis

Data were summarized with descriptive statistics for means, and the standard errors of the means were analyzed with analysis of variance (ANOVA), using the Least Square Method of the GLM procedure of the SAS [17]. The differences between the groups were analyzed via 3rd order orthogonal polynomials. All results were summarized as mean ± standard error of mean (SEM). Ordinary linear regression and Pearson correlation analyses were performed with the use of variables, namely allantoin, microbial-N, DOMI and purine derivatives excretion, and digestible organic matter digested in the rumen.

RESULTS

The dry matter (DM), organic matter (OM), crude protein (CD), neutral detergent fiber (NDF) and acid detergent fiber (ADF) values of meadow and pasture samples (g/kg DM) collected monthly are presented in *Table 1*. Levels of creatinine and allantoin, uric acid, hypoxhanthine and xanthine in spot urine samples are shown in *Table 2*. The purine derivatives index (PDC index), purine derivatives excretion, purine derivatives absorption, microbial-N and DOMI for spot urine samples are shown in *Table 3*. Monthly variations in both mean values of the PDC index and microbial-N *(Fig. 2)* and mean values of DOMI are presented in *Fig. 3*.

Table 1. *The nutrient composition of meadow and pasture samples collected monthly (g/kg DM)*
Tablo1. *Aylık olarak toplanan çayır ve mera örneklerinin besin madde kompozisyonu (g/kg KM)*

Period			DM	OM	CP	NDF	ADF
Years	Months						
2010	June		913.0	838.4	134.0	437.2	351.7
	July		931.2	866.5	148.0	476.8	337.7
	August		892.5	801.2	106.5	500.2	392.1
	September		909.3	830.9	143.7	479.4	334.9
	October		905.6	824.5	131.1	442.1	313.5
	November		913.0	837.6	107.5	468.4	345.5
	December		904.3	827.3	101.2	543.7	415.5
2011	January	1	902.1	829.4	118.0	613.0	469.6
	February	2	896.0	832.7	138.5	232.7	113.7
	March		917.5	851.3	127.5	410.8	264.0
	April		923.4	819.4	154.0	403.4	246.8
	May		906.8	840.3	135.7	451.6	344.0

1- *Mixed vetch and oat hay were used to feed sheep in January and February,* ***2-*** *Concentrate was used to feed sheep in January and February*

Table 2. *Mean concentrates (mmol /L) of allantoin, uric acid, hypoxanthine plus xanthine and creatinine in spot urine samples collected from grazing Karayaka sheep*
Tablo 2. *Merada otlayan Karayaka koyunlarından toplanan spot idrar örneklerinde allantoin, ürik asit, hipoksantin + ksantin ve kreatinin ortalama değerleri (mmol/L)*

Grazing Period		n	Allantoin	Uric Acid	Hypoxanthine +Xanthine	Creatinine	Purine Derivatives
Year	Months						
2010	June	9	7.67±0.37	1.76±0.18	0.65±0.06	6.39±0.19	10.08±0.44
	July	9	7.71±0.38	1.40±0,08	0.68±0.06	6.30±0.32	9.79±0.43
	August	10	6.17±0.18	1.25±0.06	0.61±0.06	6.75±0.23	8.02±0.18
	September	10	6.81±0.29	1.77±0.09	0.75±0.07	5.74±0.21	9.32±0.29
	October	10	8.62±0.58	1.59±0.12	0.68±0.04	6.49±0.28	10.90±0.66
	November	9	7.28±0.27	1.64±0.19	0.35±0.03	4.72±0.22	9.27±0.31
	December	10	5.04±0.22	1.96±0.10	0.32±0.02	5.44±0.27	7.32±0.21
2011	January	9	4.84±0.18	0.87±0.05	0.42±0.04	5.00±0.16	6.13±0.20
	February	8	6.31±0.31	1.29±0.13	0.53±0.03	6.64±0.28	8.12±0.31
	March	10	5.64±0.28	0.59±0.04	0.50±0.05	5.60±0.27	6.73±0.30
	April	10	5.12±0.20	1.29±0.09	0.54±0.06	5.61±0.15	6.95±0.24
	May	9	5.55±0.33	1.28±0.12	0.48±0.05	5.46±0.20	7.31±0.35
Significance of main effects							
Linear			***	***	NS	NS	***
Quadratic			***	NS	***	***	***
Cubic			***	NS	***	NS	***

*** *P<0.001,* ***NS:*** *Non Significant,* ***L:*** *Linear,* ***Q:*** *Quadratic,* ***C:*** *Cubic*

DISCUSSION

Measurement of microbial protein supply to sheep has been a major area of study in the context of their protein nutrition. An estimate of microbial protein contribution to the intestinal protein flow is incorporated into the new protein evaluation systems already being used in a number of countries. The supply of microbial protein to the animal per unit of feed ingested varied from 14 to 60 g microbial-N/kg digestible organic matter fermented in the rumen [16]. This variation is due to the influence of various factors related to the diet or rumen environment. As seen in *Table 1*, CP levels of plant samples collected monthly did not fluctuate all year around. The lowest CP levels were

Table 3. *Mean levels of purine derivatives index (PDC index), purine derivatives excretion, purine derivatives absorption, microbial-N supply and DOMI in spot urine samples collected from grazing Karayaka sheep*

Tablo 3. *Merada otlayan Karayaka koyunlarından toplanan spot idrar örneklerinde PDC indeksi, pürin türevleri ekskresyonu, pürin türevleri absorpsiyonu, mirobial-N ve DOMI ortalama değerleri*

Grazing Period		n	PDC Index	PD Excretion (mmol/d)	Purine Absorption (mmol/d)	Microbial-N Supply		DOMI g/d
Year	Months					(g of N/d)	(g of N/kg of DOMR)	
2010	June	9	32.29±1.51	16.24±0.76	19.28±0.92	14.02±0.67	20.87±0.73	1249.44±90.31
	July	9	31.90±1.02	16.04±0.52	19.06±0.62	13.86±0.45	20.86±0.78	1226.26±61.17
	August	10	24.36±0.74	12.25±0.37	14.48±0.45	10.53±0.33	21.06±0.70	776.13±44.04
	September	10	33.27±1.17	16.74±0.59	19.89±0.70	14.46±0.51	20.98±0.70	1308.28±69.59
	October	10	33.98±1.40	17.09±0.70	20.31±0.85	14.77±0.62	19.76±0.73	1350.41±83.61
	November	9	40.26±1.20	20.25±0.60	24.10±0.72	17.52±0.52	18.12±0.73	1725.41±71.46
	December	10	28.00±1.14	14.08±0.57	16.69±0.69	12.14±0.50	17.49±0.73	993.41±67.84
2011	January	9	24.90±0.69	12.53±0.35	14.81±0.43	10.77±0.31	21.24±0.70	808.77±41.49
	February	8	25.04±0.99	12.60±0.50	14.89±0.61	10.83±0.44	17.26±0.70	816.91±59.32
	March	10	24.71±0.92	12.43±0.46	14.69±0.56	10.68±0.41	17.29±0.70	797.47±54.68
	April	10	25.23±0.94	12.69±0.47	15.01±0.57	10.91±0.42	15.69±0.73	828.42±56.06
	May	9	27.14±0.96	13.65±0.48	16.18±0.59	11.76±0.43	19.34±0.70	942.39±57.47
Significance of main effects								
Linear			***	***	***	***	***	***
Quadratic			NS	NS	NS	NS	NS	NS
Cubic			***	***	***	***	*	*

**** P<0.001, * P<0.05,* ***NS:*** *Non Significant,* ***DOMR:*** *Digestible organic matter fermented in the rumen, calculated as 0.65xDOMI (g of N/kg of DOMR),* ***DOMI:*** *Digestible organic matter intake g/d*

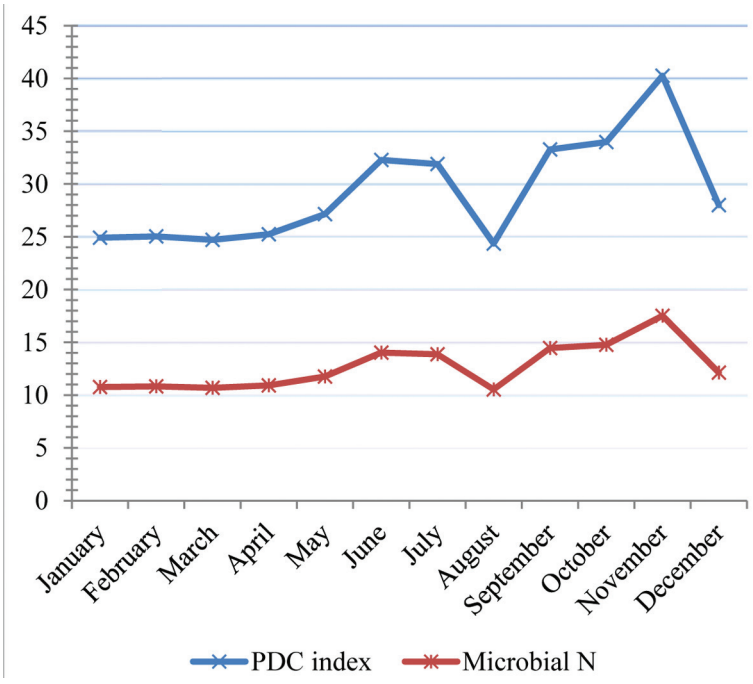

Fig 2. Monthly changes of mean values of PDC index and Microbial-N (g N/d) for grazing Karayaka sheep

Şekil 2. Merada otlayan Karayaka koyunlarında ortalama PDC index ve Mikrobial N değerlerinin aylık değişimi

observed in August and December. They are in agreement with estimated mean microbial-N and DOMI values *(Fig. 2* and *3*) for Karayaka sheep.

Creatinine is produced from creatine in muscle, and is excreted in the urine. Its excretion is correlated with the muscle mass. When expressed as 'mmol/per kg $W^{0.75}$', the daily excretion is relatively constant. The value is approximately 0.5 mmol/kg $W^{0.75}$/d in sheep [5]. The excretion of creatinine is affected minimally by the amount of protein and non-protein nitrogen consumed. In the current study, creatinine concentrations in the spot urine samples were higher in August, October and February than in other months but overall values were not significantly

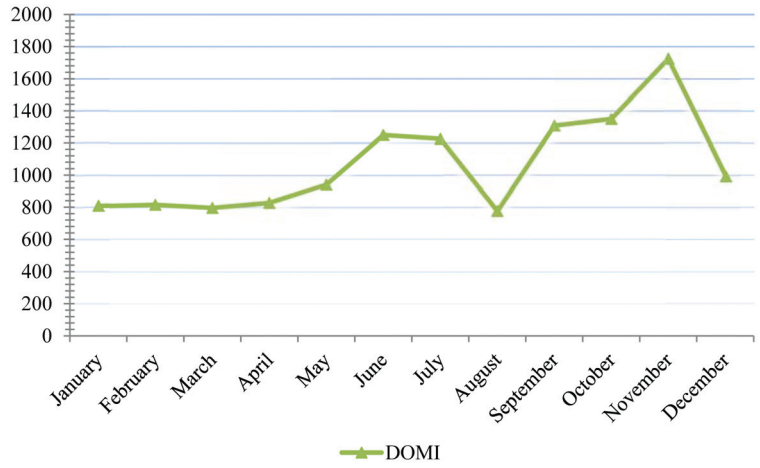

Fig 3. Monthly changes of mean values of DOMI (g/d) for grazing Karayaka sheep

Şekil 3. Merada otlayan Karayaka koyunlarında ortalama DOMI (g/gün) değerlerinin aylık değişimi

influenced by season *(Table 2)*. There was no linear or cubic relationship between creatinine excretion and month but a quadratic relationship was determined. It was also the case that season did not affect creatinine excretion in urine.

The method for estimating microbial-N production from urinary purine derivatives assumes that duodenal nucleic acids are mostly of microbial origin [15]. It has been reported that after intestinal digestion and absorption, the purine base catobolites are proportionally recovered in urine, mostly as allantoin, but also as hypoxanthine, xanthine and uric acid [18]. The present study reports 69-84%, 9-27% and 4-8%, for allantoin, uric acid and xanthine plus hypoxanthine, respectively. For the same catabolites, Chen and Gomes [11] reported the proportions to be 60-80%, 10-30% and 5-10% for allantoin, uric acid and xanthine plus hypoxanthine, respectively. Furthermore, allantoin excretion in urine was reported to be 80-85% of total purine derivatives. The profile of PD excretion in grazing Karayaka sheep was similar to that reported in previous studies [19-21]. The results obtained may indicate that the proportion of purine derivatives is independent of diet.

In the current study, the levels of allantoin in the urine of grazing sheep ranged from 4.84-8.62 mmol/L on a monthly basis across the sampling period. The study also determined a positive correlation (r=0.615, P<0.01) between the amount of allantoin excreted in urine and rumen microbial protein flowing into the small intestine, as described by the equation:

Y=0.378X+1.594

Where y is the amount of allantoin excreted in urine and x is rumen microbial protein flowing into the small intestine. This equation showed that allantoin excretion was able to enhance duodenal flow of microbial protein. Studies of cattle [22,23] and sheep [7] have indicated a high correlation between the excretion of purine derivatives and rumen microbial protein flow into the small intestine (R^2 = 0.97). In the present study, the average allantoin amounts in spot urine samples for sheep were lowest in January, April and December, whereas the highest values were determined in June, July and October. In 2010, allantoin amounts in June, July and October were higher than in other months *(Table 2)*. That phenomenon may reflect the impact of vegetation changes due to higher than average rainfall during those months.

The higher excretion of PD in October and November clearly indicates enhanced microbial protein synthesis, since significant relationships have already been reported between urinary PD excretion and the levels of nucleic acid infused in the abomasum [4,15] and duodenum [9,24]. Orellana-Boero *et al.*[25] found that the excretion of purine derivatives in the urine increased linearly (r = 0.867) with digestible organic matter intake. The principle is that duodenal purine bases are efficiently absorbed in the small intestine [24,26]. Urinary PD excretion is used to predict ruminal microbial protein synthesis. The daily excretion of purine derivatives and the microbial-N supply in grazing Karayaka sheep were found to be in the range of 12.25-20.25 and 10.53-17.52 mmol/d, respectively *(Table 3)*. These values for Karayaka sheep are within the range of those published for different sheep breeds [2,10]. The urinary PD excretion values obtained in goats [3] and wethers are similar to those observed in sheep [10,27,28]. Hence, purine derivatives in spot urine samples may provide a practical indicator of microbial protein supply status in grazing ruminants.

The estimated average monthly DOMI values *(Table 3)* in grazing Karayaka sheep were within the range of 776 to 1725 g/day. The estimation of DOMI from PDC index *(Table 3)* through the use of spot urine sampling from grazing Karayaka sheep showed that this technique may be applied in grazing animals where DOMI cannot be measured directly. Many reports have confirmed a linear relationship between allantoin excretion and both the level of feed intake and flow of nucleic acids in the duodenum [29]. Laurent *et al.*[30] determined that allantoin excretion is correlated with digestible organic matter intake, and also that allantoin excretion (r=0.54, P<0.01) can be used as an index of rumen microbial protein synthesis.

The present study also determined that digestible organic matter fermented in the rumen was converted to a similar proportion of microbial-N in all months, ranging from 16 to 21 g N/kg of digestible organic matter apparently digested in the rumen (*Fig. 2* and *3*; *Table 3*), which was similar the range reported by Yu *et al.*[31]. The higher amount of urinary allantoin reported in the present study in June, July, September, October and November was due to the increased digestible organic matter intake. The present study also determined that there were linear and cubic relationship between the PDC index, urinary excretion of purine derivatives, microbial-N and DOMI and month *(Table 3)*, and that there was a seasonal influence on these parameters.

In the current study, the estimated microbial-N values appear insufficient for adequate protein nutrition. Monthly mean values of PDC index, microbial-N and DOMI were relatively stable. However, fluctuations were observed between June and December (*Fig. 2* and *3*). The lowest values for DOMI, PDC index and microbial-N were observed in August and December (*Fig. 2* and *3*). This is due to meadow and pasture conditions reflecting the driest period of the year *(Fig. 1)*. In conclusion, protein nutrition of pastured sheep may be affected by weather changes. Spot urine sampling may serve as the basis for modeling their feed requirements. Furthermore, it may provide a basis for the preparation of balanced diets to meet the protein requirements of sheep by closely approximating the amount of rumen microbial-N flowing into the small intestine. However, further studies are required under different field conditions and with different breeds of sheep to develop the spot urine sampling technique into a model.

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Kafkas Univ Vet Fak Derg
19 (4): 569-574, 2013
DOI: 10.9775/kvfd.2012.8369

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

Effects of Graded Levels of Crude Glycerine Addition to Diets on Growth Performance, Carcass Traits and Economic Efficiency in Broiler Chickens [1][2]

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[1] *Some results of this study have been presented in 22nd International Scientific Congress as a presentation in Stara-Zagora, Bulgaria, June 7-8 2012*

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Makale Kodu (Article Code): KVFD-2012-8369

Summary

The aim of the current study was to evaluate the effects of graded levels of crude glycerine addition to diets on growth and economic performance, carcass yield, organ weights and moisture levels of meat in broiler chickens. For this purpose, a total of 270 one day old male chicks (Ross 308) were randomly divided into 3 equal groups which fed with isocaloric and isonitrogenous diets with graded levels (0%, 5% and 10%, respectively) of crude glycerine. Each of experiment group was constituted by 5 subgroups with 18 birds each. The birds had ad libitum access to feed and water until termination of the experiment at d 42. Results indicated that 5% dietary crude glycerine addition improved body weight, body weight gain and feed conversion ratio compare with birds fed with basal diet at d 42 ($P<0.01$). Similarly, dietary 10% crude glycerine supplementation also increased growth performance and improved FCR in broiler chickens at 28th and 42nd days of experiment ($P<0.05$ and $P<0.001$, respectively). Moreover, birds fed with 5% dietary crude glycerine had showed over 20% higher relative economic efficiency compare than birds fed basal diet. As a conclusion, dietary crude glycerine addition improved growth and economic performance and Feed Conversion Ratio of broilers in both supplementation levels.

Keywords: *Broiler, Carcass, Economic efficiency, Glycerine, Growth performance*

Rasyona Artan Düzeylerde Ham Gliserin İlavesinin Broylerlerde Büyüme Performansına, Karkas Özelliklerine ve Ekonomik Etkinliğe Olan Etkileri

Özet

Bu araştırmanın amacı, rasyona artan düzeyde ham gliserin ilavesinin broylerlerde büyüme ve ekonomik performans, karkas randımanı, iç organ ağırlıkları ve etin nem düzeyi üzerine etkilerinin belirlenmesidir. Bu amaçla toplam 270 adet bir günlük yaşta erkek broyler civciv (Ross 308) her biri izokalorik ve izonitrojenik olan ve artan düzeylerde (sırasıyla %0, %5 ve %10) ham gliserin içeren yemlerle beslenen üç eş gruba ayrılmıştır. Her bir deneme grubu her biri kendi içinde 18 adet civciv içeren 5'er alt gruba ayrılmıştır. Hayvanlar denemenin sonlandığı 42. güne kadar yem ve suya ad libitum olarak ulaşmışlardır. Deneme sonunda %5 düzeyinde ham gliserin ilavesinin canlı ağırlık, canlı ağırlık artışı ve yemden yararlanma oranlarını bazal rasyonla beslenen hayvanlara göre önemli ölçüde iyileştirdiği belirlenmiştir ($P<0.01$). Benzer olarak rasyona %10 düzeyinde ham gliserin ilavesi denemenin 28. ve 42. günlerinde büyüme performansını arttırmış ve yemden yararlanma oranını iyileştirmiştir (sırasıyla, $P<0.05$ ve $P<0.001$). Buna ilaveten, %5 ham gliserin ile beslenen hayvanlar bazal rasyonla beslenenlere göre %20 daha yüksek göreceli ekonomik etkinlik göstermiştir. Sonuç olarak rasyona ham gliserin ilavesi, her iki düzeyde de, broylerlerde büyüme ve ekonomik etkinlik ile Yemden Yararlanma Oranı'nı iyileştirmiştir.

Anahtar sözcükler: *Broyler, Büyüme performansı, Ekonomik etkinlik, Gliserin, Karkas*

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INTRODUCTION

In poultry industry, because of increasing prices of energy rich feedstuffs, there is remarkable effort for searching of energy alternatives for chicken diets. In this content, researchers focus on the use glycerine as a less expensive energy source in poultry diets [1]. Glycerine, also known as glycerol or glycerin, is a by-product of biodiesel production and usage of this product as an energy source for animal diets has been got attention in recent years [2-4]. Glycerine has been evaluated for poultry as a feed ingredient which provides energy for cellular metabolism [5-9]. Moreover, several studies have been performed for understanding effects of dietary glycerine addition on performance in broiler chickens [4,7-9], quails [10], and laying hens [11]. Simon et al.[7] observed that dietary glycerol addition at level of 5 and 10% had improving effect on body weight gain, feed intake and feed efficiency similar to Suchy et al.[12] who evaluated that effect of pure and raw glycerol addition to diet on production parameters and slaughter traits in male and female broiler chickens. They found that birds fed diet with glycerol had signicantly higher body weight, feed intake and carcass trait compare with birds fed with basal diet. On the other hand, Cerrate et al.[9] reported that feed intake and body weights of birds were decreased with 10% glycerine addition to diets in broilers. However authors stressed that addition of glycerine to diets at a level of 5 and 2.5% had improvement effect on breast yield as a percentage and dressed carcass of birds.

Even though pure glycerine can be used in many different applications including food, cosmetic, drug and weapon industries, and generally recognized as safe for use in animal feed usage of crude glycerine, which find a place to itself in animal feeds recently, has some concern about residual level of methanol sodium, fatty acid and moisture content in it [3,9,11]. In this point, because of contradiction results from different studies about the effect of dietary glycerine addition on performance and carcass characteristics in broiler chickens, and insufficient number of study about the usage of crude glycerine in diets of birds; the aim of present trial was evaluated the effect graded level of dietary crude glycerin on growth and economic performance, carcass yield, organ weights and moisture levels of meat in broiler chickens.

MATERIAL and METHODS

In this experiment, 270 one-day-old male Ross 308 broiler chicks were randomly divided into 3 groups according to the dietary regimen and each group was constituted by 5 subgroups of 18 birds.

The experimental protocol was approved by Local Ethics Committee of Selcuk University.

In the control group, chickens were fed with basal standard diets based on corn and soybean meal by recommendations of NRC [13], more specifically with a starter diet [23% crude proteins (CP) and 3100 kcal/kg metabolically energy (ME)] for the first 10 d of trial then with a grower diet [22% CP and 3150 kcal/kg ME] for the between 11 to 28 d of experiment, and then with a finisher diet [21% CP and 3200 kcal/kg ME] for the 3rd and the last 14 days of trial *(Table 1)*. Birds from the groups 2 and 3 received standard diets supplemented with 5% or 10% glycerol, respectively for the whole experimental period. Feed and water were provided *ad libitum*. The birds were housed in wire-bottomed pens fitted with electrical heaters during the 42 days experimental period. The temperature started at 33°C (from the 1st day to the 3rd day) and was gradually reduced (2-3°C/week) according to normal management practice. Chicks were maintained on a 24 h constant light schedule until the end of the experiment.

Body weights were determined by pen on days 1, 14, 28 and 42 and feed intakes were measured during each rearing period. Similarly, the cumulated body weight gains (expressed as g/bird and calculated body weight differences of birds from the 1st day to the 14th, 28th and 42nd days, respectively) and feed conversion ratios (expressed as g of consumed feed/weight gain in g) were calculated for each rearing period. On the day 42, 3 birds from each pen for which the body weight was closed to the mean value were slaughtered by cervical dislocation. The weight of the carcass, liver, spleen, gizzard, heart, glandular stomach and cloacal fat were recorded and all values were expressed as percentages of the carcass weight for a same bird. The duodeno-jejunal contents were collected from 3 other birds from each pen slaughtered on the d 42 and the samples were diluted with deionised water for determine pH levels of intestine.

By the end of trial, experiment groups were economically evaluated by using two procedures with a help of consideration the costs of feeds, labor, equipments, nursing and chicken meat from actual market prices; one of them was the total cost needed to obtain one-kilogram carcass weight and second was the net revenue per unit of total.

Data from present study were analyzed by Anova using SPSS 11.50 program (Inc., Chicago, II, USA). Significant differences among treatment were determined using Duncan's multiple range tests [14] with a 5% level of probability.

RESULTS

The growth performance and the feed intakes in broilers according to the dietary treatments are summarized in the *Table 2*. The body weights (BW) and the body weight

Table 1. *Feedstuffs and nutrient composition of experiment diets*
Tablo 1. *Deneme rasyonlarının yem ham madde içerikleri ve besin madde değerleri*

Ingredients	Feeding Stage								
	Starter (0 to 10 d)			Grower (11 to 28 d)			Finisher (29 to 42 d)		
Crude glycerin[1]	0	5	10	0	5	10	0	5	10
Corn	52.33	46.33	40.38	54.13	48.13	42.13	54.8	48.73	42.63
Soybean meal	32	33.05	34.00	31	32	33	31.13	32.2	33.3
Gluten	7	7	7	6.5	6.5	6.5	5	5	5
Vegetable oil	4	4	4	4.5	4.5	4.5	5.5	5.5	5.5
Limestone	1	1	1	0.9	0.9	0.9	0.9	0.9	0.9
DCP	2.25	2.25	2.25	2	2	2	2	2	2
Salt	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Vitamin mix[2]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Mineral mix[3]	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Methionine	0.35	0.35	0.35	0.2	0.2	0.2	0.1	0.1	0.1
Lysine	0.50	0.45	0.45	0.2	0.2	0.2	0	0	0
Total	100	100	100	100	100	100	100	100	100
Calculated Analyses									
CP	23	23	23	22	22	22	21	21	21
ME	3107	3106	3107	3152	3153	3153	3201	3201	3200
Ca	1.02	1.03	1.03	0.92	0.93	0.93	0.92	0.93	0.93
Avail. P	0.50	0.50	0.50	0.46	0.46	0.46	0.46	0.46	0.46
Met+sist.	1.13	1.12	1.11	0.96	0.95	0.94	0.82	0.82	0.81
Lizin	1.46	1.44	1.45	1.2	1.21	1.22	1.03	1.04	1.06

[1] Chemical composition of crude glycerin; 4.13% ash, 0.17% salt, 9.11 pH, and 0.45% methanol; [2] Vitamin premix supplied the following per kilogram of diet: vit-A 15.000 IU, vit-D_3 5.000 IU, vit-E 50 mg, vit-K_3 10 mg, vit-B_1 4 mg, vit-B_1 8 mg, vit-B_6 5 mg, vit-B_{12} 0.025 mg, niacin 50 mg, pantothenic acid 20 mg, folic acid 20 mg, biotin 0.25 mg, vit-C 75 mg, kolin 175 mg ; [3] Mineral premix supplied the following per kilogram of diet: Mn 100 mg, Zn 150 mg, Fe 100 mg, Cu 20 mg, I 15 mg, Co 0.5 mg, Se 0.2 mg, Mo 1 mg, Mg 50 mg

gains (BWG) during the first 14 d of experiment showed no significant variance among treatment groups, whereas, BW were significantly increased in birds fed with 5% and %10 crude glycerine (CG) supplemented diet compared to bird fed with basal diet at 28 and 42 days of experiment ($P<0.05$ and $P<0.01$, respectively). Furthermore, the mean of BW and BWG results cumulated over the whole experimental period (1 to 42 d) were markedly increased in graded level CG supplemented broilers ($P<0.05$ and $P<0.01$, respectively). On the other hand, although the differences were not significant among groups, the highest feed intakes (FI) for the all periods (starter, grower and finisher) were also observed in birds supplemented with 5% CG. Feed conversion ratio (FCR) was not significantly altered in the CG supplemented birds compared to the controls at d 14 while it improve by 5% and 10% CG addition at 28^{th} and 42^{nd} days of experiment ($P<0.001$ and $P<0.01$, respectively).

As shown in *Table 3*, the carcass traits (including carcass yields, moisture of breast and drumstick), the relative organ weights (100 x organ weight/carcass weight) and intestinal pH levels were summarized. Even though there were no significant variances in carcass traits according to the 3 dietary regimens, the lowest carcass yield was recorded for birds supplemented with 10% CG but differences among treatments were not significant. The relative gizzard ($P<0.05$) weights were significantly decreased in birds supplemented with 10% CG compared to birds fed with basal diet. By contrast, cloacal fat percentages (not significant) were higher in 10% CG supplemented broilers than control group birds. Intestinal pH levels showed no significant differences between treatment groups, however supplemented birds (5% and 10% glycerine, respectively) present more alkaline pH compare with birds fed with basal diet.

The results for economic evaluation of graded level of dietary CG inclusion was summarize in *Table 4*. Our finding indicated that 5% dietary CG supplementation had improving effect on relative economic efficiency (REEF) in broiler chickens. Chicks fed with 5% dietary CG showed over 20% higher results then birds fed with basal diet. On the other hand when CG level increased (to 10%) REEF of chickens decreased over 5% than birds fed without any CG addition in their diets.

Table 2. *Effects of dietary graded levels of crude glycerin (CG) supplementation on growth performance of broiler chickens*
Tablo 2. *Rasyona artan düzeylerde ilave edilen ham gliserinin (HG) broylerlerde büyüme performansına etkileri*

Treatment	Day 1	Days 1 to 14				Days 1 to 28				Days 1 to 42			
CG level (%)	BW, g	BW, g	BWG, g	FI, g	FCR[1]	BW, g	BWG, g	FI, g	FCR[1]	BW, g	BWG, g	FI, g	FCR[1]
0	46.21	362.56	316.35	427.39	1.35	1112.60[b]	1066.39[b]	1751.33	1.64[a]	2110.73[b]	2064.52[b]	3734.48	1.81[a]
5	46.12	373.03	326.92	430.28	1.32	1221.96[a]	1175.84[a]	1794.29	1.53[b]	2282.20[a]	2242.09[a]	3813.86	1.70[b]
10	46.14	359.72	313.59	408.28	1.30	1198.17[a]	1152.03[a]	1702.24	1.48[b]	2228.90[a]	2182.77[a]	3649.83	1.67[b]
SEM[2]	0.17	3.74	3.76	5.34	0.01	18.67	18.65	16.94	0.02	25.75	25.75	31.27	0.02
P	NS	NS	NS	NS	NS	*	*	NS	***	**	**	NS	**

*[1] **FCR** = Feed conversion ratio was calculated by dividing feed consumption (g) to BW gain (g) per pen basis, [2] **SEM** = Standard error of the mean, **NS** = Not significant at P>0.05; * P<0.05; ** P<0.01; *** P<0.001, [a,b] Means within a treatment and column with different subscripts differ significantly*

Table 3. *Effects of dietary graded levels of crude glycerin (CG) supplementation on carcass yield (%), meat moisture levels (%), relative organ weights (g/100 g body weight), and intestinal pH in broiler chickens*
Tablo 3. *Rasyona artan düzeylerde ilave edilen ham gliserinin (HG) broylerlerde karkas randımanı (%), et nem düzeyleri (%), göreceli organ ağırlıkları (g/100 g canlı ağırlık) ve bağırsak pH üzerine etkileri*

CG Levels (%)	Carcass Yield	Moisture of Breast Meat	Moisture of Drumstick	Intestinal pH	Cloacal Fat Pad	Liver	Spleen	Heart	Gizzard	Glandular Stomach
0	74.92	75.92	77.04	6.28	1.34	2.09	0.11	0.46	1.41[a]	0.37
5	74.59	76.35	75.74	6.30	1.42	1.84	0.09	0.42	1.34[ab]	0.33
10	69.93	76.00	76.16	6.36	1.58	1.97	0.11	0.47	1.19[b]	0.34
SEM	1.29	0.12	0.31	0.03	0.09	0.05	0.01	0.01	0.03	0.01
P	NS	NS	NS	NS	NS	NS	NS	NS	*	NS

__NS__ = Not significant at P>0.0, [a,b] Means within a treatment and column with different subscripts differ significantly

Table 4. *Effects of dietary graded levels of crude glycerin (CG) supplementation on economic efficiency in broiler chickens*
Tablo 4. *Rasyona artan düzeylerde ilave edilen ham gliserinin (HG) broylerlerde ekonomik yararlanım üzerine etkileri*

Cost of Items (Per Bird)	Level of Dietary CG (%)		
	0	5	10
Fixed costs (TL)[a]	1.52	1.52	1.52
Feed cost (TL)	4.48	4.64	4.51
Total cost (TL)	6.00	6.16	6.03
Carcass weight (CW, kg)	1.58	1.71	1.56
Cost/kg CW (TL)	3.80	3.60	3.87
Total revenue (TL)[b]	8.69	9.41	8.58
Net revenue (TL)[c]	2.69	3.25	2.55
Relative economic efficiency[d]	100.00	120.82	94.80

a- *All stable prices (including transport, bird and poultry house costs) of whole rearing period for each bird,* ***b-*** *Assuming that the selling price of one kilogram carcass weight is 5.50 TL,* ***c-*** *Net revenue per unit total cost,* ***d-*** *Relative economic costs of treatments when costs of birds fed with basal diet assume 100%*

DISCUSSION

In present study, effects of CG (by-product of biodiesel industry) on the growth performance were evaluated in broiler chickens. It was determined that addition of CG into broiler diets at level of 5% and 10% showed significant promoting effects on growth performance (BW and BWG), particularly in the growing and finisher period of rearing. The growth performance was similar during the first 14 d of trial in broiler chickens, after than it showed remarkable increased for birds fed with graded level CG supplemented diets. This result might be related with AME_n of glycerine 10% higher than corn [13] so it is a replacement of carbohydrates in diet [15]. Similarly, FCR results also showed significant variances among treatments not starter but grower and finisher rearing period of experiment. Dietary 5% and 10% CG supplementation resulted in depression of FCR values compare birds fed with basal diet. For present trial, graded level of CG addition to diet had improvement effect on FCR in birds. However, the feed intake has not significantly differed between groups for the whole experimental period but it was slightly increased (not significant) in birds consume graded level of CG in their diets at d 42, probably, palatability increasing affect of CG. These findings showed positive correlation with number of studies [4,7,12,16] which have reported dietary glycerol supplementation had positive effects on growth performance in broiler chickens. Suchy et al.[12] substituted 50% of dietary soybean oil with pure and raw glycerol in a ratio of 1:2 in male and female broilers. They observed that BW of both male and female chickens in experimental groups was significantly higher than birds in control group at d15 and d40 of experiment (P<0.05, P<0.01, respectively). But contrary to our findings in present trial, they found that dietary glycerol addition in different forms (in pure and raw) had increasing effect on FI for whole 40d rearing period in broiler chickens.

Moreover, Simon et al.[7] also observed improvement effect of dietary glycerol at levels of 5% and 10% on BW and BWG in broiler chickens. These differences between studies probably related to variances of glycerol content especially metabolizable energy density of products.

The results from present trial were in disagreement with previous studies which have reported glycerol addition had negative [5] or no effects [1,8,9] on growth parameters in birds. Lin et al.[5] concluded that substitution of dietary energy source at a level of 42.2% with glycerol had significantly detrimental effects on FI. But Simon et al.[8] observed different level of dietary glycerol addition (from 5% to 25%) had no negative effect on FI and FCR in birds. Similarly, Cerreta et al.[9] found that birds fed diets with 10% glycerine addition showed significantly less FI than those fed diets with 0 or 5% glycerine and therefore had significantly reduced BW. On a contrary, Mclea et al.[16] observed that increasing level of glycerin addition had improving effect on FCR in birds. These incompatible results from different studies may be related to variances of dietary glycerol level or different content of glycerol, particularly varied methanol and fatty acid content of glycerol.

Although the 10% of dietary CG addition tended to decreased the carcass yield in the present study, we noticed that inclusion of CG in broiler diets had no significant effect on carcass traits (including moisture of drumstick and breast meat) and relative organ weights (except gizzard) with an agreement with previous studies [9,12]. In present trial, we also observed that the relative cloacal fat weight tended to increase in broilers fed with glycerol supplemented, especially at a level of 10% CG due to overestimation of metabolically energy assigned to glycerine [18]. Similarly, Lessard et al.[6] have also found out that inclusion of 5% glycerol had increased the cloacal fat pad weight. However, the variances among treatments had no statistical meaning for this finding for present trial. On the other hand, the relative gizzard weight ($P<0.05$) was significantly depressed in glycerol supplemented birds, especially at a level of 10% CG, compared to the control birds. These findings were in accordance with previous study in which Sehu et al.[4] have also observed significant depression of relative gizzard weight with 10% dietary glycerol addition in broiler chickens.

Results from economic evaluation indicated that dietary CG inclusion, especially at a level of 5% had improvement effect on REEF in broiler chickens. However, same tendency for REEF was not observed for birds fed with 10% CG included diets. Contrary, these birds showed lesser REEF results compare than birds fed without any dietary CG inclusion, probably cause of they represent same carcass weight like as control birds with more expensive diet costs. This finding showed partly agreement with Abd-Elsamee et al.[1] results, who found that increasing level of dietary glycerine addition, particularly at a level of 6%, had improvement effect of economic efficiency in broilers.

In conclusion, the results of the current study indicate that graded level (5% and 10%) crude glycerine addition to diets, especially 5% CG, had improvement effects on growth performance (BW, BWG) and FCR in broilers chickens. Moreover, birds fed with 5% dietary CG had present over 20% higher relative economic efficiency compare than birds fed basal diet. However, all other parameters, including carcass parameters and organ weights (except gizzard), were not significantly affected by dietary CG inclusion. As a result, this experiment indicates that graded level of CG inclusion, particularly at a level of 5% CG, could be effectively used in diets with higher profitability in broiler chickens.

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Kafkas Univ Vet Fak Derg
19 (4): 575-581, 2013
DOI: 10.9775/kvfd.2012.8400

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

Cloning and Expression of *Cellulosimicrobium cellulans* β-1,3-Glucanase Gene in *Lactobacillus plantarum* to Create New Silage Inoculant for Aerobic Stability [1][2]

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Makale Kodu (Article Code): KVFD-2012-8400

Summary

In this study, the recombinant plasmid pTE353-βG was created by inserting the p353-2 cryptic plasmid region of pLP3537 into pTEG5 recombinant plasmid contains pUC18 and β-1,3-glucanase gene of *Cellulosimicrobium cellulans*. The recombinant plasmid pTE353-βG was then introduced into *Lactobacillus plantarum* by electroporation. Insert analysis of pTE353-βG digested with *Sac*I produced 1.9 kbp β-1,3-glucanase gene band on agarose gel as well as 1.9 kbp DNA encoding β-1,3-glucanase gene insert amplified on the recombinant vector via PCR indicated the integration of the gene into the plasmid. Recombinant *L. plantarum* colonies with pTE353-βG on MRS-laminarin-agar plate showed clear positive zones by Congo-red staining that revealed the expression of β-1,3-glucanase encoding gene. The β-1,3-glucanase enzyme of recombinant strain produced the same activity band with *C. cellulans* enzyme in terms of molecular weight, which showed the activity of secreted protein without any proteolytic degradation. Optimal temperature and pH values of *L. plantarum* β-1,3-glucanase have been determined 40°C and 6.0 respectively, by enzymatic analysis. These results revealed that recombinant *L. plantarum* could be considered as a silage inoculant for aerobic spoilage of silage.

Keywords: *Cellulosimicrobium cellulans, β-1,3-Glucanase, Lactobacillus plantarum, Cloning, Gene expression, Recombinant silage inoculant*

Aerobik Stabilite için Yeni Silaj İnokülantı Oluşturmak Amacıyla *Cellulosimicrobium cellulans* β-1,3-Glukanaz Geninin *Lactobacillus plantarum*'da Klonlanması ve Ekspresyonu

Özet

Bu çalışmada, rekombinant pTEG5 plazmit DNA'sına (pUC18 + *Cellulosimicrobium cellulans*'ın β-1,3-glukanaz geni) pLP3537 plazmitinin p353-2 kriptik plazmit bölgesinin aktarılması ile rekombinant pTE353-βG plazmit DNA'sı oluşturulmuştur. Rekombinant pTE353-βG plazmiti *Lactobacillus plantarum*'a elektroporasyon tekniği ile transfer edilmiştir. *Sac*I enzimi ile kesilmiş pTE353-βG'nin agaroz jelde 1.9 kbç büyüklüğünde gen bandı üretmesinin yanı sıra, β-1,3-glukanaz genini şifreleyen 1.9 kbç büyüklüğündeki DNA parçasının PCR ile rekombinant vektörden amplifiye edilmesi genin plazmite entegrasyonunu göstermektedir. pTE353-βG içeren rekombinant *L. plantarum* kolonileri MRS-laminarin-agar plağında Congo-red boyaması ile β-1,3-glukanaz geninin eksprese olduğunu gösteren açık renkli pozitif zon vermişlerdir. Rekombinant suş tarafından üretilen enzim, *C. cellulans* enzimi ile aynı moleküler ağırlıkta aktivite bandı üretmiş ve böylece enzimin herhangi bir proteolitik parçalanmaya maruz kalmadığı anlaşılmıştır. Enzimatik analizler sonucunda *L. plantarum* tarafından üretilen β-1,3-glukanaz enziminin optimum sıcaklık ve pH değerleri sırasıyla 40°C ve 6.0 olarak bulunmuştur. Bu sonuçlar, rekombinant *L. plantarum*'un silajın aerobik bozulmasını önlemek için inokülant olarak düşünülebileceğini ortaya çıkarmıştır.

Anahtar sözcükler: *Cellulosimicrobium cellulans, β-1,3-Glukanaz, Lactobacillus plantarum, Klonlama, Gen ifadesi, Rekombinant silaj inokülantı*

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INTRODUCTION

Endo-1,3-β-glucanases (EC 3.2.1.6 and EC 3.2.1.39) are widely distributed among bacteria and higher plants [1]. These enzymes catalyse the hydrolysis of β-1,3-glucan component found in the yeast cell wall and other β-1,3-glucans such as laminarin, curdlan and pachyman [2,3]. The bacterium *Cellulosimicrobium cellulans* (also known with the synonyms *Cellulomonas cellulans*, *Oerskovia xanthineolytica*, and *Arthrobacter luteus)* has been regarded as a major source of yeast-lytic enzymes, particularly endo-β-1,3-glucanases, proteases and mannanases [4]. Commercially available yeast-lytic glucanase preparations derived from this organism, namely Lyticase, Zymolyase, and Quantazyme, have been produced and widely used for yeast protoplast preparation and yeast DNA isolation [4-6]. Only one of these preparations (Quantazyme, Quantum Biotechnology, Canada) is produced recombinantly and protease-free [4,5].

The primary goal of making silage is to maximize the preservation of original nutrients in the forage crop for feeding at a later date [7]. Therefore, ensiled forages are the most commonly used feeds for ruminants all over the world [8]. *Lactobacillus plantarum* and other *Lactobacillus* species, *Enterococcus faecium* and *Pediococcus* species are most common silage inoculant bacteria and one or more of these bacteria to be included in silage inoculants [7,9]. One of these bacteria, *L. plantarum*, is the most important bacterium used in silage fermentation. However, aerobic spoilage by yeasts and moulds is a major cause of reduced nutritional value of silage and increases the risk of potential pathogenic microorganisms [10]. Various chemical additives with anti-fungal properties, such as propionic acid, sorbate, benzoate, acetic acid, ammonia, urea, some enzyme preparations and *Lactobacillus buchneri* as silage inoculant have been used to prevent or enhance of aerobic stability and decrease of spoilage [11]. However, usage of most of these additives has been restricted because of their other undesirable properties. For instance, propionic acid is difficult to handle because of its corrosive nature as well as at a pH of 4.8, about 50% of the acid being undissociated [7,11], sorbate, benzoate, and acetic acid being too costly [11]. Besides, *L. buchneri* as silage inoculant causes dry matter losses in silage material at early fermentation phase [12].

In the present study, we aimed to express the β-1,3-glucanase gene of *C. cellulans* in *L. plantarum* to create a recombinant silage inoculant to enhance the aerobic stability and decrease the spoilage by secreting the β-1,3-glucanase enzyme by *L. plantarum*.

MATERIAL and METHODS

Strains of Bacteria and Growth Conditions

Lactobacillus plantarum strain 5057 was cultured on MRS broth (Merck, 1.10661) and MRS agar (Merck, 1.10660) at 37ºC without shaking. For culturing of recombinant *L. plantarum*, both MRS-broth and MRS-agar supplemented with ampicillin (50 µg mL^{-1}). *Cellulosimicrobium cellulans* (*Oerskovia xanthineolytica*, ATCC 21606) was cultured in GYM Streptomyces medium (glucose (0.4% wt/v), yeast extract (0.4% wt/v), malt extract (1% wt/v), pH 7.2) at 28ºC with shaking at 250 rpm. Agar (1.2% wt/v) and $CaCO_3$ (0.2% wt/v) were added into GYM Streptomyces medium for preparation of GYM Streptomyces agar. Recombinant *Escherichia coli* carrying recombinant pTEG5 plasmid DNA was cultured in LB-broth (10 g bacto tryptone (Merck), 5 g yeast extract (Merck) and 10 g NaCl (Merck) per L, pH 7.5) at 37ºC with shaking at 250 rpm. Agar (1.5% wt/v) was added into LB medium for LB-agar. Both LB-broth and LB-agar were supplemented with ampicillin (50 µg mL^{-1}). For activity testing of recombinant *L. plantarum* on MRS-laminarin-agar plate, the plate was stained with Congo-red solution (0.1% wt/v Congo-red) for 15 min and then destained with 1 M NaCl solution for 15 min. Clear haloes on the MRS-laminarin-agar plate indicated the presence of β-1,3-glucanase activity [13].

Plasmids

Recombinant vector pTEG5 was previously created in the Animal Biotechnology and Genetic Engineering Laboratory, Department of Animal Science, Faculty of Agriculture, Çukurova University [14]. The recombinant vector pTE353-βG (pUC18 + β-1,3-glucanase gene + p353-2 cryptic plasmid) was created using *Escherichia coli*/ *Lactobacillus* shuttle vector pLP3537 (ampicillin resistant and erythromycin resistant; amp^R, er^R) [15] and pTEG5. pTE353-βG was used for *L. plantarum* transformation [16]. Recombinant *L. plantarum* strain carrying the recombinant plasmid pTE353-βG, was cultured in MRS broth and on MRS agar at 37°C supplemented with ampicillin (50 µg mL^{-1}). Recombinant plasmid pTEG5 was isolated from *E. coli* cells as described previously [17]. On the other hand, pTE353-βG was isolated from recombinant *L. plantarum* cells as described previously [18].

DNA Modification

The following modifying enzymes were purchased and used for DNA modifications; *Sac*I, *Sma*I, *Eco*RI, *Hind*III, and bacterial alkaline phosphatase, bacteriophage T4 DNA ligase as well as Pfu DNA polymerase (Fermentas, Vivantis and Promega Corporation). Restriction enzyme reactions were monitored by examining digestion by agarose gel electrophoresis using standard methods [19]. Linearised plasmid DNA and PCR products were excised from gels and purified using Genomic DNA Purification Kit (Fermentas).

Cloning Procedures

p353-2 cryptic plasmid region (2.4 kbp) was derived from pLP3537 vector by digestion with *Eco*RI and then ligated to *Eco*RI digested pTEG5 vector to construct the pTE353-βG recombinant plasmid.

The pTE353-βG was electrotransformed into *Lactobacillus plantarum* 5057 strain using the modified method [16] as follows: For electroporation, 50 µL of the competent *L. plantarum* cells were mixed with 1 µg of plasmid DNA and transferred to a prechilled electroporation cuvette (1 mm gap). After incubation for 2-3 min, the cells were exposed to a single electrical pulse using a Gene-Pulser (Invitrogen) set at 25 µF, 200 Ω, 1300 V (13 kV cm^{-1}) and 25 mA resulting in time constant of 5 ms. After electroporation, electrotransformed *L. plantarum* cells were diluted with 500 µL MRS broth and incubated at 32°C for 4 h. Finally the cells were plated on MRS-agar containing ampicillin (50 µg mL^{-1}) and incubated at 37°C for 48 h.

PCR and Restriction Endonuclease Analysis

The sequences of the primers used for amplifying of β-1,3-glucanase gene from the recombinant vector were 5'-AGAGCTCGTGGCACTGCACTCGTTCGAGTCT-3' (forward) and 5'-AGAGCTCGACGGGCGCGGTCAGAGCGTCCAG-3' (reverse) based on the gene sequence [20]. The PCR mixture consisted of 5 µL of reaction buffer, 1 µL of 40 mM dNTP mix (200 µM each final), 1 µL each of forward and reverse primers (20 pmol each primer), 0.5 µL of Pfu DNA polymerase (2.5 U/µL), 1 µL of 50% wt/v DMSO (1% wt/v final), and 210 ng of template in a total volume of 50 µL. The following amplification program was used: Initial denaturation step at 94°C for 2 min, then 30 cycles of denaturation at 98°C for 10 s, annealing and elongation at 68°C for 5 min. A final extension step was performed as 72°C for 5 min. PCR reaction performed with recombinant plasmid analysed by agarose gel (0.8% wt/v) electrophoresis.

For restriction endonuclease analysis, recombinant plasmid pTE353-βG was isolated from *L. plantarum* transformant. To detect the β-1,3-glucanase gene, recombinant plasmid was cleaved with *Sac*I+*Eco*RI restriction endonuclease mixture and analysed by agarose gel (0.8% wt/v) electrophoresis. Furthermore, pTE353-βG was digested by *Hind*III to obtain a single linearized band.

Electrophoretic Analysis of Extracellular Proteins

To obtain the extracellular proteins of *C. cellulans* and *L. plantarum* strains from culture supernatants, the overnight grown cells were pelleted by centrifuge. The extracellular extracts (supernatants) were mixed with 1:1 volume of 20% TCA for precipitation. After the incubation at room temperature for overnight, protein pellets were obtained by centrifuge. Air-dried proteins were dissolved in 0.1 M Tris-HCL buffer (pH 8.0).

SDS-PAGE and SDS-Laminarin-PAGE (0.2% laminarin) were done as described previously [21] with slab gels (12% wt/v acrylamide). After the electrophoresis, the gel was stained for 1 h with Coomassie blue R 250 dye in methanol-acetic acit-water solution (4:1:5 by volume) and destained in the same solution without dye [22,23]. For activity staining (zymogram analysis), SDS was removed by washing the gel at room temperature in solutions containing 50 mM Na_2HPO_4, 50 mM NaH_2PO_4 (pH 7.2), isopropanol 20% v/v for 1 h and 50 mM Na_2HPO_4, 50 mM NaH_2PO_4 (pH 7.2) for 1 h, respectively. Renaturation of enzyme proteins was carried out by keeping the gel overnight in a solution containing 50 mM Na_2HPO_4, 50 mM NaH_2PO_4 (pH 7.2), 5 mM β-mercaptoethanol and 1 mM EDTA at 4°C. The gel was then transferred onto a glass plate, sealed with film, and incubated at 30°C for 4 h. The gel was stained in a solution of Congo-red (0.1% Congo-red, 0.2 M NaOH), for 1 h, and destained in 1 M NaCl for 30 min. Clear bands indicated the presence of β-1,3-glucanase activity [24-26].

Enzyme Assay

β-1,3-Glucanase activity was assayed by adding 1 mL enzyme to 1 mL laminarin (2%, wt/v) in 0.1 M Na-phosphate buffer, pH 6.0 and incubating at 40°C for 30 min. The reaction was stopped by addition of 3 mL of 3,5-dinitrosalicylic acid reagent and $A_{540\ nm}$ was measured in a Pharmacia spectrophotometer [27].

Temperature and pH effects on enzyme activity were assayed at different temperatures ranging from 20 to 100°C and at pH values ranging from 4 to 11 for 30 min. Following buffers were used in the reactions: 50 mM Na-acetate (pH 4-6), 50 mM Na-phosphate (pH 6-8) and 50 mM Tris (pH 8-11) [28]. Enzyme assay carried out as described previously.

For the measurement of thermal stability, the enzyme was pre-incubated at temperatures between 20 to 100°C for 30 min at optimum pH. The enzyme activity was determined under standard enzyme assay condition. Enzyme assay carried out as described previously.

Supernatants of recombinant *L. plantarum* were taken at predetermined time intervals (0 min, 12, 24, 36, 48, 60, 72 h) of culturing period. Enzyme assay of supernatants carried out as described previously and enzyme production depending on culturing period determined.

RESULTS

Transformation of Lactobacillus plantarum

The first generation recombinant vector pTEG5 (pUC18 plus β-1,3-glucanase gene of *C. cellulans*) was created previously [14]. In present study, the 2.4 kbp *Eco*RI-*Eco*RI p353-2 cryptic plasmid region including replication origin of *L. plantarum* was derived from the pLP3537 and ligated to pTEG5 to create the second generation recombinant plasmid pTE353-βG *(Fig. 1)*.

The pTE353-βG recombinant plasmid was then electrotransformed into *L. plantarum* to express the β-1,3-glucanase gene. Recombinant *L. plantarum* colonies showed β-1,3-glucanase activity on MRS-agar plate supplemented with ampicillin (50 µg mL^{-1}) and laminarin (0.1% wt/v) with clear

zones around of the recombinant colonies *(Fig. 2)*.

While original *L. plantarum* with no ampicillin resistance gene did not grow on solid growth medium contain ampicillin, recombinant *L. plantarum*/pTE353-βG bearing *Amp* resistance gene on the vector grew well on the same medium (data not shown).

Recombinant pTE353-βG plasmid was isolated from recombinant *L. plantarum* strain. It was then subjected to restriction fragment length analysis together with PCR amplified DNA encoding the gene on agarose gel electrophoresis (0.8% wt/v). β-1,3-glucanase gene fragment (~1.9 kbp) amplified by PCR and restriction endonuclease digested recombinant plasmid confirmed the success of the cloning experiments. Recombinant plasmid digested with *Sac*I+*Eco*RI was yielded the same DNA fragment consisting of pUC18, p353-2 and β-1,3-glucanase gene *(Fig. 3)*.

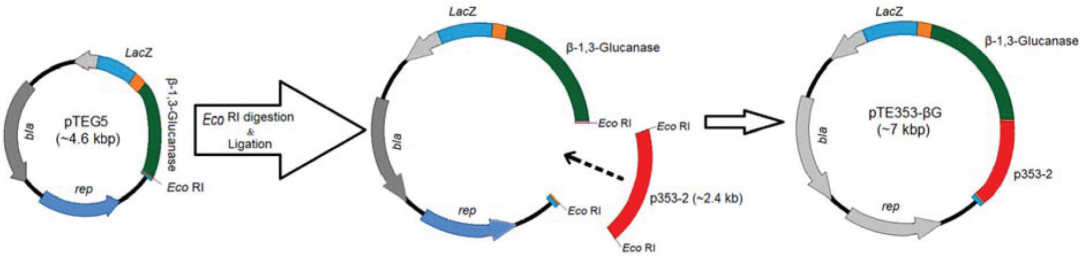

Fig 1. Construction of pTE353-βG plasmid (~7 kbp) by ligating p353-2 cryptic plasmid region including replication origin of *L. plantarum* (~2.4 kbp) into pTEG5 (~4.6 kbp)

Şekil 1. *L. plantarum* replikasyon orijinini içeren p353-2 kriptik plazmit bölgesinin (~2.4 kbç) pTEG5'e (~4.6 kbç) ligasyonu ile pTE353-βG plazmitinin (~7 kbç) oluşturulması

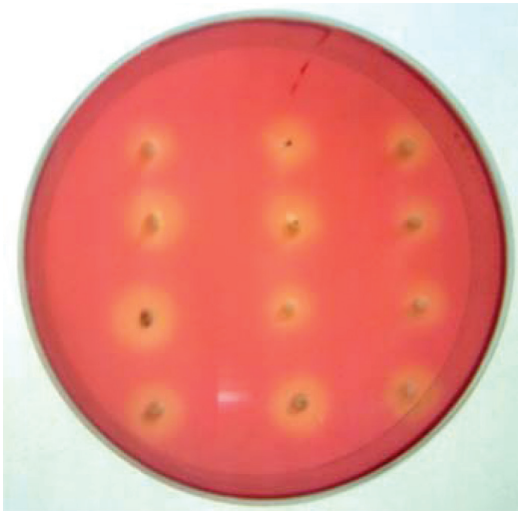

Fig 2. Recombinant *L. plantarum* colonies showing clear β-1,3-glucanase activity on MRS-agar plate with Congo red staining

Şekil 2. MRS-agar plağında Congo-red boyaması ile β-1,3-glukanaz aktivitesi gösteren rekombinant *L. plantarum* kolonileri

Culture supernatants of *C. cellulans*, *L. plantarum*/pTE353-βG, and *L. plantarum* were applied to SDS-PAGE and SDS-Laminarin-PAGE to visualize total proteins and zymogram analysis, respectively. For zymogram analysis, denaturated proteins were renaturated on SDS-Laminarin-PAGE after removing denaturating agents from the gel and then allowing to the enzyme to digest substrate, thereby producing clear zones on the gel. On zymogram analysis, only β-1,3-glucanase protein band of *C. cellulans* with 54.5 kDa in size was showed a clear zone together with extracellular protein counterpart of other recombinant *L. plantarum* strain *(Fig. 4)*. It is clearly indicated that β-1,3-glucanase gene of *C. cellulans* successfully expressed in *L. plantarum* without any significant proteolytic degradation.

Enzyme Properties

The optimum activity of the enzyme isolated from recombinant *L. plantarum* was observed at 40ºC. The mean enzyme activities were 91, 86 and 62% at 30, 50 and 60ºC

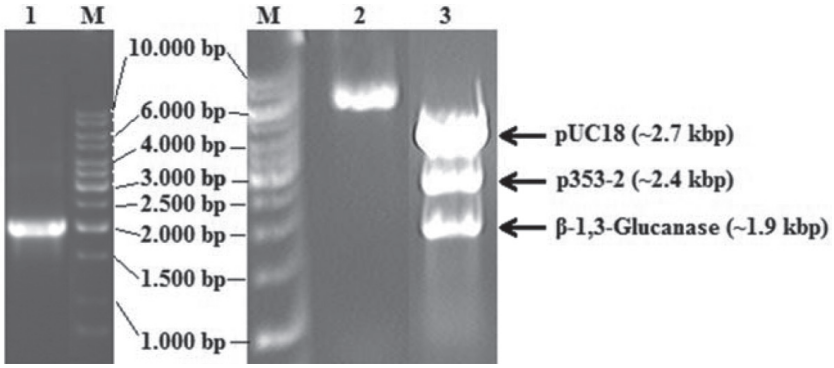

Fig 3. Restriction endonuclease and PCR analysis of pTE353-βG plasmid on agarose gel (M: 1 kbp DNA marker, 1: PCR amplified fragment of β-1,3-glucanase gene from pTE353-βG, 2: pTE353-βG/*Hind*III, 3: pTE353-βG/*Sac*I+*Eco*RI)

Şekil 3. pTE353-βG plazmitinin agaroz jelde restriksiyon endonükleaz ve PCR analizleri (M: 1 kbç DNA markır, 1: pTE353-βG'dan PCR ile amplifiye edilmiş β-1,3-glukanaz gen fragmenti, 2: pTE353-βG/*Hind*III, 3: pTE353-βG/*Sac*I+*Eco*RI)

respectively, whereas only 23% activity was retained after incubation at 70ºC for 30 min *(Fig. 5A)*. The enzyme also showed a significant relative activity (80.6%) between pH 5.0 and 7.0 with an optimum pH of 6.0 *(Fig. 5B)*. For thermal stability estimation, the retaining activity was determined at optimum pH and temperature *(Fig. 5C)*. The retained original enzyme activity obtained from 20 to 60ºC was 94% for 15 min. The enzyme was stable for 15 min between at 20 and 40ºC, while at 50, 60, 70 and 80ºC, 9, 22, 71 and 87% of the original activities were lost, respectively.

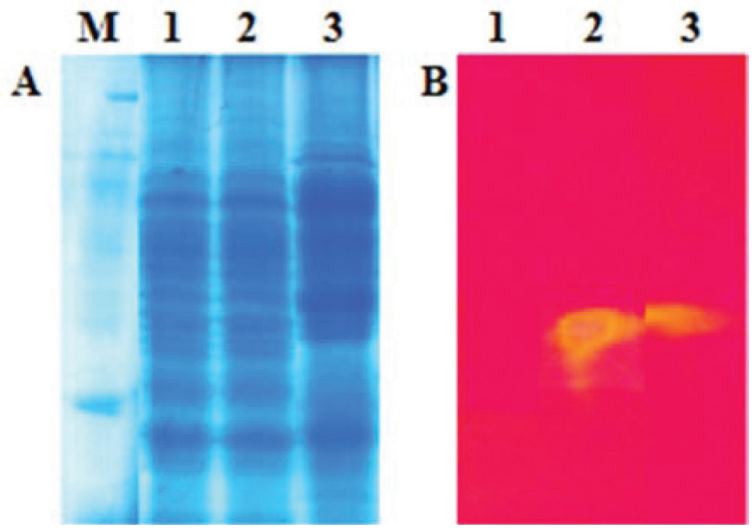

Fig 4. SDS-PAGE (A) and SDS-Laminarin-PAGE (B) analysis of recombinant and non-recombinant bacterial proteins (M: Marker, 1: *L. plantarum*, 2: *L. plantarum*/pTE353-βG, 3: *C. cellulans*)

Şekil 4. Rekombinant ve rekombinant olmayan bakteriyel proteinlerin SDS-PAGE (A) ve SDS-Laminarin-PAGE (B) analizleri (M: Markır, 1: *L. plantarum*, 2: *L. plantarum*/pTE353-βG, 3: *C. cellulans*)

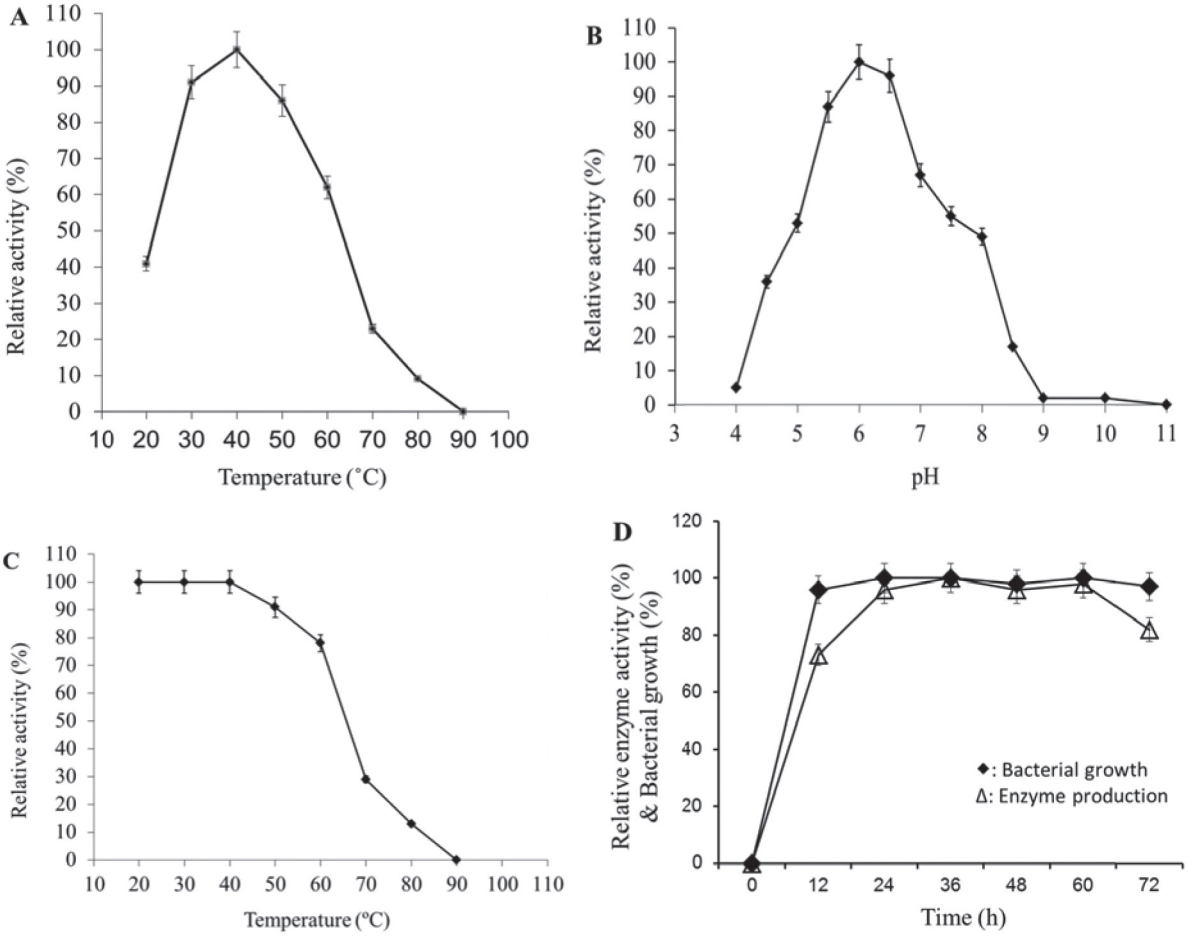

Fig 5. Enzyme properties of recombinant β-1,3-glucanase (A: Effect of temperature, B: Effect of pH, C: Thermal stability, D: Enzyme production depending on bacterial growth)

Şekil 5. Rekombinant β-1,3-glukanazın enzimatik özellikleri (A: Sıcaklığın etkisi, B: pH'nın etkisi, C: Termal stabilite, D: Bakteri gelişimine bağlı enzim üretimi)

The production of β-1,3-glucanase by the recombinant *L. plantarum* was reached maximum activity at 36 h of incubation period *(Fig. 5D)*. After 60 h of incubation period, the enzyme activity started to decrease.

DISCUSSION

With this study, β-1,3-glucanase gene of *C. cellulans* was cloned and expressed in *L. plantarum*. The enzyme secreted from *L. plantarum* was found to be active with clear zones on MRS-agar plate containing laminarin. On the other hand, zymogram analyses clearly indicated that activity bands with same size surrounded with clear zones confirming the expression of *C. cellulans* β-1,3-glucanase gene in *L. plantarum* successfully without any significant proteolytic degradation.

β-1,3-Glucanase gene of *C. cellulans* (ATCC 21606) was well studied and enzymatic properties were revealed [29]. Furthermore, the nucleotide sequence of the gene was determined [20]. In our previous study, the DNA fragment of gene was generated by PCR from *C. cellulans* genome, ligated into *Sma*I digested pUC18 and then first generation recombinant vector pTEG5 was created [14]. In the present study, p353-2 cryptic plasmid region (2.4 kbp) including replication origin of *L. plantarum* was derived from *Escherichia coli*/*Lactobacillus* shuttle vector pLP3537 then ligated into *Eco*RI digested pTEG5 and second generation recombinant vector pTE353-βG was created. The pTE353-βG recombinant vector was then successfully transferred into *L. plantarum* 5057.

Silage can spoil rapidly if exposed to air during storage or feed out stage of silage. Although a common misconception is that molds are responsible for spoilage of silage, yeasts are the primary microorganisms that cause aerobic spoilage and heating [7]. Some chemical additives and inoculant microorganisms were used to increase the aerobic stability and to prevent the spoilage of silage. Molecular studies on silage inoculants have been increased and then some genes were cloned in lactic acid bacteria for improvement for DM loses.

Aerobic deterioration of silage is a complex process which depends on many factors and usually it is initiated by aerobic yeasts [30]. Various chemical additives with antifungal properties, such as propionic acid, sorbate, benzoate, acetic acid, ammonia, urea, some enzyme preparations and *Lactobacillus buchneri* as silage inoculant have been used to prevent or enhance of aerobic stability and decrease of spoilage [11]. However usages of most of these additives have been restricted because of their other undesirable properties.

Previously, several genes from other microorganisms were cloned and expressed in *L. plantarum* such as α-amylase [31-34], cellulase, xylanase and endoglucanase [8,35], endo-1,4-β-glucanase [36], levanase [37], β-galactosidase [38] and chitinase [39]. Besides, herbal β-1,3-glucanase genes from soybean [40], jujube fruit [41] and rice [42] were cloned previously. On the other hand, the *C. cellulans* β-1,3-glucanase gene was cloned and expressed in *Bacillus subtilis* and *E. coli* [1-3,20,43]. But this is the first report to the best of our knowledge in which the β-1,3-glucanase gene from *C. cellulans* was cloned and expressed in *L. plantarum* to create new silage inoculant for better aerobic stability.

In our study, the optimum temperature of the recombinant enzyme was found with a wide range between 30-60°C. Although the gene has been cloned in several bacteria including *E. coli* and *B. subtilis*, temperature properties have not been investigated. On the other hand, temperature optimums of the β-1,3-glucanases from strain DSM 10297 and TK-1 were reported as 40 and 60°C, respectively [4]. Our finding is in agreement with optimum temperatures of the enzymes from the strains DSM 10297 and TK-1. On the other hand, optimum pH of our recombinant enzyme was found as 6.0 with a significant relative activity (80.6%) between pH 5.0 and 7.0. The present study indicates that, this enzyme prefers slightly acidic pH for optimal activity. Similar pH conditions for activity of native β-1,3-glucanase from *C. cellulans* have been reported previously [29]. pH values of various silages observed in other studies were reported as 4.3-4.5 (alfalfa silage with 30-35% DM), 4.7-5.0 (alfalfa silage with 45-55% DM), 4.3-4.7 (grass silage with 25-35% DM), 3.7-4.2 (corn silage with 35-40% DM), and 4.0-4.5 (high moisture corn silage with 70-73% DM) [7]. Our enzyme has been protected almost 40 and 55% of its activity in 4.0 and 5.0 pH conditions, respectively. With our results, the recombinant enzyme could be considered as a solution partially for aerobic spoilage at feed out stage. Besides, considering the development of *L. plantarum* at 30°C, this temperature value is quite appropriate for activation of the recombinant enzyme. Our enzyme has been suffered the loss of activation over 60°C for 30 min. So, under the unsuitable silage conditions with 60°C, our enzyme began to denature.

In conclusion, the β-1,3-glucanase gene of *C. cellulans* cloned in *L. plantarum*. Recombinant *L. plantarum* expressing β-1,3-glucanase encoding gene could be considered as a silage inoculant for aerobic spoilage of silage.

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Kafkas Univ Vet Fak Derg
19 (4): 583-587, 2013
DOI: 10.9775/kvfd.2012.8416

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

The Effects of D-Ala^2, D-Leu^5-Enkephalin (DADLE)'s Continuous Perfusion on Erythrogram and Biochemical Profile in Rats [1]

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[1] *This study is has been supported by the Research Fund of Istanbul University (Project No: 102/15052003)*

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Makale Kodu (Article Code): KVFD-2012-8416

Summary

In this experimental study it was aimed to investigate the effects of chronic continuous DADLE administration on erythrogram and the biochemical profile (ALP, ALT, AST, LDH, glucose, urea, creatinine, albumin, total protein). The study was carried on 48 adult male Wistar rats. DADLE was administered via osmotic mini-pumps, implanted subcutaneously. After the 28-day treatment, plasma glucose levels increased and urea levels decreased significantly in experimental group animals compared to the control group. Differences between the groups were not found to be statistically significant with respect to the other investigated parameters. So we conclude that chronic continuous administration of DADLE via osmotic mini-pumps did not exert any detrimental effect on erythrogram and the biochemical profile in rats.

Keywords: *DADLE, Opioid, Osmotic pump, Erythrogram, Biochemical profile*

D-Ala^2, D-Leu^5-Enkephalin (DADLE)'in Sıçanlarda Sürekli Perfüzyonunun Eritrogram ve Biyokimyasal Profile Etkisi

Özet

Bu deneysel çalışmada DADLE'nin kronik sürekli uygulamasının bazı eritrositer parametreler (eritrogram) ve biyokimyasal profil (ALP, ALT, AST, LDH, glikoz, üre, kreatinin, albümin, total protein) üzerine olan etkilerinin araştırılması amaçlanmıştır. Çalışmada 48 adet yetişkin erkek Wistar sıçan kullanılmıştır. DADLE deri altına implante edilen ozmotik mini pompalar ile uygulanmıştır. Yirmisekiz günlük uygulama sonrası deney grubu hayvanlarının plazma glikoz düzeyleri artarken üre düzeyleri düşmüş ve bu değişikliklerin kontrol grubu ile karşılaştırıldığında istatistiksel önemde olduğu görülmüştür. İncelenen diğer parametreler açısından gruplar arasındaki farklar istatistiksel olarak önemli bulunmamıştır. Bu nedenle DADLE'nin ozmotik mini pompalar ile kronik ve sürekli uygulanmasının sıçanlarda eritrogram ve biyokimyasal profil açısından herhangi bir zararlı etki oluşturmadığı sonucuna varılmıştır.

Anahtar sözcükler: *DADLE, Opioid, Ozmotik pompa, Eritrogram, Biyokimyasal profil*

INTRODUCTION

Hibernation is an adaptive and protective strategy for a group of animal species and makes it possible for them to survive extended periods of food deprivation and extreme ambient conditions [1,2]. Animals in the hibernating state can tolerate body temperatures close to zero [3] and excessively decreased blood flow to vital organs [4,5].

Dawe and Spurrier [6] showed that blood from hibernating animals can induce hibernation when transfused into active euthermic individuals and suggested for the first time the existence of a triggering substance in the blood. Furthermore they showed that this hibernation induction trigger (HIT) is not speciesspecific [7]. Later it has been found that HIT is present in the albumin fraction of plasma [8] and opioid receptors are involved in the induction of hibernation [9]. The synthetic peptide DADLE (D-Ala^2,D-Leu^5-Enkephalin; $C_{29}H_{39}N_5O_7$) is a stable analogue of the endogenous delta opioid encephalin [10,11]. It passes blood-brain barrier after systemic application [12] and has a selective agonistic

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property on delta-opioid receptors [10]. DADLE has been found to induce hibernation in hibernant animals after continuous infusion [13].

The fascinating tissue protective adaptation seen during hibernation attracted substantial research interest on HIT and DADLE, so a wide range of studies have been conducted investigating the potential protective efficacy of them [4,14]. Indeed it has been demonstrated that HIT and DADLE prolong the survival of isolated organs (heart and liver) in a multiorgan preservation preparation almost 6-fold, which could have crucial implications for organ transplantation research [15,16]. Summers et al.[17] found that DADLE treatment improves hemodynamic stability and prolongs survival in an animal model of severe hemorrhagic shock. Another *in vivo* study showed that parenteral administration of DADLE protects the liver of rats against ischemia reperfusion injury [18]. Further studies pointed out the protective efficacy of DADLE on the nervous system [10,11,19]. Recently, Borlongan et al.[20] showed that DADLE pre-treatment, administered parenterally, prevents cell death processes and behavioral symptoms caused by experimentally induced stroke in rats.

The aforementioned results together manifest that DADLE has substantial protective efficacy in various cells and tissues and may be regarded as a promising novel therapeutic agent. Nevertheless it is known that prevalence of anemia is highly correlated with opioid use [21], and it has been reported that opioids cause membrane alterations of red blood cells [22]. Besides activation of different opioid receptor subtypes may exert different effects on target cells [23]. To the best of our knowledge no study has so far investigated the side effects of DADLE on erythrogram or the biochemical profile. So the aim of the present study was to investigate the potential side effects of long-term DADLE infusion on some erythrocyte and biochemical parameters.

MATERIAL and METHODS

Animals

Male Wistar albino rats weighing 250-275 g were used for the study. They were randomly allocated to 2 groups, each consisting of 24 rats, as control and experimental groups. All animals were housed in polycarbonate cages with wood shavings bedding in a climate-controlled animal room with 12/12 h light/dark cycle. They were fed standard laboratory chow and tap water ad libitum. All experimental procedures were approved by Istanbul University Veterinary Faculty's Animal Experiments Local Ethics Committee (Approval no. 2003/65)

DADLE Application

Rats were anesthetized using xylazine/ketamine (10/75 mg/kg). Osmotic mini pumps (Alzet, USA) containing DADLE (Sigma-Aldrich, Germany) and physiologic saline solution were implanted subcutaneously into the interscapular region of experimental and control group animals, respectively. Experimental group animals received approximately 2.5 µl (±10%) of DADLE solution (0.5%) per hour, depending on the mean pumping rate of osmotic pumps during 28-day experimental period.

Samples and Tests

At the end of the experimental period all animals were exsanguinated via cardiac puncture under general anesthesia with xylazine/ketamine (10/75 mg/kg, Bayer, Turkey). Blood samples were collected into dry and EDTA tubes. Erythrogram parameters were determined using anticoagulated blood samples by using an electronic cell counter (Medonic 570, Boule Medical, Sweden) . Sera were obtained from blood samples collected into dry tubes and were used for the determination of biochemical profile including following parameters: alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose (GLU), creatinine (CREA), lactate dehydrogenase (LDH), albumin (ALB), total protein (TP) and urea (URE) using an automatic biochemical analyzer (Abbott Architect, Abbott Laboratories, USA).

Statistical Analysis

All statistical analyses were performed with statistical software SPSS (version 11.5.2.1) using the independent samples t-test. All results are reported as means ± SEM. Significance was accepted at $P<0.05$ level.

RESULTS

Hematological parameters are shown in *Table 1*. There were no statistically significant differences between groups.

The data on biochemical parameters are shown in *Table 2*. Glucose level increased and urea level decreased in DADLE treated group compared to the control group, and both differences were found to be statistically significant ($P<0.05$). There were no statistically significant differences regarding other parameters.

Table 1. *Hematological parameters*
Tablo 1. *Hematolojik parametreler*

Parameter	Control (n=24)	DADLE (n=24)
Red blood cells (10^6/mm^3)	7.91±0.11	8.08±0.09
Hemoglobin (g/dl)	12.27±0.16	12.44±0.12
Hematocrit (%)	40.98±0.54	41.49±0.40
MCH (pg)	15.52±0.15	15.43±0.12
MCHC (g/dl)	29.96±0.11	30.03±0.08
MCV (fl)	51.81±0.42	51.40±0.41
RDW (%)	21.62±0.31	21.29±0.32

MCH: *mean corpuscular hemoglobin;* ***MCHC:*** *mean corpuscular hemoglobin concentration;* ***MCV:*** *mean corpuscular volume;* ***RDW:*** *red blood cell distribution width*

Table 2. *Biochemical parameters*
Tablo 2. *Biyokimyasal parametreler*

Parameter	Control (n=24)	DADLE (n=24)
ALP (U/L)	387.61±14.63	409.13±22.40
ALT (U/L)	79.17±3.31	84.75±3.01
AST (U/L)	154.17±12.07	164.83±16.59
LDH (U/L)	362.86±9.96	392.29±16.65
Urea (mg/dl)	37.57±0.74*	34.54±0.88*
Albumin (g/dl)	3.19±0.05	3.17±0.03
Glucose (mg/dl)	161.65±4.07*	178.71±4.42*
Creatinine (mg/dl)	0.40±0.01	0.40±0.01
Total protein (g/dl)	6.92±0.06	6.95±0.07

ALP: *alkaline phosphatase;* ***ALT:*** *alanine aminotransferase;* ***AST:*** *aspartate aminotransferase;* ***LDH:*** *lactate dehydrogenase; * Differences between the means of control and experimental groups are statistically significant (P<0.05)*

DISCUSSION

To the best of our knowledge there are no studies examining the effects of long-term DADLE administration on erythrogram. In the present study 28-day continuous infusion of DADLE did not reveal any significant effect on the investigated parameters related to erythrocytes. Although studies focusing on the side effects of DADLE on hematological parameters are lacking, the effects of other opioids such as morphine on erythrocytes and related parameters have been investigated in various studies. Nie et al.[24] found that acute and chronic *in vivo* and *in vitro* application of morphine, a mu-opioid receptor agonist, increased the osmotic fragility of red blood cells and shortened their lifetime by affecting the cell membrane. Zeiger et al.[22] explained the high anemia prevalence among chronic opioid users with increasing levels of opioid receptors on red blood cells due to chronic opioid usage and subsequently increasing deformability of erythrocytes. Even though there is evidence indicating the presence of kappa- and mu-opioid receptors on the erythrocyte membrane [22,25], we could not encounter any finding about the delta opioid receptors in the literature. The abovementioned detrimental effects of opioids on erythrocytes were attributed both to the ligand-receptor interactions [22] and to the direct effect of opioid on erythrocyte membrane [24]. So the absence of any effect of DADLE on red blood cells and related parameters can be due to the lack of delta opioid receptors on erythrocyte membran or could be interpreted that chronic DADLE administration at this dose does not have any side effects on erythrocyte membran directly.

Because of its crucial role in biotransformation and metabolism of most opioids as well as various other drugs, liver is predisposed to toxic injury, especially in chronic exposure to these drugs [26-28]. Indeed it has been reported that parenteral administration of various opioids caused hepatic damage indicated by increased liver enzymes [29]. However to the best of our knowledge there are no studies investigating the adverse effects of chronic DADLE treatment on liver. Notwithstanding with the reports about detrimental effects of opioids on liver, Yamanouchi et al.[18] stated that acute DADLE treatment exerted a hepato-protective effect against acute liver damage and decreased serum ALT level in rats. In the present study chronic DADLE treatment via osmotic mini-pumps showed neither a beneficial nor a detrimental effect on liver enzymes.

Lactate dehydrogenase is a stable cytosolic enzyme present in a wide variety of mammalian tissues and cells, and leaks out of the cells into the bloodstream as a result of cell membrane damage [30]. The increase of LDH level in extracellular fluid (e.g., plasma, serum) is a sensitive but nonspecific marker of cell and tissue damage including liver, kidney, heart and muscle [31,32]. There are various *in vitro* studies in literature, investigating the effects of DADLE on several tissues and cells and it has been reported that DADLE exerted protective effect on these tissues and cells indicated by a decrease of LDH level [33-36]. Dalargin (D-Ala^2,Leu^5,Arg^6-enkephalin), another delta opioid receptor (DOR) agonist similar to DADLE, has been found to have a decreasing effect on LDH level in a traumatic shock model in rats [37]. Atıcı et al.[28] reported that long-term opioid treatment caused hepatic and renal damage in rats as indicated by a serum LDH increase. Nevertheless we could not find any reports about the *in vivo* effects of chronic DADLE treatment on LDH level. In the present study long-term DADLE treatment did not alter the plasma LDH activity, and this finding can be interpreted as an absence of detrimental effects on tissues including liver and kidney.

There was no significant difference between the creatinine levels of experimental and control groups in the present study. Besides, plasma urea level of DADLE treated animals was significantly ($P<0.05$) lower than that of the control group animals. Nevertheless both parameters were in normal range. Since the DADLE treatment did not alter the creatinine levels of both groups, it can be argued that the glomerular function was not altered by long-term treatment. So the aforementioned decrease in urea level of the experimental group can be based either on the decrease of protein catabolism or on an impairment of urea synthesis. The first possibility of altered protein metabolism by DADLE can be eliminated considering that there was no difference between plasma albumin and total protein levels of groups, and both parameters were in normal range. In this context it can be suggested that the decrease in the urea level of the experimental group is based on its impaired synthesis in liver. However we could not find any data about the effects of long-term DADLE treatment on urea synthesis in the literature to compare and discuss this possibility.

A wide range of studies has been conducted to investigate the effects of opioids on glucose homeostasis [38-44].

It has been known for several decades that parenterally administered morphine causes hyperglycemia [45]. Bailey and Flatt [42] reported that mu receptor agonist DAMGO (D-Ala², N-Me-Phe⁴, Gly⁵-ol-Enkephalin) and delta receptor agonist DADLE dose dependently increased plasma glucose levels in rats. Beta endorphin, which exhibits high affinity for delta opioid receptors [46], has been found to significantly increase plasma glucose level [47]. Nonetheless Gunion et al.[39] reported that only mu-opioid receptor stimulation resulted in hyperglycemia in rats whereas delta opioid receptor sub-type was ineffective. In our present study long-term DADLE treatment produced a significant (P<0.05) hyperglycemia in the experimental group. This increasing effect of DADLE on plasma glucose level may be due to its interaction with delta opioid receptors as suggested before [40-42].

In summary 28-day continuous infusion of DADLE via osmotic mini-pumps in our present study did not cause any significant alterations on erythrocyte parameters and biochemical profile except for a decrease in the plasma urea level and an increase in the glucose level. The finding of hyperglycemia is in accordance with previous data. Even though there is no relevant data in the literature to compare and discuss the significant decrease in the plasma urea level that we have observed, the levels of experimental and control groups were in normal range. Considering that the inspected biochemical parameters related to liver and kidney functions and the erythrocyte parameters were unaffected by DADLE, it would be reasonable to suggest that DADLE does not have any major detrimental effects at this dose level. Because of its promising tissue protective effects, DADLE deserves further study as a therapeutic agent, and our results could contribute to the further evaluation of chronic effects of DADLE treatment on various tissues, organs and systems.

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Kafkas Univ Vet Fak Derg
19 (4): 589-594, 2013
DOI: 10.9775/kvfd.2012.8456

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

Parasitic Aortitis due to *Onchocerca armillata* in Slaughtered Cattle in the Southeastern Region of Turkey

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Makale Kodu (Article Code): KVFD-2012-8456

Summary

In this study, total of 400 aorta suspected with parasitic aortitis was investigated between the years 2011-2012 in slaughtered cattle in the Southeastern of Turkey. Particularly arcus aorta and its surroundings were investigated. Macroscopically 32 of 400 (8%), both macroscopically and microscopically 43 (10.75%) cattle were diagnosed as aortic onchocercosis. The aim of this study is to investigate prevalance and pathology of parasitic aortitis in detail caused by *Onchocerca armillata* in the Southeastern region of Turkey.

Keywords: *Cattle, Onchocerca armillata, Parasitic aortitis*

Türkiye'nin Güneydoğusu'nda Mezbahalarda Kesilen Sığırlarda *Onchocerca armillata*'nın Neden Olduğu Parazitik Aortitis

Özet

Bu çalışmada, Türkiyenin Güneydoğusu'nda 2011-2012 yılları arasında mezbahalarda kesilen 400 sığır aortasında gözlenen parazitik aortitis olguları incelendi. Aortaların alınış yeri arcus aorta ve çevresi idi. İncelemeler sonunda 400 hayvandan 32 tanesinde (%8) makroskobik, 43 tanesinde ise (%10.75) hem makroskobik, hem de mikroskobik olarak aortik onkoserkozis tespit edildi. Bu çalışmanın amacı Türkiye'nin Güneydoğusu'nda *Onchocerca armillata* adlı parazitin neden olduğu parazitik aortitis olgularının prevalansı ve patolojisinin ayrıntılı bir biçimde incelenmesidir.

Anahtar sözcükler: *Sığır, Onchocerca armillata, Parazitik aortitis*

INTRODUCTION

Onchocerca armillata a parasite found in the wall of the aorta of cattle [1,2] buffalo [3], sheep [3-5], goat [6] and camels [7]. *Onchocerca armillata* is mostly observed in the south of the Asian regions and African continent closer to the equator [8-10]. In recent years, onchocercosis was also reported in the Tanzania [11], Cameroon [12], Venezuela [13] and in the Greece [14]. *Onchocerca* spp. have been reported at skin, tendons, aponeurosis, testicular and breast tissues as well [8]. The life cycle and vectors of the *Onchocerca armillata* are unknown. However, black flies and mosquitoes are thought to be vectors [12-14]. Onchocercosis often do not show any significant clinical symptoms. Sometimes the bulls show neural symptoms and sometimes microfilaria localized at ocular regions causes recurrent ophthalmitis [8,14]. *Onchocerca armillata* mostly settles in the arcus aorta region. However, can also be found in brachiocephalic truncus, cervical and brachial arteries and the abdominal aorta up to the iliac bifurcation regions [1,8]. Affected aortic intima layers have undulant appearance. Numerous tunnels and nodules are found in the media. These nodules show protrusions towards the intima and media layers. Survived and matured microfilaria can pass the circulation [2]. Parasitic tunnels are filled with a thin connective tissue without inflammatory cells. Granulomatous nodules present around the dead, degenerate or calcified parasite larvae, contain dominantly eosinophil leukocytes [8,11,15,16]. Granulomatous foci in the

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vessel wall can be resorbed over time. Aneurysms can be observed in very old animals due to calcified nodules [1,15,17]. In recent years, antiparasitic disenfestation decreased the frequency of the onchocercosis [13].

The aim of this study is to examine the prevalence and pathology of parasitic aortitis that caused by *Onchocerca armillata* in the Southeastern region of Turkey.

MATERIAL and METHODS

This study was conducted in Sanliurfa, Diyarbakır, Hatay and Gaziantep provinces of Turkey between the years 2011-2012. A hundred sample from each province, from a total of 400 cattle aorta, was collected from slaughterhouses. The aortic samples were taken 2 cm above and below including arcus aorta and were fixed in 10% neutral buffered formalin. Fixed aorta lumens were opened and investigated. At suspected places of intima, adventitia or random regions of arcus aorta was trimmed parallel to the long axisbn of the vessel and passed through all the layers. Then, tissue samples embedded in paraffin by routine methods. Sections were cut 5-6 µm in thickness and were stained with hemato-xylin and eosin (HE), Masson's trichrom and Von kossa.

RESULTS

Macroscopical Findings

Macroscopically 32 of 400 (8%) cattle were diagnosed as aortic onchocercosis. The affected vessel walls, in general, thickened, crusty in consistency and lost flexibility. In a few cases the vessel walls were in appearance of rubber tube and their lumens were severely narrowed.

The intima, media and adventitial layers of the aorta were evaluated in more detail. Accordingly;

Tunica intima: Affected areas revealed tough and an irregular surface. Some of nodules were yellowish gray in color *(Fig 1. A)*. The nodules of varying sizes (0.5-4.0 cm), especially in areas close to the bifurcation, projecting to the vessel lumen, hard in consistency, bright and white surface, cross-sectional areas of calcification felt creaking *(Fig. 1-B)*. Cut surfaces were dry and in caseous structure, which was cleaved easily and surrounded by a tough connective tissue to the size varies from lentils to nuts *(Fig. 1-A,F)*. In some areas, upturned edges formed crater-like foci which causes shrinkage of the surface with the star-like connective tissue induration *(Fig. 1-A)*. In cross-sections, caseous foci, easily cleaved and yellowish gray in color were also observed *(Fig. 1-F)*.

Tunica media: The cross section of the affected areas had tough and an irregular surface. Thickening of the medial layer was noticed in areas where parasitic granulomas were observed. In some areas, dark red-brown old hemorrhage areas hard in consistency were observed along with the long axis. Their cross-sections showed caseous and/or calcified parasite nodules and tunnels *(Fig. 1-E,F)*.

Tunica adventitia: Parasitic nodule formations were observed rarely at the adventitial layer. These nodules bud-like, located sometimes single, sometimes combined with each other in groups of two to three. In this layer the diameter of the granulomas were quite large (the largest of the 4 cm) compared to the intimal layer. Granulomas that over-flowing out of the vessel were hard in consistency, and often surrounded by a broad connective tissue *(Fig. 1-C)*. In cross-sections the presence of large caseation and small amount of calcified areas were noticed *(Fig. 1-D)*.

Histopathological Findings

Fourty three of 400 (10.75%) cattle were diagnosed as aortic onchocercosis both macroscopically and micro-scopically. Histopathologically, 11 of 43 (2.75%) parasitic aortitis were diagnosed with no macroscopic findings. In general, varying degrees of tissue reactions were investigated in the aorta. The majority of parasitic tunnels were located in the intima and media *(Fig. 2-B)*. These tunnels contained dense haemorrhagia without inflammatory cell infiltration *(Fig. 2-A)*. However, calcium deposits were noticed in some regions of these tunnels *(Fig. 2-A,B,C)*. There are round or ellipsoidal cavities containing one or more intact parasitic sections, some with microfilariae. Worms and its structure surrounding thin cuticula appeared to reside within a round or ellipsoidal cavity, with a space between the worm section and the host-derived lining. Some parts of the parasitic cysts were limited by a thin connective tissue without any inflammatory cell infiltration *(Fig. 2-B,C,D)*. A thin halo was found between larvae and the connective tissue capsule *(Fig. 2-B,D)*. Muscle fibers around the parasitic cysts and tunnels were hyalinised. Masson's trichrome stain revealed presence of connective tissue at the cyst capsule. Some areas appeared basophilic with the H&E further stained by von Kossa revealed calcium deposits around and in the parasitic cysts and tunnels.

Details of inflammatory cell infiltration, calcification, sclerosis and parasitic granulomas are given in *Table 1*.

Inflammatory reactions were examined in 3 stages; acute, subacute, and chronic granulomatous aortitis.

Acute aortitis: Mostly started at adventitial vessels, sporadic eosinophils, neutrophils and macrophage infiltrations were spread in a linear style from intimal vessels to muscle bundles. In some areas, dilated blood vessels, rounded endothelial cells, inflammatory oedema and hyalinization of muscle bundles were detected around the parasitic cysts.

Subacute aortitis: A great number of eosinophils, a few number of macrophages, lymphocytes and plasma cell infiltrations were observed in the surroundings of medial and adventitial layers and that were moving towards to the

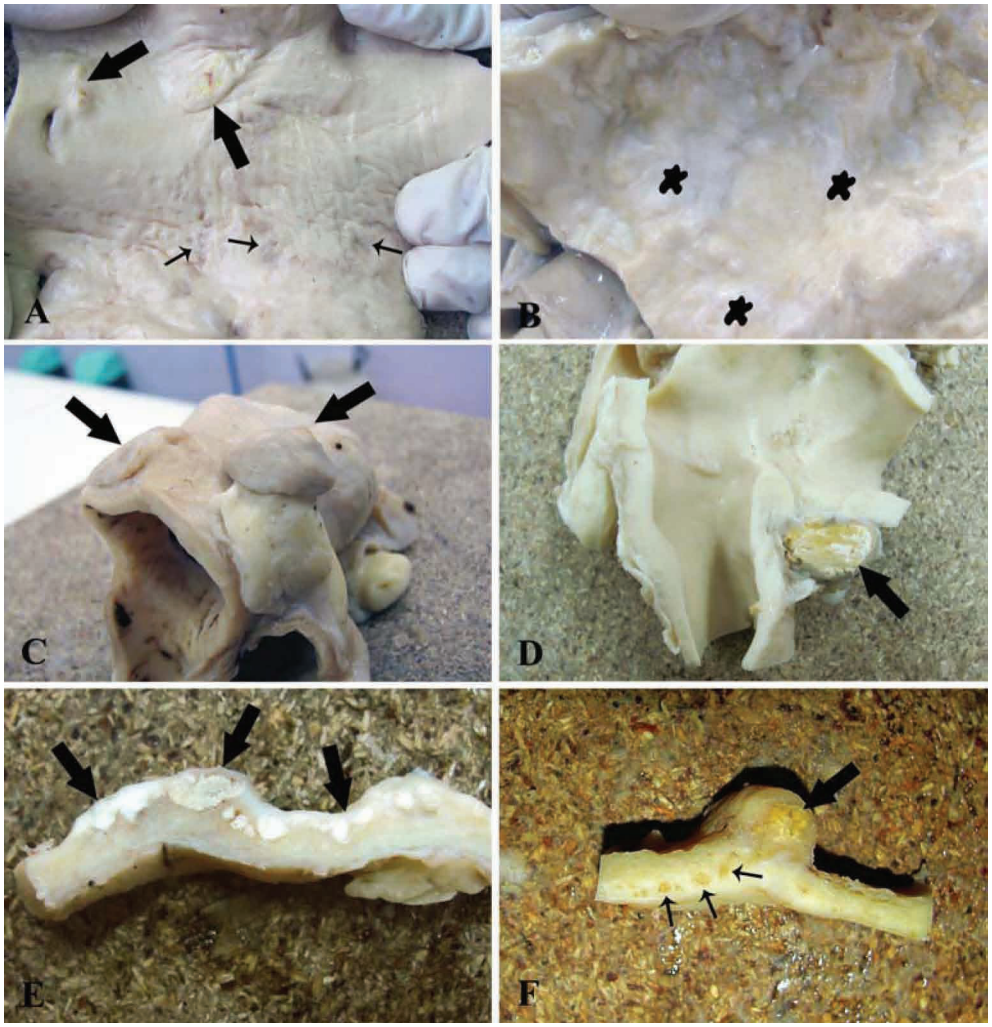

Fig 1. Macroscopic appearance of parasitic aortitis. **A-** Parasitic nodules in the lumen of the aorta *(thick arrows),* and areas of scar in appearance of star *(thin arrows),* **B-** Irregular and opaque appearance of calcified areas in the lumen of the aorta *(stars),* **C-** Parasitic granulomas, surrounded by tough connective tissue, extending outward the aorta serosa *(arrows)*, **D-** Cross sections of parasitic granuloma in serosal surface *(arrow),* **E-** Calcium deposits along with long axis of intimal surface *(arrows),* **F-** Parasitic tunnels advancing up to intima, adventitia and serosa *(thin arrows),* demarcated caseified parasitic granuloma in the serosal surface *(thick arrow)*

Şekil 1. Parazitik aortitisin makroskobik görünümü. **A-** Aorta lümenine doğru çıkıntı yapan parazit nodülleri *(kalın oklar)* ve yıldız görünümündeki sikatriks alanları *(ince oklar),* **B-** Aorta lümeninde düzensiz ve opak görünümdeki kalsifikasyon alanları *(yıldızlar),* **C-** Aorta serozasından dışa doğru taşan etrafı sert bağ doku ile sarılı paraziter granulomlar *(oklar),* **D-** Serozal yüzeydeki paraziter granulomun kesit yüzü *(ok)*, **E-** İntima yüzeyinde uzun eksen boyunca ilerleyen kalsiyum birikimleri *(oklar)*, **F-** İntimadan adventisya ve serozaya kadar ilerleyen kazeifiye parazit tünelleri *(ince oklar),* serozada demarke durumdaki kazeifiye parazit granulomu *(kalın ok)*

dead and caseous parasitic cysts. This cellular infiltration was generally localised around the old or calcified tunnels *(Fig. 2-F)*. Some areas showed new formation of blood vessels with presence of few fibroblasts.

Chronic granulomatous aortitis: The chronic granulomatous aortitis was the most common type of inflammation. Hyperchromatic and multinucleated giant cells were observed around the dead, degenerated or calcified parasitic cyst walls. A large number of eosinophilic infiltration was present between giant cells. This infiltration was supported by dense macrophages, lymphocytes, and plasma cells *(Fig. 2-F)*. The outermost composed from granulation tissue that including fibroblasts, fibrocytes and newly formed blood vessels. Muscle bundles were hyalinised *(Fig 2-E)*. In some areas, inflammatory cell infiltration was decreased while sclerosis was increased. In eight cases, arteriosclerotic foci with presence of clefts at their centers composed of lymphocytes, macrophages, fibroblasts, and fibrocytes were found. Some cases did not show any inflammatory cell infiltration around the parasitic cysts (8 cases), some of them were calcified (28 cases) and some others revealed inflammatory cell infiltration (26 cases). On the other hand eosinophils and mononuclear cell infiltration were observed without any sign of parasitic cysts in eight cases. The inflammatory reaction against to cysts and calcified cysts formations was observed at the highest rate in the Diyarbakir province *(Fig. 3)*.

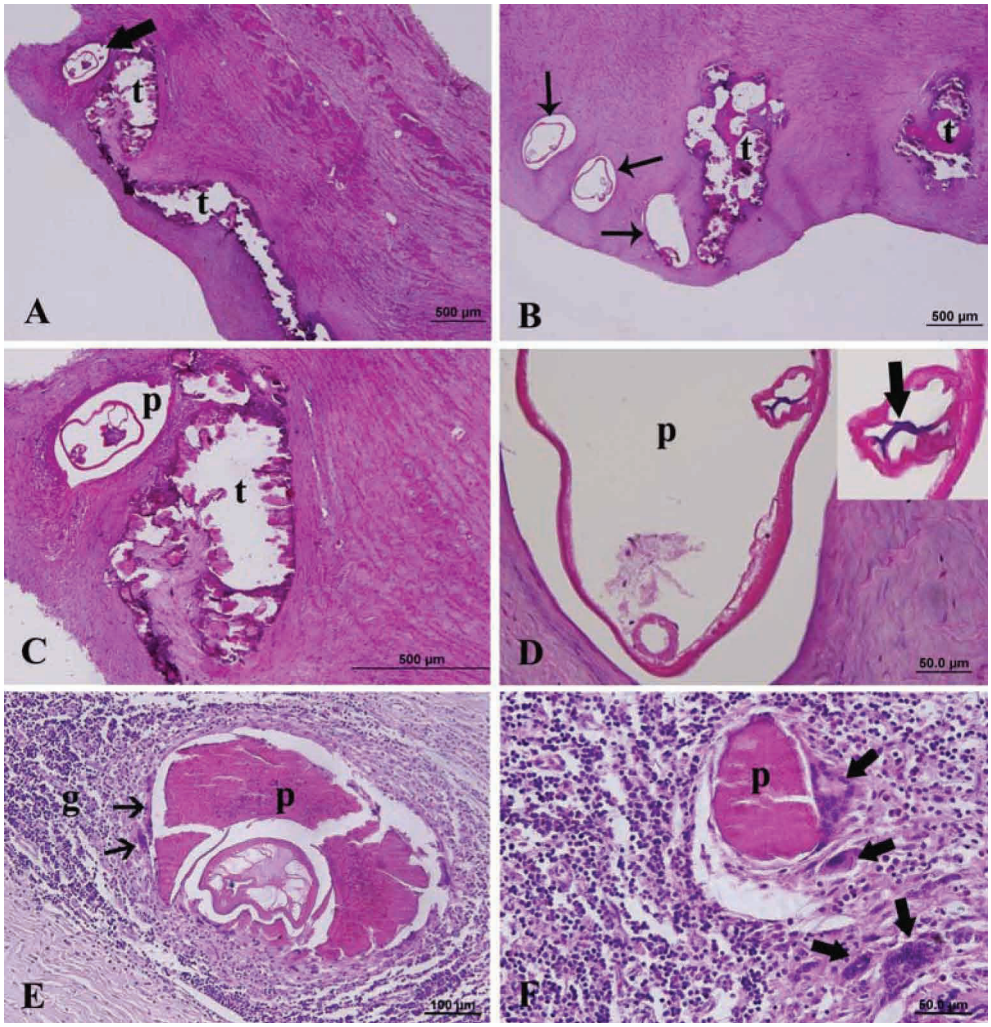

Fig 2. Microscopical aspects of parasitic aortitits. **A-** Calcified tunnels continue along with long axis of intimal surface (t). Parasitic cyst demarcated by a thin connective tissue without any inflammatory reaction *(arrow),* H&E X 40, **B-** Parasitic cysts surrounded by a thin connective tissue and halo in the intimal layer *(arrows)* and parasite tunnels (t) containing hyaline tissue and calcium deposits, H&E X 40, **C-** Calcified wide tunnel (t) progressing in different directions towards the layer of media and parasitic cyst (p) demarcated by the connective tissue, H&E X 100, **D-** An active parasitic cysts (p) surrounded by a large halo and a thin connective tissue, H&E X 100, the closer view of the mikroflaria *(arrow, enlarged figure)*, **E-** Central part of parasitic granuloma is partly caseified in the medial layer, (p) surrounded by giant cells *(arrows)* and connective tissue, H&E X 400, **F-** Completely caseified parasite (p), surrounded by multinucleated giant cells *(arrows),* eosinophils and mononuclear cell infiltration, H&E X 400

Şekil 2. Parazitik aortitisin mikroskobik görünümü. **A-** İntimada uzun eksen boyunca devam eden kalsifiye olmuş tüneller (t). Etrafı ince bir bağ doku ile sınırlandırılmış yangı hücresi bulundurmayan paraziter kist *(ok),* H&E X 40, **B-** İntimada etraflarından ince bir bağ doku ve halo ile çevrili paraziter kistler *(oklar)* içerisinde hyalinize doku ve kalsiyum birikimleri bulunduran parazit tunelleri (t), H&E X 40, **C-** Media katmanına doğru değişik yönlerde ilerleyen kalsifiye olmuş geniş tunel (t) devamında etrafı bağdoku ile sınırlı parazit kisti (p), H&E X 100, **D-** Etrafı ince bir bağ doku ve geniş bir halo ile çevrili aktif parazit kisti (p), H&E X 100, içerisindeki aktif mikroflaria'nın yakından görünümü *(ok, büyütülmüş resim),* **E-** Media katmanında, merkezi kısmen kazeifiye olmuş, çevresinde dev hücreler *(oklar)* ve bağdoku ile sarılı paraziter granülom (p), H&E X 400, **F-** Tamamen kazeifiye parazit (p) etrafında çok çekirdekli dev hücreleri *(oklar),* eozinofil lökositler ve mononükleer hücre infiltrasyonları, H&E X 400

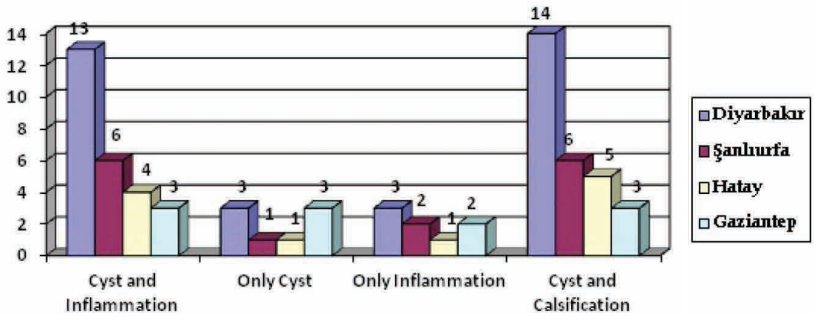

Fig 3. Evaluation of parasitic cysts according to the provinces

Şekil 3. Parazitik kistlerin illere göre değerlendirmesi

Table 1. *Histopathological aspects of parasitic aortitis due to Oncocerca armillata*
Tablo 1. *Onchocerca armillata'nın neden olduğu paraziter aortitisin histopatolojik değerlendirmesi*

Number	Case Number	Inflammation			Sclerosis			Calcification			Parasitic Cyst		
		I	M	A	I	M	A	I	M	A	I	M	A
1	D-18	-	-	-	+	-	-	-	+	-	-	-	-
2	D-68	MEG	ME	M	+	-	-	+	-	-	+	+	-
3	D-23	M	-	-	+	-	-	+	-	-	+	-	-
4	D-58	-	-	ME	-	-	-	-	-	-	-	-	-
5	D-28	ME	M	ME	-	+	-	-	-	-	-	+	+
6	D-17	-	-	-	+	-	-	+	-	-	+	-	-
7	D-75	ME	ME	ME	+	+	-	+	+	-	+	-	-
8	D-7	-	M	M	-	-	-	-	-	-	-	-	-
9	D-12	-	-	M	-	-	+	+	-	-	-	-	+
10	D-69	-	MD	M	+	+	-	-	-	-	+	+	-
11	D-72	ME	ME	MEG	-	+	-	+	+	+	+	+	+
12	D-86	ME	-	MEG	-	+	+	-	+	+	-	+	+
13	D-67	M	M	-	+	-	-	+	-	-	+	-	
14	D-4	ME	MG	M	+	+	+	+	+	-	+	+	-
15	D-3	MG	M	M	+	-	+	+	-	-	+	-	-
16	D-56	-	MG	M	-	+	+	-	+	-	-	+	-
17	D-39	-	M	M	-	-	-	-	-	-	-	-	-
18	D-21	-	M	M	+	-	+	+	+	-	+	+	-
19	D-29	ME	MEG	ME	+	+	-	-	+	-	-	+	-
20	D-44	ME	-	ME	+	-	-	+	+	-	+	+	-
21	D-30	-	M	M	-	-	-	-	-	-	-	-	+
22	S-10	-	-	ME	-	-	+	-	-	-	-	-	+
23	S-75	-	ME	ME	-	-	-	-	-	-	-	-	-
24	S-14	M	-	-	-	-	-	+	-	-	+	-	-
25	S-65	-	ME	M	-	-	-	-	-	-	-	-	-
26	S-64	M	MG	MG	-	+	+	-	+	-	-	+	+
27	S-21	MEG	MEG	MEG	+	+	+	+	+	+	+	+	+
28	S-90	MEG	MEG	MEG	+	+	+	+	+	+	+	+	+
29	S-51	M	-	M	+	-	+	+	-	-	+	-	+
30	S-17	-	ME	-	-	+	-	-	-	-	+	-	+
31	GA-52	-	-	-	+	-	-	+	-	-	+	-	-
32	GA-65	-	-	M	-	-	-	-	-	-	-	-	-
33	GA-1	ME	-	-	+	-	-	+	-	-	+	-	-
34	GA-27	-	ME	-	-	-	-	-	-	-	+	+	-
35	GA-78	-	-	-	-	-	-	-	-	-	-	-	+
36	GA-30	ME	ME	-	-	-	-	-	-	-	-	-	-
37	GA-18	ME	ME	-	-	-	-	-	-	-	+	-	-
38	GA-17	-	-	-	-	-	-	+	+	-	+	+	-
39	H-26	ME	ME	MEG	-	+	+	+	+	+	+	+	+
40	H-86	ME	-	-	+	+	-	+	+	-	+	+	-
41	H-36	-	-	-	+	-	-	+	-	-	+	-	-
42	H-23	M	M	-	+	-	-	+	-	-	+	-	-
43	H-48	ME	MEG	MEG	-	+	+	+	+	+	+	+	+

DISCUSSION

Aortic onchocercosis seen in different parts of the world [3,10,15,18]. Previous studies reported high incidence of onchocercosis in the aorta (50-100%) [1,4,15]. Use of anti-helmentic drugs and pesticides decreased the presence of *Onchocerca* spp. in the both vectors and hosts [13]. In this study, 43 (10.75%) of 400 animals from four different

provinces showed the presence of aortic onchocercosis. This means that antihelmentic drugs were not effectively used in Turkey.

Recent studies showed Onchocercosis have still seen in different parts of the world [11,13,14,17]. Alibasoglu et al.[1] reported that onchocercosis was seen in the southeastern provinces of Turkey in cattle, was not seen in western Anatolia provinces, and it was concluded that the disease was seen in tropical climates. However, according to recent study conducted by Beytut et al.[6] the parasite was also seen at Kars province where the climate is severely cold than that of southern regions, also some other cases reported from Greece [14]. This means that Onchocercosis can also be seen in cold places. It is known that *Onchocerca* spp. is carried by black flies and mosquitoes [8,14]. As these flies can be found all over the world it is hard to establish a direct relationship with climate and the incidence of the disease.

Onchocerca armillata is a parasite originated from *Onchocerca* spp. It is known that flarial forms are found in bloodstream and adult forms are reside in around arcus orta. There is any report about parasitic aortitis caused by other *Onchocerca* species which is located around arcus aorta [1,8,10]. In this study, we decsribed parasitic aortitis due to *Onchocerca armillata* around arcus aorta

In our country the prevalence and pathology of aortic onchocercosis have been only studied by Alibaşoğlu et al.[1] and Beytut et al.[6]. Alibaşoğlu et al.[1] examined the aortas of animals around Cukurova and Western Anatolia regions and they seen intensive infestation in the Cukurova region. Materials of the present study were selected from the cities that are close together and on the same line in the Southeast provinces. Between these cities aortic onchocercosis was mostly seen in Diyarbakir province. This was followed by Sanliurfa, Gaziantep and Hatay provinces. According to *Fig. 3*; only cysts, the inflammatory reaction against to cysts and calcified cyst formations were occurred at the highest rate in the Diyarbakir province. Only the cyst formation and inflammation were observed in all the provinces with almost equal intensity. The data obtained from the present study indicates that antihelmentic and pesticide drugs are not effectively used in the Diyarbakir and Sanliurfa provinces.

Ogundipe et al.[10] reported that in the parasitic cysts, live, dead and calcified tissues were located close to each other. In the present study the finding of degenerated or calcified cysts located close to each other were compatible with Ogundipe et al.[10] and other studies [1,10,16]. Similarly, degenerated or calcified cysts were surrounded by the parasitic granulomas that contains giant cells, eosinophils, macrophages, lymphocytes and fibroblasts were also compatible with previous findings [10].

The absence of any inflammatory reaction around tunnels, and some parasitic cysts was remarkable. However, in some cases, these parasite tunnels were found to be calcified. It was previously reported by many researchers that no inflammatory reaction occur against to live and vibrant forms of parasite [1,15,16]. Some of the information in the literature show that a substance released from live cuticula of parasite body prevents reaction against to the parasite [15,17].

With this study, the prevalence and pathology of *Onchocerca armillata* in cattle was examined in detail in the Southeastern region of Turkey.

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Kafkas Univ Vet Fak Derg
19 (4): 595-599, 2013
DOI: 10.9775/kvfd.2012.8474

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

The Effects of Dietary Rosemary (*Rosmarinus officinalis L.*) Oil Supplementation on Performance, Carcass Traits and Some Blood Parameters of Japanese Quail Under Heat Stressed Condition

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Makale Kodu (Article Code): KVFD-2012-8474

Summary

In this study, the effects of rosemary (*Rosmarinus officinalis L.*) oil supplementation to diet were investigated on performance, carcass traits and some blood parameters of Japanese quails exposed to a high ambient temperature of 34°C. A total of 180 fifteen-day-old quails were divided into 6 treatments consisting of 10 birds of 3 replicates. All groups were balanced according to initial live weight and gender. Birds were kept in wire cages in temperature-controlled room at either 22°C for 24 h/d (thermo neutral-TN) or 22°C for 16 h/d and 34°C (heat stress-HS) for 8 h/d (from 9:00 to 17:00) during the study. Trial was conducted as a 2x3 factorial arrangement. Birds were fed either a basal (control) diet (TN and HS) or the basal diet supplemented with 125 or 250 ppm of rosemary oil. The highest final live weight was observed in 250 ppm rosemary oil under the TN condition and the lowest was in control group of HS. HS condition affected negatively on quail live weight ($P<0.05$), but the effect of rosemary oil on this parameter was not significant ($P>0.05$). Live weight gain and feed intake were not significantly different among the treatment groups ($P>0.05$). Feed conversion ratio was better in rosemary oil groups than control groups in both environmental conditions ($P<0.01$). Heat stress deteriorated carcass yield ($P<0.01$). The highest hot and cold carcass yield (g/100 g of body weight) was observed in 250 ppm rosemary oil added group under the TN condition, but this difference did not significant. Birds kept in HS conditions had greater glucose level than hens kept in TN conditions ($P<0.01$). Rosemary oil decreased blood glucose level, especially in 250 ppm group of HS ($P<0.05$). Total, HDL, LDL cholesterol and triglyceride levels were not significantly different among the treatment groups ($P>0.05$). In conclusion, rosemary oil supplementation reduced the negative effects of heat stress. Rosemary oil could be considered as a potential natural feed additive, following further studies.

Keywords: *Rosemary oil, Performance, Carcass traits, Blood parameters, Quail, Heat stress*

Sıcaklık Stresi Altındaki Japon Bıldırcınlarında Karma Yeme İlave Edilen Biberiye (*Rosmarinus officinalis L.*) Yağının Performans, Karkas Özellikleri ve Bazı Kan Parametreleri Üzerine Etkileri

Özet

Bu çalışmada, karma yeme ilave edilen biberiye (*Rosmarinus officinalis L.*) yağının yüksek çevre sıcaklığına (34°C) maruz bırakılan Japon bıldırcınlarında performans, karkas özellikleri ve bazı kan parametreleri üzerine etkileri araştırılmıştır. Toplam olarak 180 adet 15 günlük bıldırcın, her tekerrürde 10 bıldırcının bulunduğu 3 tekerrürlü 6 gruba ayrılmıştır. Tüm gruplar başlangıç canlı ağırlıkları ve cinsiyet bakımından dengelenmiştir. Bıldırcınlar, çalışma süresince sıcaklık kontrollü odalarda 22°C'de 24 saat/gün (termo nötral-TN) ve 22°C'de 16 saat/gün 34°C'de 8 saat/gün (9:00-17:00) (sıcaklık stresi-HS) tel kafeslerde barındırılmıştır. Deneme 2x3 faktöriyel deneme düzenine göre yapılmıştır. Bıldırcınlar temel yem (kontrol) (TN ve HS) ve temel yeme 125 ve 250 ppm biberiye yağı ilave edilen karma yemler ile beslenmiştir. En yüksek canlı ağırlık TN bölümünde 250 ppm biberiye yağı ile beslenen grupta, en düşük canlı ağırlık ise sıcaklık stresi uygulanan tarafta kontrol grubunda gözlenmiştir. Sıcaklık stresi (HS) bıldırcınların canlı ağırlığını olumsuz yönde etkilemiş ($P<0.05$), biberiye yağının bu parametre üzerine etkisi önemsiz bulunmuştur ($P>0.05$). Canlı ağırlık artışı ve yem tüketimi bakımından araştırma grupları arasında önemli farklılıklar tespit edilmemiştir ($P>0.05$). Yemden yararlanma oranı her iki çevre koşulunda da biberiye yağı ilave edilen gruplarda, kontrol gruplarından daha iyi bulunmuştur ($P<0.01$). Sıcaklık stresi karkas verimini kötü yönde etkilemiştir ($P<0.01$). En yüksek sıcak ve soğuk karkas randımanı (g/100 g canlı ağırlıkta) TN şartlarında 250 ppm biberiye yağı ile beslenen grupta gözlenmiş, fakat bu farklılık istatistiksel olarak önemli çıkmamıştır. Sıcaklık stresi grubundaki bıldırcınların kan glikoz seviyesi termo-nötral gruba göre daha yüksek tespit edilmiştir ($P<0.01$). Sıcaklık stresi uygulanan tarafta özellikle 250 ppm biberiye yağı verilen grupta kan glikoz seviyesi düşmüştür ($P<0.05$). Toplam HDL, LDL kolesterol ve trigliserit düzeylerinde deneme grupları arasında önemli farklılık tespit edilmemiştir ($P>0.05$). Sonuç olarak, biberiye yağı ilavesi sıcaklık stresinin olumsuz etkilerini azaltmıştır. Biberiye yağı müteakip çalışmalardan sonra, potansiyel bir doğal yem katkı maddesi olarak düşünülebilir.

Anahtar sözcükler: *Biberiye yağı, Performans, Karkas özellikleri, Kan parametreleri, Bıldırcın, Sıcaklık stresi*

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INTRODUCTION

Heat stress is one of the most important factors adversely affecting overall poultry production in tropical countries [1]. Heat stress has been associated with decreases on weight gain, feed intake, feed efficiency and digestibility of nutrients of birds [2]. The ideal temperature for poultry is 10-22°C for optimum body weight and 15-27°C for feed efficiency.

Different natural agents are used to minimize the harmful effects of heat stress on performance of poultry. For example some vitamins [3,4], minerals [5] and substances with anti-oxidant character such as tomato powder [6], Turkish propolis [7], different essential oils [8] and oil mix (*Thymus serpyllum, Laurus nobilis L., Myrtle oil, Foeniculum vulgare, Salvia officinalis*) [9].

Rosemary, (*Rosmarinus officinalis L.*), of the family Labiatae, is an aromatic shrub with an intense pleasant smell reminiscent of pine wood [10]. The essential oil volatile composition of rosemary has been the subject of considerable research in recent years. The principal volatile compounds in rosemary are camphor and 1,8-cineole, followed by borneol, verbenone, a-pinene and camphene [11-13]. Rosemary's oils from natural populations showed high variations in their antimicrobial and anti-oxidant activity [14,15].

The aim of this study was to evaluate the effects of dietary Rosemary oil (*Rosmarinus officinalis L.*) supplementation on the performance, carcass traits and some blood parameters in Japanese quail reared in thermo-neutral (TN) condition and exposed to high ambient temperatures (HS) of 22 and 34°C, respectively.

MATERIAL and METHODS

Experimental Design and Diet Regimens

A total of 180 fifteen-day-old Japanese quails *(Coturnix coturnix japonica)* obtained from a commercial company (Deva-Yum Marketing Company, Elazig, Turkey) were used after Firat University Animal Ethical Committee approval (Official form date and number: 20.01.2012 and 2012/06). The experiment was conducted at the Poultry Unit of Veterinary Faculty, Firat University, between February 15 to March 14. The birds which were exposed to two different ambient temperature [thermo-neutral (TN) and heat stress (HS)] and three different concentrations of rosemary oil (0, 125 and 250 mg/kg), were divided into 6 treatments consisting of 10 birds of 3 replicates according to 2x3 factorial order. All groups were balanced according to initial live weight and gender. Birds were kept in wire cages in temperature controlled room at either 22°C for 24 h/d (TN) or 22°C for 16 h/d and 34°C (HS) for 8 h/d (from 9:00 to 17:00 h). At both temperatures, birds were fed either a basal diet or the basal diet supplemented with 125 or 250 ppm rosemary oil. The rosemary oil was mixed in a carrier (zeolite), which was then added at one kg per hundred kg to the basal diet. For the rosemary oil treatments, 125 or 250 mg of commercial rosemary oil were added per kg of feed. The concentrations of the volatile components in rosemary oil were shown *Table 1*. Diets and fresh water were offered *ad libitum*. Light was provided continuously (24 h) throughout the experiment. Ingredients and chemical composition of the basal diet were shown in *Table 2*. The basal diets contained 23.87% CP and 2897 kcal/kg of ME.

Table 1. *The concentration of the volatile components in rosemary oil*
Tablo 1. *Biberiye yağının içindeki uçucu bileşenlerin konsantrasyonu*

Volatile Components	**Concentration** (%)
1,8 Cineole	39.31
Camphor	14.69
α-Pinene	13.85
β- Pinene	9.87
Camphene	6.17
Limonene	3.17
P-Cymene	2.58
Borneol	2.33
Myrcene	2.02
α-Terpineol	2.28
Bornyl Acetate	1.46
Others	2.27

Table 2. *Ingredients and chemical composition of standard diet*
Tablo 2. *Standart karma yemin bileşimi ve kimyasal kompozisyonu*

Feed Ingredients	**%**	**Nutritional Composition**	**%**
Maize	29.03	Dry matter	88.25
Wheat	25.00	Crude protein	23.87
Soybean meal (48 CP)	34.29	Crude fibre	2.55
Corn Gluten	4.10	Ether extract	4.75
Vegetable oil	2.92	Ash	5.45
Dicalcium phosphate	2.02	Calcium ****	1.00
Ground limestone	0.87	Available phosphorus****	0.79
$NaHCO_3$	0.12	Methionine ****	0.40
Salt	0.28	Lysine ****	1.18
DL-Metiyonin	0.02	ME, kcal/kg****	2897
Vitamin mix *	0.25		
Mineral mix**	0.10		
Additive***	1.00		

** Vitamin premix supplied per 2.5 kg; Vitamin A 12.000.000 IU; vitamin D_3 2.000.000 IU; vitamin E 35.000 mg; vitamin K_3 4.000 mg; vitamin B_1 3.000 mg; vitamin B_2 7.000 mg; Niacine 20.000 mg; Calcium D-pantotenat 10.000 mg; vitamin B_6 5.000 mg; vitamin B_{12} 15 mg; Folik Asit 1.000 mg; D-Biotin 45 mg; vitamin C 50.000 mg; Choline chloride 125.000 mg; Canthaxanthin 2.500 mg; Apo Karotenoik Acid Ester 500 mg, ** Mineral premix supplied per kg; Mn 80.000 mg; Fe 60.000 mg; Zn 60.000 mg; Cu 5.000 mg; Co 200 mg; I 1.000 mg; Se 150 mg, *** Group Rosemary 0 (1.000 g zeolit); Group Rosemary 125 (12.5 g rosemary oil+987.5 g zeolit); Group Rosemary 250 (25 g rosemary oil + 975 g zeolit), **** Calculated*

Feed intake and BW were determined at weekly intervals. The weight gain and feed conversion of birds were then calculated.

At the end of the study (43th day) six males and females quail from each group with an average body weight near the group average were slaughtered and blood samples were collected. Blood samples were centrifuged at 2260 × *g* for 5 min, and sera were collected. Following slaughtering, hot and cold carcass characteristics were evaluated according to Institute of Turkish standards rules [16].

Chemical Analysis

Serum cholesterol, triglyceride, and glucose concentrations were measured using a biochemical analyzer (Olympus AU-600) at University of Firat, Faculty of Medicine, Department of Biochemistry. Chemical composition of feed ingredients (dry matter, crude protein, ash and ether extract) were analyzed according to the AOAC [17] procedures and crude fiber was determined by the methods of Crampton and Maynard [18].

Statistical Analysis

Data were subjected to two-way anova by using GLM (General Linear Model) prosedure. Significant differences were further subjected to Duncan's multiple range test (SPSS [19]). The results were considered as significant when *P* values were lower than 0.05.

RESULTS

The effects of dietary rosemary oil on performance of quails are given in *Table 3*. As shown in *Table 3*, the highest final live weight was observed in 250 ppm rosemary oil under the TN condition, the lowest was obtained in control group of HS condition. Heat stress decreased live weight of quails (P<0.05), supplementation of rosemary oil on live weight was not significant and dose of rosemary oil was not important among the groups of both TN and HS conditions. Live weight gain and feed intake were not significantly different among the treatment groups both in TN and HS conditions (P>0.05). The worst feed conversion ratio was calculated in control groups of both TN and HS conditions. Feed conversion ratio was improved in rosemary oil groups of 125 and 250 ppm in both conditions (P<0.01). The highest hot and cold carcass yield (g/100 g of body weight) was observed in 250 ppm rosemary oil under the TN condition. The lowest was in control group of HS. Under HS condition, deterioration of carcass yield was found significant (P<0.01).

Heat stress affected blood glucose level (P<0.01). Birds kept in HS conditions had greater glucose level than hens kept in TN conditions *(Table 4)*. Rosemary oil decreased blood glucose level especially in 250 ppm rosemary oil group (P<0.05). Total, HDL, LDL cholesterol and triglyceride levels were not significantly different among the treatments (P>0.05).

DISCUSSION

In the present study, rosemary oil supplementation to diet had significant effects on the measured values under thermo-neutral (TN) and heat stress (HS) conditions in growing Japanese quails; it improved performance, positively. The improved performance of rosemary oil groups could be due to these positive effects of rosemary oil on digestive system. In agreement with these results, HERNANDEZ et al.[20] reported that a supplementation of essential oil extract (EOE) from oregano, cinnamon and pepper improved apparent whole tract and ileac digestibility of the nutrients in broilers. JANG et al.[21] showed that a supplementation of a blend of commercial essential oils

Table 3. *Effects of rosemary (Rosmarinus officinalis L.) supplementation in diet on performance, hot and cold carcass yield in Japanese quail reared under heat stress*

Tablo 3. *Sıcaklık stresi altındaki Japon bıldırcınlarında karma yeme ilave edilen biberiye (Rosmarinus officinalis L.) yağının performans, sıcak ve soğuk karkas üzerine etkileri*

Traıts	Rosemary Oil, ppm						Main Effects of Heat Stress and Feed Additive	
	HS			TN				
	0	125	250	0	125	250	HS	FA
Initial Live Weight, *g*	58.15±0.85	58.10±0.30	58.10±0.50	58.50±1.00	58.20±0.20	58.10±0.40	NS	NS
Final Live Weight, *g*	192.67±3.88	200.60±4.05	202.54±3.55	206.20±5.80	205.56±3.92	214.11±3.66	*	NS
Live Weight Gain, *g/bird/day*	4.64±0.12	4.91±0.40	4.98±0.16	5.09±0.17	5.08±0.13	5.38±0.12	NS	NS
Feed Intake, *g/bird/day*	17.04±0.06	17.15±0.12	17.28±0.15	18.61±0.10	17.76±0.63	18.60±0.37	NS	NS
Feed Conversion Ratio, *g feed/g gain*	3.68±0.01^{a}	3.50±0.04^{b}	3.47±0.01^{b}	3.66±0.03^{A}	3.50±0.05^{B}	3.46±0.01^{B}	NS	**
Hot Carcass, *g hot carcase wt/100 g live wt.*	64.73±0.75	65.97±1.03	66.55±1.92	69.92±1.29	69.31±0.90	70.64±1.15	**	NS
Cold Carcass, *g cold carcase wt/100 g live wt.*	62.70±0.86	64.32±1.09	64.24±1.96	67.71±1.19	67.85±1.27	69.04±1.04	**	NS

NS: *Non significant, * P<0.05,* ******* *P<0.01,* ^{a,b,A,B}***:*** *Mean values with different superscripts within a column differ significantly,* ***HS:*** *Heat stres,* ***TN:*** *Thermo-Neutral,* ***FA:*** *Feed Additive*

Table 4. *Effects of rosemary (Rosmarinus officinalis L.) supplementation in diet on serum glucose and lipid levels in Japanese quails reared under heat stress*

Tablo 4. *Sıcaklık stresi altındaki Japon bıldırcınlarında karma yeme ilave edilen biberiye (Rosmarinus officinalis L.) yağının serum glikoz ve lipit seviyelerinin üzerine etkileri*

Traits, mg/dl	Rosemary Oil, ppm						Main Effects of Heat Stress and Feed Additive	
	HS			TN				
	0	125	250	0	125	250	HS	FA
Glucose	289.67±5.53^{a}	273.67±7.27^{ab}	257.33±7.01^{b}	215.80±8.34^{B}	251.17±8.30^{A}	201.80±5.63^{B}	**	*
Total Cholesterol	209.00±32.75	175.00±21.99	165.33±16.71	152.67±29.01	154.60±25.95	149.83±18.56	NS	NS
Triglyceride	919.50±176.44	913.00±90.93	881.83±231.30	680.20±155.41	741.00±243.22	614.33±97.58	NS	NS
HDL Cholesterol	69.33±9.97	69.67±3.52	70.80±7.04	75.00±4.80	82.80±9.64	80.00±5.29	NS	NS
LDL Cholesterol	95.40±12.08	60.83±13.13	60.33±9.03	54.80±10.19	60.00±7.22	58.83±8.51	NS	NS

NS: *Non significant, * P<0.05,* ** *P<0.01,* ^{a,b,A,B}***:*** *Mean values with different superscripts within a column differ significantly,* ***HS:*** *Heat stres,* ***TN:*** *Thermo-Neutral,* ***FA:*** *Feed Additive*

combined with lactic acid increased trypsin and pancreatic amylase activity in broilers. Rosemary's oils obtained from natural plants showed high variations of their antimicrobial and antioxidant activity [14,15]. Previous studies reported that dietary antioxidants, such as vitamin C, E, flavonoids, and phenolic can reduce oxidative damage in animals which is generated by different stress sources [22,23]. Jamroz and Kamel [24] who observed improvements of 7.7% in feed conversation ratio fed a diet supplemented with a plant extract containing capsaicin, cinnamaldehyde and carvacrol in broilers. Improved feed conversion in groups with supplemented rosemary oil may be due to the combined effects of all these active ingredients in a positive manner.

At the inspection of the carcass characteristics *(Table 3),* there was significant effect of HS on this parameter. In stressed conditions, elevated concentrations of glucocorticoids exert catabolic effects. This demolition decreases the rate of muscle synthesis and thus results in muscle wasting and retardation in growth [25,26]. Supplementation of rosemary oil to diet did not affect the carcass yields at the present study. However, Simsek et al.[8] reported that adding anise oil in the ration had positive effects on the carcass yield in broilers. Also, Alcicek et al.[27] showed that supplementation essential oil (Herbomix™) in the ration had positive effects on the carcass yield in broilers. Possibility, the doses of rosemary oil could not have enough action on catabolic effects of glucocorticoids via anti-oxidant activity [28].

Effects of rosemary (*Rosmarinus officinalis L.*) oil supplementation in diet on blood levels of lipids and glucose were investigated in the present study *(Table 4)*. We observed that birds kept in HS conditions had greater glucose level than hens kept in TN condition and dietary rosemary oil had a positive effect on blood glucose level. This finding indicates that the rosemary oil might be producing its hypoglycemic activity by a mechanism independent from insulin secretion by the inhibition of endogenous glucose production or the inhibition of intestinal glucose absorption [29,30]. In a previous study, it has been suggested that 50% ethanol extract of *Rosmarinus officinalis*, in part, due to intestinal α-glycosidase (AGc) inhibitory activity of its active compound might play a role in controlling dietary glucose uptake in the small intestinal track [31]. In agreement with current results, BAKIREL et al.[32] investigated potential effect of ethanolic extract of *Rosmarinus officinalis* leaves on glucose homeostasis in rabbits. Results of that study showed that ethanolic extracts of leaves of *Rosmarinus officinalis* reduced blood glucose level in normoglycemic and glucose-hyperglycemic rabbits.

In conclusion, rosemary oil supplemented to diet especially at a level of 250 ppm level supplemented diet had positive effects on performance and blood glucose level. The rosemary oil could therefore be considered as a potential natural feed additive for growing quails, due to increasing consumer's demand for healthy animal production after further studies carried out in different stress conditions.

Acknowledgement

The authors thank to Mr. Fahris KILIC for providing the rosemary oil.

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Kafkas Univ Vet Fak Derg
19 (4): 601-606, 2013
DOI: 10.9775/kvfd.2012.8517

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

The Relationships Among Some Udder Traits and Somatic Cell Count in Holstein-Friesian Cows [1]

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[1] *This study was supported by Adnan Menderes University (The Research Projects Funding Unit No: BMYO-11001)*

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Makale Kodu (Article Code): KVFD-2012-8517

Summary

This study was carried out to investigate the relationships between somatic cell count (SCC) and some udder traits in 129 head Holstein-Friesian cows (1st and 5th month of lactation) raised at 3 dairy farm in Kamislar Village in Bozdogan county of Aydin province, Turkey. Farms were visited monthly between July 2011 and June 2012, and about 1108 milk samples and different udder measures were taken in 50±20 days, 90±20 days, 130±20 days of lactation of each cow studied. Mean log_{10}SCC were calculated as 4.9±0.003 (87.923). The overall results showed that dairy farms and month of year groups had a significant effect on log_{10}SCC ($P<0.01$). Positive correlations among fore teats perimeter and log_{10}SCC (0.11) in farm A and among and log_{10}SCC distance between fore teats (0.12) and distance between rear teats (0.10) in farm B, were found at the level of $P<0.01$, respectively. The correlation between rear teats perimeter and log_{10}SCC (-0.12) was found to be negative and statistically significant in farm C ($P<0.01$). Consequently, udder traits relevant to minimization or elevation of SCC should be carefully evaluated in selection studies.

Keywords: *Holstein-Friesian, Cow, Somatic Cell Count, Udder Traits*

Siyah Alaca İneklerdeki Somatik Hücre Sayısı ile Bazı Meme Özellikleri Arasındaki İlişkiler

Özet

Bu araştırma, Aydın ili Bozdoğan ilçesi Kamışlar Köyü'ndeki 3 süt sığırcılığı işletmesinde yetiştirilen 129 baş Siyah Alaca ineğin (laktasyonun 1. ve 5. ayları arasında) somatik hücre sayısı ile bazı meme özellikleri arasindaki ilişkileri incelemek amacıyla yapılmıştır. İşletmelere Temmuz 2011 ve Haziran 2012 tarihler arasında, ayda bir gidilerek laktasyonun 50±20 gün, 90±20 gün, 130±20. günlerinde yaklaşık 1108 adet süt örneği ile farklı meme ölçüleri alınmıştır. Ortalama log_{10}SHS 4.9 ± 0.003 (87.923) olarak hesaplanmış, log_{10}SHS üzerine işletme ve ayın istatistiki olarak önemli bir etkisi olduğu tespit edilmiştir ($P<0.01$). A işletmesinde ön meme başı çapı ile log_{10}SHS arasındaki (0.11) ve B işletmesinde log_{10}SHS ile ön meme başları arası mesafe (0.12) ve arka meme başları arası mesafe (0.10) arasındaki korelasyonlar $P<0.01$ düzeyinde bulunmuştur. C işletmesinde log_{10}SHS ile arka meme başı çevresi (-0.12) arasındaki korelasyon negatif ve istatistik bakımdan önemli çıkmıştır ($P<0.01$). Çalışmada, SHS'nı azaltan ya da arttıran meme özelliklerinin seleksiyon çalışmalarında dikkatlice değerlendirilmesi sonucuna varılmıştır.

Anahtar sözcükler: *Siyah-Alaca, İnek, Somatik hücre sayısı, Meme özellikleri*

INTRODUCTION

Milk yield improvement has been well known as the main purpose of most dairy breeders, besides it has also well been documented that single-trait selection for milk production may prone cattle to decreased immunity (resistance against disease) and reproduction efficiency [1-3]. Udder diseases, predominantly mastitis as one of the most frequent and economical cow disease, may possess increasing costs on milk producers [3-6] and finally lead to obligatory culling [7].

Reported heritability for clinical mastitis is very low in general and may vary from 0.02 [2] to 0.05 [8]. As for this, correlated traits may be used for indirect selection for improvement of mastitis resistance, majorly somatic cell score (SCC - log-transformed somatic cell count), besides within some selected conformation traits [3]. In a previous study genetic correlations of the same traits among different lactations were found positive (moderate to high), suggesting that SCC and clinical mastitis may be considered

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as the same traits for different lactations. In addition genetic correlations of clinical mastitis and SCC varied from 0.37 to 0.68 [7], with the average correlation close to 0.70 [2,9], for the first and third lactations, respectively, showing that clinical mastitis and SCC present different features of udder health [5]. The heritability of SCC is also low, varying from 0.06 [10] to 0.13 [11].

Cell numbers involved in milk are closely related to inflammation and udder health. SCC in raw and heat-treated milk from a healthy mammary gland according to EU Directive (Health and Hygiene Directive 92/46/EEC), is lower than 400.000 cells/ml, SCC in milk and milk products is lower than 500.000 cells/ml [12].

Somatic cell count, a useful predictor of intramammary infection involving leucocytes [13], is totally accepted as for the standard measurement of milk quality worldwide. Therefore SCC is readily available in almost dairy farmer at least monthly [14]. Widely distributed data are now available worldwide on large population of cows regarding factors influencing SCC in milk. A variety of reviews or relevant studies have addressed issues regarding SCC, their variety and probable use for detecting the quality of milk [14-26]. An elevated SCC in milk has a negative influence on the quality [21,27-31]. Subclinical mastitis has always been recognized as a result of low milk production, changes related to milk consistency (density), diminished probability of adequate milk processing, lowered protein and higher risk for milk hygiene [13]. Similar confounding were reported by [32] indicating a positive relation among SCC and milk yield for the first lactation, and a negative relation for the processing lactations. Clinical mastitis is more likely to be included in a breeding in contrast to SCC, whereas moderate to strong genetic correlations between clinical mastitis and SCC may suggest that selecting diminished SCC may improve mastitis resistance [33].

The relationships between different udder traits, milk yield and SCC were investigated by one researcher. The genetic correlations among udder height, rear udder height, cleft depth, udder levelness and SCC were found -0.41, -0.44, 0.20 and 0.21, respectively [34].

Some authors investigated the farm effects on SCC [35-40]. Many others reported that the effects of stage of lactation, month and parity on SCC were considered [5,14,29,31,37,39,41-49].

The objective of this study was to investigate the relationship between some udder traits and SCC in Holstein-Friesian cows raised at selected dairy farms in Bozdogan county, Aydin province.

MATERIAL and METHODS

The survey was conducted with 129 head Holstein-Friesian cows were determined at 1st-5th months of lactation and managed at 3 dairy farms in Kamislar Village in Bozdogan county of Aydin province, Turkey. The general features of the dairy farms are shown in the *Table 1*. Farms were visited monthly from July 2011 to June 2012 and were collected about 1108 milk samples and different udder and teat of each cows were measured into 3 lactation stage (50±20 days, 90±20 days, 130±20 days of lactation). In this study, cows were separated 4 parity groups. The cows having the fourth and larger lactation numbers were included in Parity 4.

Collection of Milk Samples

The milk samples from each cow were withdrawn from each teat into tubes among both the morning and evening milking samples. Morning milking was stored in a cooler box and then was immediately transferred to the laboratory for analyzing on the same day. Evening milk samples were kept in a refrigerator and analyzed the next day immediately. The milk samples were treated according to the directions given previously [50] and Standard Methods for the Examination of Dairy Products [51]. SCC in the samples was determined by direct microscopic SCC method [52]. Milk samples were spread on 2 microscope slide areas, with 5×20 mm^2 in size. Slides were arranged

Table 1. *General features of the dairy farms*
Tablo 1. *Süt sığırı işletmelerinin özellikleri*

General Features	**Farm A**	**Farm B**	**Farm C**
N cows (N=129)	51	42	36
Barn type	Open Concrete-dirt surface Free stall	Open Concrete-dirt surface Free stall	Open Concrete-dirt surface Free stall
Animal sill	Using/Hay sill	Using/Hay sill	Using/Hay sill
Milking system	Milking parlor (1x10)	Milking parlor (1x9)	Milking parlor (2x5)
Milking machine cleaning	Rightly	Rightly	Rightly
Pre-milking/post-milking udder cleaning and disinfection	Water- Disinfection Using	Water- Disinfection Using	Disinfection Using
Feeding	During milking Roughage + Mixed feed	During milking Roughage + Mixed feed	During milking Roughage + Mixed feed
Touring Area	In barn	In barn	In barn

according to Direct Microscopic Somatic Cell Count (DMSCC) and kept at 37°C in an incubator. Then, milk samples on slides were painted in the manner of the technique of a dye solution involving methylene blue. The dye solution was prepared with combined of 0.6 g of certified methylene blue chloride to 54 mL of 96% ethyl alcohol, 40 mL of tolien and 6 mL glacial acetic acid. The counting was fulfilled in 20 fields under a 100x immersion objective in each slide and averages were calculated. The multiplication of the microscope factor with this average values was corresponded to SCC in per mL of milk [53].

Udder and Teat Measurement

The distance from fore and rear teats to the floor (DFTF and DRTF, respectively) and vertical length of fore and rear teats (FTL and RTL, respectively), the distance between fore, rear and side teats (FTD, RTD and STD, respectively) and the fore and rear teat perimeter (FTP and RTP, respectively) were measured by using a ruler. The fore and rear teat diameter (FTDM and RTDM, respectively) was measured at the mid-point of each teat by the help of calipers. All measures were performed after and before morning and evening milking.

The SCC values were transformed to $\log_{10}$ for normality and homogeneity of variances in the SCC data. The statistical analyses were performed using least squares method in the General Linear Model (GLM) procedure of Minitab package program [54]. The means were compared by Duncan's multiple range test [55]. The statistical model used for the analysis is as follows:

$$Y_{ijklm}=m+s_i+a_j+p_k+lp_l+e_{iklm}$$

Where; y_{ijklm}: i. farm, j. month, k. parity, l. lactation period, m. cow's logarithmic SCC, μ : population mean, s_i : i. farm's effect (i: 1, 2, 3), a_j : j. month's effect (j: 1, 2,…,12), p_k: k. parity's effect (k: 1, 2, 3, 4), lp_l : l. lactation period's effect (1: 50±20 days, 2: 90±20 days, 3: 130±20 days), e_{ijklm}: residual random error.

In this study, the correlation coefficients were calculated between udder measures and $\log_{10}$SCC. To compute correlations between $\log_{10}$SCC and udder measures, Pearson's correlation analysis were used. Other statistical analysis was performed using SPSS statistical packet program [56].

RESULTS

Basic Statistics

The mean values of 305-day milk yield were ranged from 4142 to 4557 kg in farm A, from 3930 to 4847 kg in farm B and from 4344 to 5100 kg in farm C. According to analyses, differences between lactations were non-significant in each farm (P>0.05).

The mean values of udder traits were changed between 1.98 cm and 1.87 cm for FTDM, 1.82 cm and 1.86 cm for RTDM, 5.86 cm and 6.30 cm in for FTL, 5.24 cm and 5.51 cm for RTL, 8.04 cm and 8.10 cm for FTP, 7.96 cm and 8.15 cm for RTP, 14.71 cm and 15.62 cm for FTD, 7.78 cm and 7.84 cm for RTD, 12.26 cm and 12.75 cm for STD, 52.80 cm and 53.91 cm for DFTF, 53.45 cm and 53.95 cm for DRTF in farm A.

The mean values of udder traits were changed between 2.13 cm and 1.93 cm for FTDM, 1.92 cm and 1.90 cm for RTDM, 5.91 cm and 6.43 cm for FTL, 5.31 cm and 5.79 cm for RTL, 8.12 cm and 7.83 cm for FTP, 8.04 cm and 8.28 cm for RTP, 15.30 cm and 15.42 cm for FTD, 8.69 cm and 8.80 cm for RTD, 12.04 cm and 12.34 cm for STD, 52.23 cm and 50.99 cm for DFTF, 51.74 cm and 51.65 cm for DRTF in farm B.

The mean values of udder traits were 1.88 cm and 1.81 cm for FTDM, 1.79 cm and 1.81 cm for RTDM, 5.63 cm and 6.13 cm for FTL, 5.07 cm and 5.67 cm for RTL, 8.06 cm and 7.24 cm for FTP, 7.80 cm and 7.10 cm for RTP, 13.98 cm and 13.45 cm for FTD, 7.66 cm and 7.40 cm for RTD, 11.72 cm and 11.78 cm for STD, 52.86 cm and 54.23 cm for DFTF, 53.94 cm and 55.66 cm for DRTF in farm C.

The least square means and standard error of means of $\log_{10}$SCC are shown in *Table 2*.

The results were shown that $\log_{10}$SCC was reached the highest value in July and August and also was reached the lowest values between April and June. The values of $\log_{10}$SCC were decreased from parity 1th to 3rd and were increased to parity 4.

The differences between farms were found statistically significant for $\log_{10}$SCC (P<0.01) and the analysis revealed that the effects of months were also statistically significant for $\log_{10}$SCC (P<0.01). The differences between parity and lactation periods were found statistically non-significant for $\log_{10}$SCC (P>0.05).

Correlations Between SCC and Udder Traits

The correlations between $\log_{10}$SCC and udder measures are presented in *Table 3*. The correlation among $\log_{10}$SCC and udder traits generally tended to negatively and was only found positive and statistically significant correlation between $\log_{10}$SCC and FTDM (0.11) in farm A (P<0.05). The correlations among $\log_{10}$SCC-FTD (0.12) and $\log_{10}$SCC-RTD (0.10) were found positively and statistically significant (P<0.01). The correlations between $\log_{10}$SCC and udder measures were also found negatively and the highest correlation values was determined to -0.12 ($\log_{10}$SCC-RTP) and statistically significant in farm C (P<0.01).

DISCUSSION

In the given circumstances (twice a day milking), SCC<100.000 cell/mL in milks were obtained from udder

Table 2. *The least square means and standard error of means of log_{10}SCC (cell/ml)*

Tablo 2. *log_{10}SHS'nın en küçük kareler ortalamaları ve standart hataları (hücre/ml)*

Factors	N	X±Sx	
		log_{10}SCC	Re-transformation SCC
Farm		**	
A	448	4.90^{b}±0.003	80.687
B	360	5.09^{a}±0.007	123.956
C	300	4.94^{b}±0.004	87.923
Months		**	
1 (January)	142	4.96^{c}±0.070	92.083
2 (February)	140	4.94^{c}±0.060	88.919
3 (March)	136	4.94^{c}±0.005	87.673
4 (April)	106	4.92^{c}±0.002	83.999
5 (May)	66	4.92^{c}±0.060	84.123
6 (June)	62	4.92^{c}±0.004	84.661
7 (July)	46	5.16^{a}±0.033	146.812
8 (August)	46	5.06^{b}±0.030	115.401
9 (September)	60	4.99^{bc}±0.019	99.052
10 (October)	82	5.00^{bc}±0.016	100.582
11 (November)	104	5.01^{bc}±0.014	103.137
12 (December)	118	4.94^{c}±0.005	87.909
Parity		N.S	
1	354	5.00±0.005	93.854
2	328	4.99±0.007	99.583
3	172	4.94±0.006	88.876
4	254	4.96±0.006	91.853
Lactation period		N.S	
1 (50 ± 20)	518	5.00±0.005	100.257
2 (90 ± 20)	386	4.95±0.004	90.783
3 (130 ± 20)	204	4.93±0.003	85.930
The overall mean	**1108**	**4.94±0.003**	**87.923**

N.S: *Non-significant, * (P<0.05), ** (P<0.01),* ***a,b,c:*** *Means with different superscripts in each line are different*

Table 3. *The correlations between log_{10}SCC and udder traits in farms*

Tablo 3. *İşletmelerde meme özellikleri ile log_{10}SHS arasındaki korelasyonlar*

Traits	Farms		
	A	B	C
FTDM	0.11*	0.03	-0.08
RTDM	-0.04	-0.02	-0.01
FTL	-0.08	-0.05	-0.03
RTL	-0.04	-0.05	-0.07
FTP	-0.04	-0.01	-0.11
RTP	-0.06	-0.30	-0.12*
FTD	-0.01	0.12**	-0.07
RTD	-0.04	0.10*	0.02
STD	-0.05	0.09	-0.01
DFTF	-0.04	-0.04	-0.01
DRTF	-0.01	0.22	0.09

** (P<0.05), ** (P<0.01),* ***FTDM:*** *Fore teat diameter,* ***RTDM:*** *Rear teat diameter,* ***FTL:*** *Fore teat length,* ***RTL:*** *Rear teat length,* ***FTP:*** *Fore teat perimeter,* ***RTP:*** *Rear teat perimeter,* ***FTD:*** *Fore teat distance,* ***RTD:*** *Rear teat distance,* ***STD:*** *Side teat distance,* ***DFTF:*** *Distance from fore teat to floor,* ***DRTF:*** *Distance from rear teat to floor*

lobes that healthy and uninfected was reported [57]. In this study, SCC was determined as 4.94±0.003 (87.923) cell/mL. The vales of mean SCC (4.94±0.003 (87.923) in milk was lower than threshold value (500.000 cell/mL) that was acknowledged according to EU Directive. The mean values of SCC were found lower from findings of some researchers [29,36,37,45,46] and were found higher from findings of some researchers [47]. These circumstances is an indicator of conform to criteria of milk quality in farms in this study.

In the present study, the effect of farm on SCC was determined statistically significant (P<0.01). This result was found similar to findings of some other authors [33,35,36,38,39] and was showing differences with findings of some authors [37,44,46]. Also, the effects of month on SCC was determined statistically significant (P<0.01). The SCC values was found higher in summer months than winter months and the results of environmental temperature and hormonal mechanism of cows were reported from some researchers [29,36,40]. In this study, the effect of measuring month on SCC was found statistically significant (P<0.01), the highest value of SCC was determined on July-August and the lowest value of SCC on April, May and June. This result was found similar with findings of some researchers [36,38,48] and was found different from some authors [47]. The primary cause of differences in results may be heat stress from calving cow in summer season.

When analyzed to variations of SCC in accordance with parity, the highest value of SCC was determined in first parity and was found similar to values in other parity. The effect of parity on SCC was found statistically non-significant (P>0.05). This result was similar with findings of some researchers [46] and was found different from findings of some other authors [5,36,42,45,47].

In the present study, the effect of lactation stage on SCC was found statistically non-significant (P>0.05), while the effect of lactation stage on SCC was found statistically significant by some researchers [5,13,29,30,41-47].

In the present study, the positive correlation between SCC and FTP was found statistically significant in Farm A (P<0.01). The correlations SCC–FTD and SCC-RTD were found positive and statistically significant in Farm B (P<0.01) (respectively 0.12 and 0.10). The correlation between SCC and RTP was found negative and significant (P<0.01). These results were found similar with findings of some researchers [9,47]. Finally, correlations between SCC and

other udder traits were found statistically non-significant (P>0.05), especially relationships between SCC and FTL were found similar with findings of some researchers [58].

The results from this study indicate that some important findings about the correlations between SCC and some udder traits were obtained in farms. The values of mean SCC (4.94±0.003 (87.923) cell/mL) in milk was lower than threshold value (500.000 cell/mL) that was acknowledged according to EU Directive. The dairy cows are approved to physiologically for SCC in farms. In the sense of udder healthy optimum management programs should be carried to seasonal. Hereby, udder traits relevant to minimization or elevation of SCC should be carefully evaluated in selection studies.

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Kafkas Univ Vet Fak Derg
19 (4): 607-612, 2013
DOI: 10.9775/kvfd.2012.8518

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

Effects of Acrylamide Treatment on Oxidant and Antioxidant Levels in Rats

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Makale Kodu (Article Code): KVFD-2012-8518

Summary

The aim of this study was to investigate serum total antioxidant status (TAS), total oxidant status (TOS) and ischemia-modified albumin (IMA) levels in long term acrylamide (ACR) given rats, compared to control rats. In total, 25 male and 25 female Wistar rats were involved in this experiment. Animals in each sex were segregated into three groups. Two of them were treatment groups and one of them was control group. Each treatment group consisted of ten animals and each control group consisted of five animals. ACR was administered to the treatment groups at 2 and 5 mg/kg/day via drinking water for 90 days. In the end of the experiment, serum samples were analyzed for IMA, TAS, TOS and albumin levels with the spectrophotometric method. Serum IMA and adjusted IMA levels were significantly higher at concentrations of 2 mg/kg and 5 mg/kg in the male rats when compared with those of the control male rats. Serum TAS levels significantly decreased at concentrations of 5 mg/kg in the male rats when compared with those of the control rats. We also observed a significant increase in the levels of serum TOS at concentrations of 5 mg/kg in the male rats. There were no significant differences between serum IMA, TAS, TOS and albumin levels at concentrations of 2 mg/kg and 5 mg/kg in the female rats. Our findings show that long term treatment with 2 mg/kg and 5 mg/kg doses of ACR led to a significant depletion of serum TAS levels and overproduction of serum TOS and IMA levels, consequently, to an increase in oxidative stress.

Keywords: *Acrylamide, Total antioxidant status, Total oxidant status, Ischemia-modified albumin, Oxidative stress*

Ratlarda Akrilamid Kullanımının Antioksidan ve Oksidan Değerleri Üzerine Etkisi

Özet

Bu çalışmada, uzun süre akrilamid verilen sıçanlar üzerinde total antioksidan durum (TAS), total oksidan durum (TOS) ve iskemi modifiye albumin'in (IMA) serum düzeylerinin nasıl değiştiğinin araştırılması amaçlanmıştır. Çalışmada 65-75 g ağırlığında ve yaşları 3-4 haftalık 25 erkek ve 25 dişi Wistar cinsi sıçanlar kullanılmıştır. Hayvanlar 90 gün boyunca standart sıçan yemi ile beslenmişlerdir. Bununla beraber, günlük tüketecekleri içme suyuna 2 mg/kg/gün ve 5 mg/kg/gün dozunda akrilamid ilave edilmiştir. Akrilamid uygulaması sonrası hayvanlar anestezi altında servikal dislokasyonla öldürülmüş ve serumlarında IMA, TAS, TOS ve albumin düzeyleri spektrofotometrik yöntem ile ölçülmüştür. 2 mg/kg ve 5mg/kg akrilamid verilen erkek sıçanlara ait serum IMA düzeyleri kontrol grubuna göre önemli derecede yüksek bulunmuştur. Ayrıca, 5mg/kg akrilamid verilen erkek sıçanlara ait serum TAS düzeyleri kontrol grubuna göre önemli derecede düşük ve serum TOS değerleri önemli derecede yüksek bulunmuştur. 2 mg/kg ve 5mg/kg akrilamid verilen dişi sıçanlara ve kontrol grubuna ait serum IMA, TAS, TOS ve albumin düzeyleri arasında istatistiki açıdan önemli bir fark bulunamamıştır. Bu sonuçlara bağlı olarak bulgularımız, akrilamidin oksidatif stresi artırdığını göstermektedir.

Anahtar sözcükler: *Akrilamid, Total antioksidan durum, Total oksidan durum, İskemi modifiye albumin, Oksidatif stres*

INTRODUCTION

Acrylamide (C_3H_5NO) (ACR) is a small hydrophilic molecule that polymerizes readily in the presence of an initiator because of the double bond between the first and second C-atoms, which makes it a versatile industrial chemical [1]. Polymers of ACR (polyacrylamide) are extensively used in modern chemical technology for a variety of purposes [2]. In the last years, relevant amounts of ACR have been identified in heat-treated carbohydrate-rich foods

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such as fried potatoes, cookies, bread, cold breakfast cereal, biscuits and crackers [3,4]. It was shown that ACR might be formed through the Maillard reaction from amino acids (e.g. asparagine) and reducing sugars (e.g. glucose) [3]. Maillard reaction is proposed the major pathway for the formation of ACR in food [5,6]. Several different reaction routes have been proposed for the further reaction to ACR such as decarboxylation of the Schiff base [6-8], decarboxylated Amadori product [6,7], formation of a 3-Aminopropionamide [8,9], Strecker degradation of asparagine [5].

The biological consequences of ACR exposure have chiefly centered on neurotoxicity ever since this effect was observed in humans occupationally exposed to this compound. Subsequently, experimental exposure of rodents to ACR has also revealed a carcinogenic mode of action for this chemical [10]. It has been reported that most investigations of ACR toxicity have mainly focused on their genotoxic and carcinogenic properties; however, accumulating evidences seem to indicate that ACR also possesses cytotoxic properties by affecting the redox status of the cells [11].

Oxidative stress results from an imbalance between the production of free radical and antioxidant activity, leads to damage of biological macromolecules and disruption of normal metabolism and physiology [12]. Oxidative stres in the cells or tissues refers to enhanced generation of reactive oxygen species (ROS) and/or depletion of antioxidant defense system. ROS can attack the polyunsaturated fatty acid in the biological membranes and induce free radical chain reactions, leading to the enhancement of lipid peroxidation [13]. The methods used for the determination of oxidative stress are generally of board range. An often used and easily detectable parameter for the serum anti-oxidative and oxidant properties are the total antioxidant status (TAS) and total oxidant status (TOS) [14,15]. It has been showed that the high concentrations of ACR exert deteriorating effects on antioxidant enzyme activities, lipid peroxidation process and haemolysis in human erythrocytes *in vitro* [16].

Ischemia-modified albumin (IMA) is a novel marker of tissue ischemia [17]. IMA is serum albumin in which the N-terminus has been chemically modified [18]. The diagnostic albumin Co^{2+} binding test is based for IMA on the observation that the affinity of serum albumin for Co^{2+} is reduced after N-terminus modifications [18]. Proposed mechanisms for the conversion of serum albumin to IMA include hypoxia, acidosis, superoxide-radical injury, energy-dependent membrane distruption and exposure to free iron and copper [19]. Nowadays, IMA is accepted as a marker of oxidative stress. It has been proposed that ROS such as superoxide (O_2^-) and hydroxyl ($^{\cdot}OH$) radicals generated during ischaemia modify the N-terminus of serum albumin resulting in IMA formation [18].

Studies describing the role of oxidative stress in ACR toxicity are limited, although the main pathways for metabolism of ACR occur either through conjugation with reduced glutathione (GSH) or via epoxidation to glycidamide [16]. Also, to our knowledge, the effects of intermediate doses of ACR on serum IMA, TAS and TOS levels in rats have not been investigated yet. Therefore, the present experiment was carried out to investigate the effects of 2 and 5 mg/kg/day doses of ACR on serum IMA, TAS and TOS levels in rats.

MATERIAL and METHODS

Chemicals

ACR (Cas 79-06-1) was received from Sigma Aldrich Chemical Company. The material was a white odourless crystalline solid, with a chemical purity of>99% (for electrophoresis). The test material was prepared weakly and stored at room temperature. All other chemicals used were of analytical grade.

Animals and Husbandry

Male and female weaned Wistar rats, weighing 65-75 g and aged 3-4 weeks old, were obtained from Selcuk University Experimental Medicine Research and Application Centre (Konya, Turkey). The rats were housed in wire-topped opaque polycarbonate cages and maintained under constant environmental conditions with a 12 h light/dark schedule. The environmental temperature was 20°C and humidity was 50%. Commercial food pellets and drinking water were provided *ad libitum*. The protocols of the animal experiments were approved by the internal ethical commitee of the Selcuk University (number: 2010-98).

Dosing

We choosed the doses of ACR applied as 2 and 5 mg/kg/day in our study since not to occure a characteristic sign of ACR-induced neurotoxicity such as hindlimb foot splaying [20,21].

Experimental Design

In total, 25 male and 25 female Wistar rats were involved in this experiment. Animals in each sex were segregated into three groups. Two of them were treatment groups and one of them was control group. Each treatment group consisted of ten animals and each control group consisted of five animals. ACR was administered to the treatment groups at 2 and 5 mg/kg/day via drinking water for 90 days. Tap water was administered to five animals of control group in the same manner as in the treatment groups. After 24 h from the last application all animals were anaesthesized with ketamine in combination with a sedative. Blood samples were collected from the cardiac puncture of all rats under anaesthesia. After collecting blood samples rats were sacrificed by cervical dislocation.

Blood samples were obtained after suitable centrifugation and samples were stored frozen at -80°C until the day of analysis.

Measurement of Total Antioxidant Status

TAS of serum were determined using an automated measurement method which was based on the bleaching of the characteristic color of a more stable 2,2'-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) radical cation by antioxidants [14]. The results were expressed in mmol Trolox equivalents/L.

Measurement of Total Oxidant Status

TOS of serum were determined using a novel automated measurement method.[15] Oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar H_2O_2 equivalent per liter (μmol H_2O_2 equiv./L).

Measurement of Ischemia-Modified Albumin Levels

IMA level was measured by a colorimetric assay developed by Bar-Or et al.[17] based on measurement of unbound cobalt after incubation with patient serum. Increased amounts of IMA results in less cobalt binding and more residual unbound cobalt available for complex with a chromogen [dithiothreitol (DDT)], which can be measured photometrically. The procedure was as follows:

50 μL of 0.1% cobalt chloride was added to 200 μL of serum, gently mixed, and waited 10 min for adequate cobalt-albumin binding. Fifty microliters of DTT, at a concentration of 1.5 mg/ml, was added as a colorizing agent and the reaction was stopped 2 min later by adding 1.0 mL of 0.9% NaCl. The colored product was measured at 470 nm and compared to a serum-cobalt blank without DTT and reported in absorbance units (ABSU).

Adjusted IMA was calculated as (individual serum albumin concentration/median serum albumin concentration of the population)×IMA ABSU value. This formula was applied to correct IMA values for serum albumin (Median serum albumin concentration of each group of the subjects were used separately) [22].

Measurement of Albumin Levels

Serum albumin levels were measured by commertially available kits based on routine methods on the Synchron LX System (Beckman Coulter, Fullerton CA).

Statistical Analysis

All data are expressed as mean±standart deviations (SD). Statistical analyses were done using SPSS v. 16.0 (SPSS Inc.,IL, USA). Groups of data were compared with an analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Differences were considered significant at a probability level of $P<0.05$.

RESULTS

Serum IMA, adjusted IMA, TAS, TOS and albumin levels of the groups are presented in *Table 1*. As seen from the table, serum IMA and adjusted IMA levels were significantly higher at concentrations of 5 mg/kg ($P<0.001$ for IMA and $P<0.01$ for adjusted IMA) and 2 mg/kg ($P<0.01$) in the male rats when compared with those of the control male rats. However, there were no significant differences between the same parameters at concentrations of 5 mg/kg and 2 mg/kg in the female rats and the control female rats *(Fig. 1)*. Serum TAS levels significantly decreased at concentrations

Table 1. *Serum IMA, adjusted IMA, TAS, TOS and albumin levels of the groups (All values are mean±standart deviations)*
Tablo 1. *Grupların serum IMA, ayarlanmış IMA, TAS, TOS ve albumin düzeyleri (Bütün değerler ortalama±standart sapma)*

Parameter	Group	Control	ACR 2 mg/kg	ACR 5 mg/kg
IMA (ABSU)	Male	0.53±0.09	0.71±0.07[b]	0.72±0.07[a]
	Female	0.64±0.09	0.69±0.06	0.73±0.03
Adjusted IMA (ABSU)	Male	0.54±0.09	0.71±0.07[b]	0.73±0.07[b]
	Female	0.63±0.1	0.70±0.08	0.73 ± 0.02
TAS (mmol Trolox equivalents/L)	Male	3.65±1.2	3.34 ± 1.2	1.77±1.1[c]
	Female	3.69±1.5	3.67±1.1	3.31±1.0
TOS (μmol H_2O_2 Eq/L)	Male	8.88±2.3	12.67±2.0	14.72±5.0[c]
	Female	9.95±1.9	11.18±2.5	14.28±4.1
Albumin (g/dL)	Male	2.72±0.1	2.66±0.2	2.63±0.1
	Female	3.17±0.1	3.06±0.4	2.88±0.3

IMA- *ischemia-modified albumin;* ***TAS-*** *total antioxidant status;* ***TOS-*** *total oxidant status;* [a] *P<0.001,* [b] *P<0.01,* [c] *P<0.05 compared with control group*

of 5 mg/kg (P<0.01) in the male rats when compared with those of the control rats. But, this difference was not found at concentrations of 5 mg/kg in the female rats. On the other hands, there were no significant differences between serum TAS levels at concentrations of 2 mg/kg in the male and female rats and the control rats *(Fig. 2)*. We also observed a significant increase in the levels of serum TOS at concentrations of 5 mg/kg (P<0.05) in the male rats. No differences were found in serum TOS levels between at concentrations of 5 mg/kg in the female rats and control rats. Also, no differences were found in serum TOS levels between at concentrations of 2 mg/kg in the male and female rats and control rats *(Fig. 3)*. There were no significant differences between serum albumin levels of the groups. In addition, no sign of sickness and decreased activity of the rats or mortality was observed during the study.

DISCUSSION

ROS are produced in metabolic and physiological processes, and harmful oxidative reactions may occur in organisms, which remove them via enzymatic and non-enzymatic antioxidative mechanisms. Under some conditions, the increase in oxidants and decrease in anti-oxidants cannot be prevented, and the oxidative/anti-oxidative balance shifts toward the oxidative status [23]. Several reports have reported that ACR promotes the generation of ROS and the depletion of anti-oxidants [13,24].

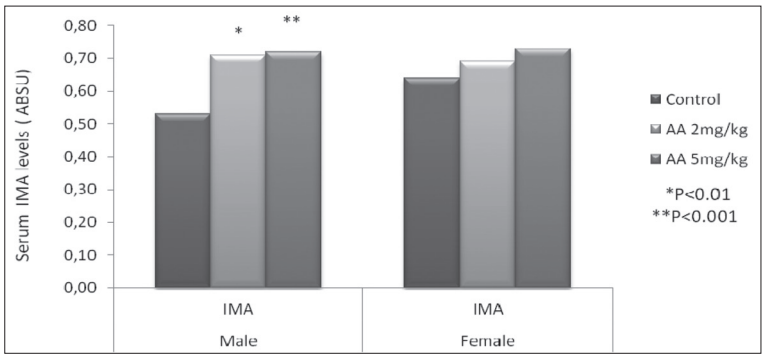
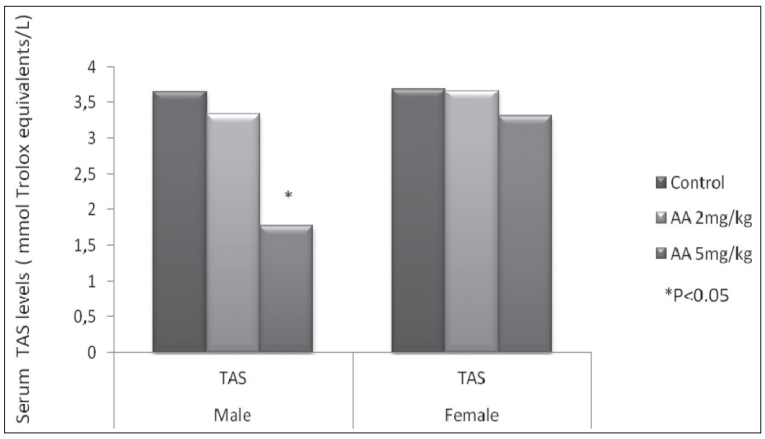


Fig 1. Serum ischemia-modified albumin (IMA) levels in all groups

Şekil 1. Bütün gruplarda serum iskemi-modifiye albumin (IMA) düzeyleri

Fig 2. Serum total antioxidant status (TAS) levels in all groups

Şekil 2. Bütün gruplarda serum total andioksidan durum (TAS) düzeyleri



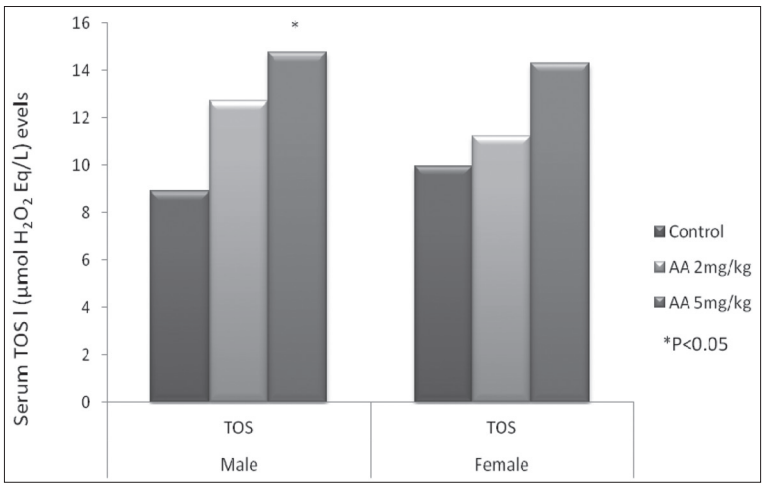


Fig 3. Serum total oxidant status (TOS) levels in all groups

Şekil 3. Bütün gruplarda total antioksidan (TOS) durum düzeyleri

The present study shows that ACR given via drinking water to male rats induced to increase in TOS and IMA levels and decrease in TAS levels.

Conjugation with GSH catalyzed by glutathione-S-transferase is the main pathway of ACR metabolism and detoxification [25]. GSH is one of the essential compounds for the maintenance of the cell integrity because of its reducing properties and participation in the cell metabolism [26]. ACR is capable of interacting with vital cellular nucelophiles possessing –SH, $–NH_2$ or –OH. Therefore, it reacts with glutathione in a similar manner and forms glutathione *S*-conjugates, which is the initial step in the biotransformation of electrophiles in to mercapturic acids [27]. Decreasing GSH content in the tissues with the increase of ACR concentration could be due to increased formation of *S*-conjugates between ACR and GSH [28]. ACR can be reactive unlike the above mentioned way. Firstly, ACR can undergo radical-mediated polymerization. Secondly, it can be metabolized to an epoxide derivate, glycidamide, presumably by cytochrome P4502E1, being readily reactive toward DNA and other macromolecules [16].

In the literature, our finding supported in studies which made with different oxidative stress marker after ACR -treatment. For example, Allam et al.[29] have reported that ACR administered either prenatally or perinatally has been shown to induce significant increase of thiobarbituric acid reactive substances (TBARS) and oxidative stres (significant reductions in total thiols, superoxide dismutase (SOD) and peroxidase activities) in the developing cerebellum of rats [29]. Alturfan et al.[30] have found that 8-OHdG levels as an indicator of oxidative DNA damage, significantly increased in ACR group. In addition, they demonstrated that in ACR-treated rats, GSH levels decreased significantly while the malondialdehyde (MDA) levels, myeloperoxidase activity and collagen content increased in the tissues suggesting oxidative organ damage. Yousef et al.[28] have found that the concentration of TBARS, and the activities of glutathione *S*-transferase and SOD in plasma, liver, testis, brain, and kidney were increased in ACR -treated male rats [28]. Catalgol et al.[16] have demonstrated that *in vitro* treatment of ACR results in haemolysis and induction of lipid peroxidation and changes the activities of antioxidant enzymes at high doses in human erythrocytes.

The results of the present study indicate that serum IMA levels increase in ACR-treated rats, compared to control rats. Albumin is the most abundant serum protein, and is a powerful extracellular antioxidant. The biochemical mechanism modifying the N-terminal region of albumin during ischemia is unclear, but reperfusion after an ischemic event may damage serum albumin as much as, if not more than, ischemia itself [31]. These modifications to albumin may involve hypoxia, acidosis, or free radical damage, most of which occur within minutes [23]. According to our hypothesis, IMA is increased in conditions that produce free radicals, such as ACR -treatment. Naruszewicz et al.[32] have reported that the first time that chronic ingestion of dietary ACR might induce oxidative stress in humans through leukocyte activation and increased production of ROS [32]. In addition, they have reported that a significant increase in the oxidized low-density lipoprotein, interleukin-6, and high sensitivity C-reactive protein concentrations occurred, with all these factors recognized as capable of enhancing atherosclerosis progression [32]. According to their hypothesis, long-term ingestion of high ACR doses with food may cause chronic inflammation and contribute to the development of early atherosclerosis as well as increase the risk of coronary artery disease. IMA was considered to be related as a potential cardiovascular risk factor [18]. Previous studies have found that IMA levels increased in acute coronary syndromes, during percutaneous coronary intervention, and myocardial ischemia [17,33,34]. Our finding supports Naruszewicz et al.'s [32] hypothesis. Because, long term intermediate doses ACR -treatment, which increased serum IMA and TOS levels, may be risk for cardiovascular diseases.

On the other hand, a significant increase serum IMA and TOS levels and decrease TAS levels did not observed in female rats. We think that estrogen may be cause this situation. The hormone estrogen, (estradiol) provides natural anti-oxidant properties because it increases the expression of antioxidant enzymes and decreases troublesome NADPH oxidase enzyme activity and superoxide production. Estrogens have a phenolic hydroxyl group at position 3 and a methyl group at position 13. The presence of this phenol group gives estrogen its antioxidant property by neutralizing oxygen free radicals. The estrogens estriol and estradiol confer significant antioxidant activity [35].

In our study, ACR administered rats grew normally and gained body weight over a 12-week period, and there were no significant differences in the average body weight compared with control rats. This finding is in accordance with that of Park et al.[36]. However, previous studies indicated that rats exposed to ACR showed a decrease in body weight [37,38]. According to our hypothesis, there were no changes in body weight in intermediate doses.

As a result, our findings show that long-term treatment with intermediate-ACR doses led to a significant depletion of serum TAS levels and overproduction of serum TOS and IMA levels especially in male rats, and cause oxidative stress.

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Kafkas Univ Vet Fak Derg
19 (4): 613-618, 2013
DOI: 10.9775/kvfd.2013.8406

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

Effect of Hyaluronic Acid/Carboxymethylcellulose and Flunixin Meglumine Combination on the Prevention of Postoperative Intraabdominal Adhesions: An Experimental Study in Rabbits [1]

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[1] *This study was presented in "IV th Congress of Veterinary Gynecology (International Participation), November 4-7, 2010, Belek, Antalya - TURKEY"*

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Makale Kodu (Article Code): KVFD-2013-8406

Summary

Despite the development in the knowledge concerning pathophysiology of abdominal cavity and improvement in surgical techniques, intraabdominal adhesions are one of the most common complications in surgery. The aim of this study was to determine the effects of hyaluronic acid/carboxymethylcellulose (HA/CMC) and flunixin meglumine combination on the prevention of postoperative intraabdominal adhesions in rabbits. All the surgery were made under aseptic condition under general anesthesia. A 2x2 cm area of parietal peritoneum was resected on the abdominal wall after median laparotomy. Twenty-four adult, male rabbits were divided into four groups equally. Groups were evaluated as C (control), S (HA/CMC), F (flunixin meglumine), and SF (HA/CMC and flunixin meglumine combination). No medication was given to the rabbits in group C. In S and SF groups, 3x3 cm of HA/CMC applied directly to peritoneal defect. In F and SF groups, flunixin meglumine was administred 1.1 mg/kg for 5 days intraperitoneally. Relaparotomy was performed 10 days after surgery. As a result of macroscopical and histopathological evaluations, the scores of adhesion in the S, F, and SF groups were significantly lower than those of the control group ($P<0.05$). Adhesion scores in SF group were lower as compared to S and F groups ($P<0.05$). It is observed that HA/CMC and flunixin meglumine combination can be used more effective on the prevention of postoperative intraabdominal adhesions in rabbits.

Keywords: *Intraabdominal adhesion, Hyaluronic acid/carboxymethylcellulose, Flunixin meglumine, Rabbit*

Postoperatif İntraabdominal Adezyonların Önlenmesinde Hyaluronik Asit/Karboksimetilselüloz ve Fluniksin Meglumin Kombinasyonu'nun Etkisi: Tavşanlarda Deneysel Bir Çalışma

Özet

Abdominal boşluğun patofizyolojisi konusunda bilgilerin artmasına ve cerrahi tekniklerin gelişmesine rağmen intraabdominal adezyonlar, cerrahideki en önemli sorunlardan biridir. Bu çalışmada, tavşanlarda postoperatif intraabdominal adezyonların önlenmesinde hyalüronik asit/karboksimetilselüloz (HA/KMS) ve fluniksin meglumine kombinasyonunun etkisinin değerlendirilmesi amaçlandı. Tüm operasyonlar genel anestezi eşliğinde aseptik koşullar altında yapıldı. Median laparotomiden sonra abdominal duvar üzerinden 2x2 cm'lik bir parietal periton alanı rezeke edildi. Çalışmada 24 adet yetişkin erkek tavşan 4 gruba eşit olarak ayrıldı. Gruplar; C (kontrol), S (HA/KMS), F (fluniksin meglumine), ve SF (HA/KMS ve fluniksin meglumine kombinasyonu) olarak değerlendirildi. Kontrol grubundaki olgulara herhangi bir tedavi uygulanmadı. S ve SF gruplarındaki olguların defektli alanları 3x3 cm'lik HA/KMS ile örtüldü. F ve SF gruplarına, fluniksin meglumine 1.1 mg/kg dozunda intraperitoneal olarak 5 gün süre ile verildi. Postoperatif 10. günde tekrar laparotomi yapıldı. Makroskobik ve histopatolojik değerlendirmeler sonucunda; S, F, and SF gruplarındaki adezyon skorları kontrol grubundakine oranla önemli derecede düşük bulundu ($P<0.05$). SF grubundaki adezyon skorları, S ve F grupları ile karşılaştırıldığında daha düşük olduğu saptandı ($P<0.05$). Tavşanlarda postoperatif intraabdominal adezyonların önlenmesinde HA/KMS ve fluniksin meglumine kombinasyonun daha etkili olduğu gözlendi.

Anahtar sözcükler: *İntraabdominal adezyon, Hyalüronik asit/karboksimetilselüloz, Fluniksin meglumine, Tavşan*

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INTRODUCTION

Adhesions are fibrous bands that cause separate organs to adhere to one another and can be classified into congenital, inflammatory, and postoperative. Postoperative adhesions are considered to be an inevitable result of surgery. The incidence of postoperative adhesion ranges from 66 to 93% after operations [1-3]. Surgical trauma, tissue ischemia, hemorrhage, infection, and foreign materials play an important role in etiology of postoperative adhesions. Postoperative intraabdominal adhesions cause chronic abdominal/pelvic pain, female infertility, and small bowel obstruction. Also, fibrous adhesions make prolong operation time, operation difficulty, increased bleeding, intestinal rupture, and morbidity in repeated operations [1-5].

When there is an injury in mesothelial surface of the peritoneum, it triggers an inflammatory reaction characterized by increased neutrophil leucocytes, fibroblasts, macrophages, and mesothelial cells. There is increase in vascular permeability and release of fibrin-rich exudate. Peritoneal mesothelial cells have a critical duty in maintaining the intraperitoneal balance between fibrinolysis and formation. Plasminogen is an important protein in the process of fibrinolysis. Early fibrinolysis, within 5 days, encourages healing of the peritoneum without adhesion formation. Inadequate fibrinolysis processes lead to persistent fibrin structures that subsequently mature into fibrous tissue, followed by organization into rigid, persistent fibrous adhesions containing blood vessels and nerve fibers [1-6].

Many different techniques, agents, and materials have been tried to reduce adhesions [1-3,6-9]. Several pharmacological drugs including corticosteriod, non-steroid antiinflammatory drugs (NSAIDs), melatonin, progesterone, aprotinin, methylen blue, heparin, mitomycin C, vitamin E, and tPA have been used to prevent adhesions [1,2,8,10,11]. NSAIDs inhibit prostaglandin and thromboxane synthesis by changing cycloxygenase activities. Flunixin meglumine (non-selective COX inhibitors) are commonly used to treat animals suffering from various diseases and has been proved to prevent the formation of peritoneal adhesions in studies [1,2,7,9-12]. Mechanical barriers such as carboxymethylcellulose, hyaluronic acid, icodextrin, polyethylene glycol, fibrin glue, oxidized regenerated cellulose, expanded polytetrafluoro ethylene, and hyaluronic acid/carboxymethylcellulose (HA/CMC) have been applied to prevent adhesion by mechanically minimizing the development of fibrin between serosal surfaces [1,2,6,13]. HA/CMC is a biosynthetic material that have been widely used in human and animals. In developed countries, HA/CMC is accepted as an important antiadhesive barrier. Unfortunately, none of these treatments is ideal [1-3,5,8,9,14-16].

Although several substances have been tried to eliminate adhesions, these have had a limited success in the intraabdominal adhesions. A current interest to overcome adhesions have been focused on various combination of drugs [2,4,8,9,17-23]. Therefore, it was to investigate the effect of HA/CMC, flunixin meglumine and their combination on the prevention of postoperative intraabdominal adhesions in rabbits.

MATERIAL and METHODS

Animals

Twenty-four rabbits weighing approximately 2.8-3.6 kg were used in this study. The rabbits were hospitalized for 2 weeks with a deprivation of food for 12 h immediately before surgery. The animals were kept under standart conditions and had free access to water and pellets. The study protocol was approved by Animal Ethic Committee of the Veterinary Faculty, Firat University (2006/7).

Anesthesia

The animals were anesthetized with 10 mg/kg of xylazine HCl (Rompun®, Bayer, İstanbul, Turkey) and 40 mg/kg of ketamine HCl (Ketalar®, Eczacıbaşı, İstanbul, Turkey) intramuscularly.

Surgical Procedure

All the surgery were performed under aseptic conditions, including an iodine scrub, drapping and use of a steril technique. Powder-free gloves were used for the operation. Following complete aseptic preparations, a 5-6 cm of incision was performed for midline laparotomy. A 2x2 cm area of parietal peritoneum was removed on the right abdominal wall. The animals were divided into four groups, each containing 6 rabbits: group C (control), group S (HA/CMC), group F (flunixin meglumine), and group SF (HA/CMC and flunixin meglumine combination). No medication was applied to the control group. HA/CMC (Seprafilm®, Genzyme, USA) 3 cm x 3 cm were placed on the deperitonized area of abdominal wall in S and SF groups. Flunixin meglumine (Finadyn®, Eczacıbaşı, İstanbul) was given 1.1 ml/kg for 5 days in F and SF groups intraperitoneally. The abdomen was closed with continuous sutures with 2/0, and skin was closed with interrupted 2/0 polypropilene sutures (Monofilament polyglytone, Caprosyn™, USA).

All animals were sacrified on the 10th days after operation. After performing a U-type incision to the anterior abdominal wall, the evaluation was macroscopically classified according to adhesion scoring system described by the Majuzi and colleagues [24]. The degree of adhesion formation was evaluated with the following adhesion scores: 0=no adhesion, 1=light adhesions can be easily removed through blunt dissection, 2=moderate adhesions which need sharp dissections (lower than 50%) for the separation, 3=severe adhesions which need

sharp dissections (more than 50%) for the separation, 4=presence of serosal injury, 5=presence of full-thickness injury.

Histopathological Evaluations

Peritoneal tissue samples containing adhesion were excised and the samples were fixed in 10% formaldehyde solution. After dehydration, the specimens were embedded in paraffin blocks. Then, 5 µm sections in thickness were prepared using a microtome and stained with Hematoxylin and Eosin (H&E). Histopathological evaluations were performed under a light microscope by one pathologist who was blind to groups according to semiquantitative scoring system described by Hooker and colleagues [25]. The histopathological adhesions were categorized as Grades 0–III based on the presence and extent of fibrosis and inflammation. Grade 0 was defined as no fibrosis, grade I as mild fibrosis, grade II as moderate fibrosis, and grade III as severe fibrosis. The grade of inflammation was assessed according to grade I on this scale represents a mild inflammatory reaction with giant cells, occasionally scaretted lymphocytes, and plasma cells, grade II represents moderate reaction with giant cells and increased admixed lymphocytes, neutrophiles, eosinophiles and plasma cells, and grade III represents a severe inflammatory reaction with microabscesses present.

Statistical Analysis

The group data were analyzed using the Kruskal-Wallis nonparametric test. Differences among the groups were evaluated using Mann-Whitney U test. Differences with a value of $P<0.05$ were considered to be statistically significant. Data were analyzed by SPSS software program (Version 14.0; SPSS Inc, Chicago IL).

RESULTS

Most of the animals tolerated the surgery well. No death was observed throughout the study period. There was no evidence of any of complication in the animals. The results of macroscopic and histopathologic evaluation are summarized in *Table 1*.

There were no adhesions in Grade 0, 1 or 2 in the control group. The adhesions in the control group were mostly Grade 3 or 4. In one case in control group, adhesion

Table 1. *The statistical results of adhesion, fibrosis, and inflammation scores*
Tablo 1. *Adezyon, fibrozis ve yangı skorlarının istatistiksel sonuçları*

Parameters	Groups				P value
	C	S	F	SF	
Macroscopic	3.56±0.89[a]	1.83±0.75[b]	2.00±0.89[b]	1.05±0.89[c]	0.002
Fibrosis	2.50±0.55[a]	1.50±0.55[b]	1.33±0.52[b]	1.18±0.00[b]	0.003
Inflammation	2.50±0.55[a]	1.52±0.55[bc]	1.23±0.52[b]	0.93±0.41[c]	0.002

Superscripts a-c indicate that means in the same line without a common superscript differ significantly (P<0.05)

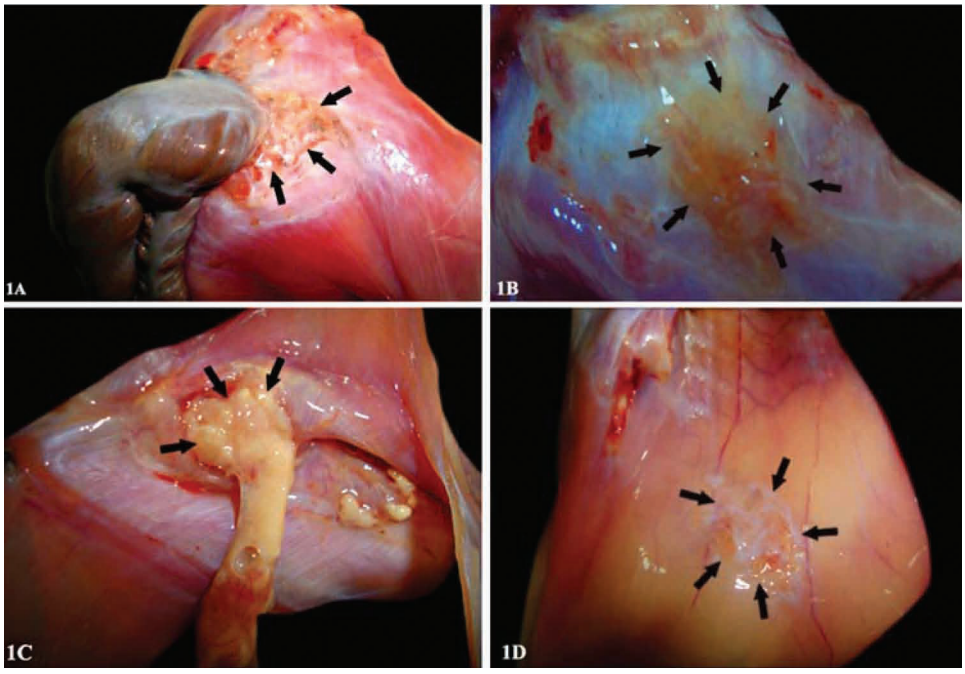

Fig 1. Macroscopical evaluations: **A)** Presence of full-thickness injury and large area of fibrosis *(arrows)* with score 5 in C group, **B)** Slight adhesion *(arrows)* with score 1 in S group, **C)** Severe adhesion which need sharp dissections (more than 50%) for the separation with score 3 in F group, **D)** No adhesion occurence with score 0 in SF group

Şekil 1. Makroskobik değerlendirmeler. **A)** C grubundaki 5. derece adezyonlu geniş fibrozis *(oklar)* ve tam kat adezyon varlığı, **B)** S grubundaki 1. dereceli hafif adezyon *(oklar)* görünümü, **C)** F grubundaki 3. derecedeki ayrılma için keskin diseksiyona (%50 den daha fazla) ihtiyaç gösteren ciddi adezyonun görünümü, **D)** SF grubundaki adezyonun yokluğu (0 derece)

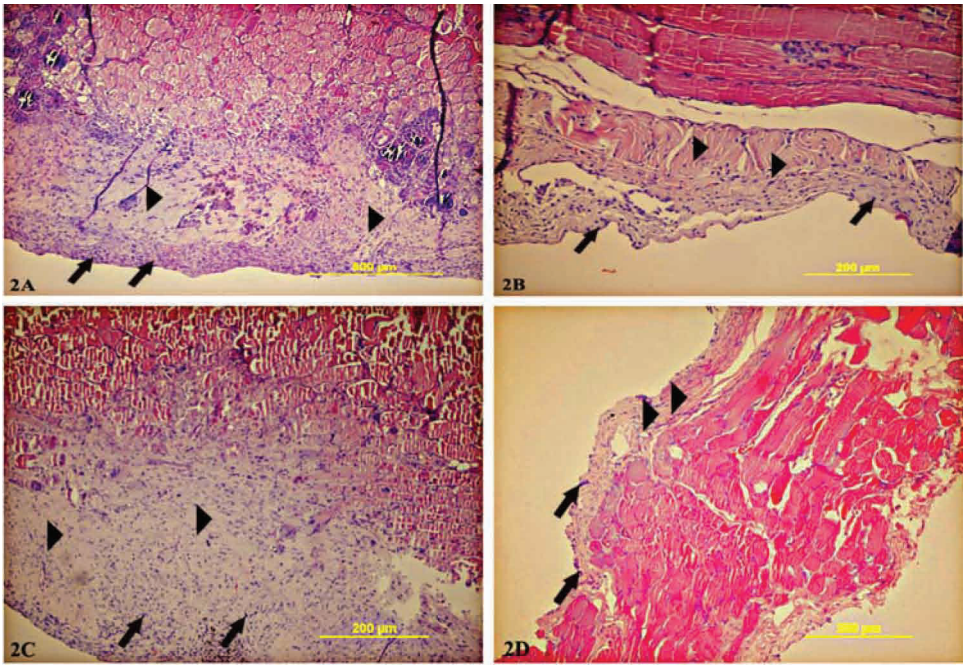

Fig 2. Histopathological evaluations. **A)** Severe fibrosis *(arrow heads)* and inflammatory changes *(arrows)* in C group, Grade III, **B)** Mild fibrosis *(arrow heads)* and mild inflammatory changes *(arrow)* S group, Grade 1, **C)** Severe fibrosis *(arrow heads)* and inflammatory changes *(arrows)* in F group, Grade III, **D)** Mild fibrosis *(arrow heads)* and no inflammatory changes *(arrows)* in SF group

Şekil 2. Histopatolojik değerlendirmeler. **A)** C grubundaki şiddetli fibrosis *(ok başı)* ve yangısal değişiklikler *(ok),* **B)** S grubundaki hafif fibrozis *(ok başı)* ve yangısal değişiklikler *(ok),* **C)** F grubundaki şiddetli fibrozis *(ok başı)* ve yangısal değişiklikler *(ok),* **D)** SF grubundaki hafif derecedeki fibrosis *(ok başı)* ve yangısal bulgu yokluğu *(ok)*

in Grade 5 was recorded *(Fig. 1-A)*. On the other hand, there were no adhesions in Grade 4 or 5 in the S, F, and SF groups. Most adhesion scores in the S and F groups were grade 1, 2, and 3 *(Fig. 1-B-C)*. Adhesion scores in the SF group were seen 2 cases in Grade 1 and 2. No adhesion was noted in 2 cases in the SF group *(Fig. 1-D)*. Statistical analysis indicated that adhesion scores were significantly lower in S, F, and SF groups when compared to the control group ($P<0.05$). A statistically significant difference was not observed between groups S and F ($P>0.05$). Group SF showed the lowest incidence of adhesions as compared to S and F groups macroscopically ($P<0.05$).

Severe fibrosis and inflammatory changes were seen in control group *(Fig. 2-A)*. Minimal fibrosis and low degree of inflammation were observed in the S, F, and SF groups *(Fig. 2 B-C)*. Statistical evaluation of fibrosis scores showed significant difference in S, F, and SF groups when compared to the control group ($P<0.05$). On the other hand, there was not significant difference between scores of fibrosis in SF group compared to that of S and F groups ($P>0.05$). However, there was a significant difference between the degrees of adhesion and inflammatory changes in SF group compared to S group ($P<0.05$), *(Fig. 2-D)*.

DISCUSSION

Despite the development in the knowledge concerning pathophysiology of abdominal cavity and improvement in surgical techniques, postoperative intraabdominal adhesions are significant health problem with major adverse effects on quality of life, use of health care resources, and financial costs after laparotomy [1-4,7-9,18,21].

There are many experimental models for the development of inflammatory and postoperative adhesions including the local peritoneal model, the damaged uterine horn, introduction of foreign objects in the abdominal and pelvic cavity, the direct mechanical intestinal damage, the ileal transection, the thermal damage, the large bowel anastomosis, the bacterial contamination, and the scraping models [16,17,19,20,22]. In this study, the parietal peritoneal model was chosen due to the similarities of the physiopathology after abdominal surgery. Since, it was observed that all cases in the control group occurred different degrees of adhesion.

Various studies have pointed out that the adhesion-forming process and mesotheliozation are completed after 7 to 14 days following surgery [10,17,23,26]. Therefore, it was chosen to wait 10 days before performing relaparotomy because other studies had shown high adhesion scores at the period of this time [4,5,27]. There have many adhesion scoring systems described by researchers in experimental models [4-6,16,17,22]. Macroscopical and histopathological evaluations were made according to semiquantitative scoring systems that had been described by Mazuji et al.[24] and Hooker et al.[25]. To date mentioned evaluations which have been used by many reseachers [4,22,23] is simple and suitable scoring systems for use in experimental studies.

The peritoneal healing process differs from skin healing process, whose differences actually came from the reepithelization, and consequences of fibrin depositon. Intraabdominal adhesions are formed as a result of tissue inflammation, fibrin deposition, fibrin organization, collagen formation and maturation [2,3,6,7]. Surgical trauma initiates a widespread inflammatory response in the peritoneum that is associated with the recruitment and activation of inflammatory cells, and the secretion of proinflammatory

mediators. Peritoneal inflammation leads to the formation of an inflammatory exudate. The increase of vessel permeability produces an outpouring of serosangineuous excudate rich in inflammatory cells. These cells promotes the formation of a fibrin-rich matrix at the sites of peritoneal injury that leads to the formation of fibrinous adhesions and eventually permanent adhesions [1,-3,7,22].

It is important to use minimal invasive surgery techniques and various agents to diminish the post-operative adhesions. These techniques and equipments have focused mainly on limiting tissue injury, like keeping the tissues moist, removing of foreign materials, avoiding of desication/ischemia, and using of laparoscopy. The roles of pharmacogical drugs are diminishing inflammatory response, enhancing fibrinolysis, preventing collagen deposition and maturation [1-4,6]. NSAIDs decrease vascular permeability, platelet aggregation, plasmine inhibitor and coagulation, and enhance macrophage function [1,2,7]. Some researchers [7,10,11,22] have demonstrated that NSAIDs have been used to prevent the adhesion formations by blocking the initial stage of inflammatory response. An optimal barrier should be non-toxic, biodegradable, easily appliable, without compromising wound healing, remain active in the presence of body fluids, and dissolved after 1 to 2 weeks. HA/CMC has been used to reduce adhesion formation after the surgery. It becomes a hydrophilic gel within approximately 24 hours after placement. It is absorbed within 7 days and cleared completely from the body within 28 days [1-4,14-16]. In our study, it was used the effect of flunixin meglumine 1.1 mg/kg on the postoperative adhesions and HA/CMC covered on the peritoneal defect. These results, as expected under guidance of previous result of the studies [7,10,11,14-18], have supported that both HA/CMC and flunixin meglumine decreases adhesion formation, as evaluated by the scoring of adhesion, fibrosis, and inflammatory compared with the control group (P<0.05), *(Table 1)*.

The physical barriers should focus on more widespread adhesion prevention without compromising peritoneal fibrinolytic activity. HA/CMC is accepted as one of the most effective barrier to prevent intraabdominal adhesions. However, their efficacy may be limited to the site of application. HA/CMC does not address the complex nature of adhesion formation and has hydroflotation and sliconization effect on the applicated area [6-8,9,21,28]. An current approach to increase the efficacy of barriers or drugs could be improved the synergistic effect of various combinations without causing significant complications [4,9,19-23,26,28]. Lim et al.[8] found that the coadministration of HA/CMC with neurokinin 1 receptor antagonist not only increases the efficacy of the barrier at the site of application, but also significantly reduces the adhesions formation at distal unprotected sites. Attar et al.[18] have reported that combined use of HA/CMC and melatonin were found to be effective reducing the adhesion formation in a rat uterine horn model. In this study, adhesion scores of the HA/CMC and melatonin groups were similar, and significantly lower than those of the control group. Also, adhesion scores in the combination group were lower than those in the control, HA/CMC, and melatonin groups. Altun et al.[23] recently showed that low molecular weight heparin and octreotide were almost have the same effect for prevention of adhesion formation. In this work, it was observed that the scores of adhesion and inflammation in SF group were significantly lower than that in S and F groups (P<0.05), *(Tablo 1)*. So that based on this results, it was concluded that they were effective in combination rather than the separate using. The result of HA/CMC and flunixin meglumine combination in this study was paralel to those of other combination [8,9,18,23,26].

Numerous articles on the combination of some agents used adhesion prevention have been published but knowledge on this subject is limited and contradictory [17,18,29-31]. Şahin and Sağlam [19] researched heparin to the carboxymethylcellulose at laparotomy, whereas Başbuğ et al.[20] added hyaluronic acid plus heparin, Tayyar et al.[26] tried heparin to the amniotic membrane to cover injured rabbit uterine horns. These combinations seem to be more effective in reducing adhesion formation rather than the use of heparin, hyaluronic acid, carboxymethylcellulose and amniotic membrane alone. Conversely, some researchers [17,27,29-32] revealed that there was no difference between the groups in the combined application of various drugs (HA/CMC+atorvastatin, dimethyl sulfoxide+synovial fluid, low molecular weight heparin+hyperbaric oxygen, medroxyprogesterone acetate+heparin, HA/CMC+vitamin E, vitamin E+selenium) on the prevention of intra-abdominal adhesions.

In conclusion, HA/CMC and flunixin meglumine reduce significantly the prevalence of adhesion development separately. However, HA/CMC and flunixin meglumine combination have reduced more effectively the formation of postoperative intraabdominal adhesions. Further and more detailed investigations could be useful and necessary using additional examinations.

Acknowledgements

The author would like to thank to Dr. Yesari ERÖKSÜZ for pathological evaluations, Dr. Cemal ORHAN for his help in statistical analysis, and Dr. Süleyman AYDIN for checking the English of the manuscript.

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Kafkas Univ Vet Fak Derg
19 (4): 619-627, 2013
DOI: 10.9775/kvfd.2013.8442

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

Risk Factors Associated with Passive Immunity, Health, Birth Weight and Growth Performance in Lambs: II. Effects of Passive Immunity and Some Risk Factors on Growth Performance During the First 12 Weeks of Life [1]

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[1] *This study was supported by TUBITAK (Project code; TOVAG 110 O 847)*

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Makale Kodu (Article Code): KVFD-2013-8442

Summary

The primary objective of this study was to evaluate the relationship between passive immunity and growth performance in lambs in neonatal and post-neonatal periods. The study also evaluated the effect of gender, type of birth, birth weight and health of lambs, lambing season and dam's age on growth performance. This study included two crossbred Akkaraman flocks (n=301) in Kars, Turkey. The results disclosed that serum IgG concentration determined at 24th of birth (SIgG-24) was significantly associated with growth performance in both periods (P<0.001) but this relationship was poor (R^2=0.148 to 0.198). Neonatal growth performance was influenced only by birth weight, health status, SIgG-24 and type of birth (R^2=0.527 to 0.721 P<0.001) on multiple stepwise regression analyses. The study also disclosed that post-neonatal growth performance was affected by all variables (P<0.001 R^2=0.471 to 0.472). This study suggested that passive transfer cause a significant amount of variation in growth performance for lambs from birth to 12 weeks of life, a conclusion that has not previously been identified. It also disclosed positive association among growth performance, birth weight, type of birth, gender, health status of lambs, dam's age and lambing season. The growth performance was rendered by dam's age (≤2 years old), twin lambs, female lambs, winter lambing, illness, and birth weight of ≤3 kg. These factors should be taken into consideration and dealt with in order to increase growth performance so that which farm productivity and profitability is maintained.

Keywords: *Lamb, Passive immunity, Growth performance, Risk factors*

Kuzularda Pasif İmmünite, Sağlık, Doğum Ağırlığı ve Büyüme Performansı İle İlişkili Risk Faktörleri: II- Pasif İmmünite ve Bazı Risk Faktörlerinin İlk 12 Haftalık Büyüme Performansı Üzerine Etkisi

Özet

Bu çalışmanın temel amacı kuzularda neonatal ve sonraki periyotta pasif immünite ve büyüme performansı arasındaki ilişkinin belirlenmesidir. Çalışmada ayrıca kuzunun cinsiyeti, doğum tipi, doğum ağırlığı ve sağlığı ile birlikte kuzulama sezonu ve anne yaşının büyüme performansı üzerine etkisi değerlendirildi. Çalışma Türkiye'de Kars'ta iki Akkaraman melezi sürüde (n=298) yürütüldü. Sonuçlar doğumdan sonra 24. saat serum IgG konsantrasyonu ve her iki dönem büyüme performansı arasında önemli (P<0.001) fakat zayıf (R^2=0.148-0.198) bir ilişki olduğunu göstermiştir. Çoklu adımsal regresyon modeli ile neonatal büyüme performansının sadece doğum ağırlığı, sağlık durumu, SIgG-24 ve doğum tipinden etkilendiği (P<0.001R^2=0.527-0.721) belirlendi. Aynı metot ayrıca post-neonatal büyüme performansının tüm değişkenlerden etkilendiği göstermektedir (P<0.001 R^2=0.471-0.472). Bu çalışma kuzularda pasif immunitenin ilk 12 haftalık dönem büyüme performası ile önemli oranda varyasyon gösterdiğini işaret etmektedir. Bu sonuç daha önce değerlendirilmemiştir. Çalışmada ayrıca doğum ağırlığı, doğum tipi, cinsiyet, sağlık durumu, anne yaşı ve kuzulama sezonunun da büyüme performansı üzerine etkili olduğu belirlendi. Kuzularda anne yaşının ≤2 olması, ikiz, dişi ve kış sezonunda doğması, hastalığa maruz kalması ve doğum ağırlığının ≤3 kg olmasıınn büyüme performansını azalttığı saptandı. Bu faktörler büyüme performansını artırmak için dikkatle ele alınması gerekmektedir. Böylece çiftlik verimliliği ve karlılığı arttırılabilir.

Anahtar sözcükler: *Kuzu, Pasif immünite, Büyüme performansı, Risk faktörleri*

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INTRODUCTION

Most of the outputs from livestock consist of food production in the form of meat, milk and other products. The profitability of sheep farm is significantly limited by cosideable growth performance, low market weight and ill-health [1-5]. Therefore, it is important for the sheep industry to be well informed about the external, controllable factors that affect live weight. For this reason, much work has been done on the effects of genetic and non-genetic factors on growth performance. The growth rate of lambs, particularly during the early stages, is influenced by several factors including breed, the ewe's diet, milk yield, parity or age of the ewe, type of birth, gender, birth weight and environmental factors such as the lambing season [1,2,4,6]. Thus, there is a need for evaluating the effects of animal-related and environmental factors on growth traits if adequate level of growth performance is to be attained. Nonetheless, not enough research has been carried out in Turkey to determine the relationship between risk factors and growth performance in lambs [7-9].

Recent studies indicate that passive immunity may be a significant factor that affects growth performance [10]. Lambs are born agammaglobulinemic at birth due to syndesmochorial placentation. Therefore, immunity during the neonatal period depends on the passive transfer of maternal IgG through the colostrum. This process is referred as passive transfer of immunity (PTI), determined by measuring serum IgG concentration (SIgGC) [10-13]. If neonatal lambs are unable to obtain and absorb a sufficient amount and quality of colostral IgG in the first 12 h of birth, secondary immunodeficiency disorder in other words Failure of Passive Transfer (FPT) develops. FPT results in hypogammaglobulinemia and predisposes neonataes to diseases [10-16]. There exists sufficient amount of knowledge on the association between PTI and the risk of illness and death in neonatal lambs [10,12,14,15], but not enough has been reported about the potential long-term implications of passive transfer. PTI appears to help predict health and productivity in juvenile ruminants, both before and after the neonatal period [10,11]. Studies to date have indicated that there appear a significant linear correlation between passive immunity and growth performance in different periods during the first 6 months of life in beef [17], dairy heifer [18] and buffalo [19] calves, as well as in dairy lambs [10,20] and dairy goat kids [21,22]. Furthermore, an association between PTI and first lactation, milk yield and milk fat content in dairy heifer calves [23] and pre- and post-weaning growth performance in crossbreed calves have been found [24,25]. Never-theless, very little information is available on how passive transfer affects growth performance in lambs, especially after the neonatal period.

To the authors' knowledge, no reports have been published on the predictive value of passive transfer for the growth performance of lambs in long term period. Therefore, the primary objectives of this study was to determine the relationship between passive immunity and growth performance in different periods of the first 12 weeks of life and the secondary purpose of this study was to evaluate the effect of gender, type of birth, birth weight, health of lambs, lambing season and dam's age on growth performance in lambs of two crossbred Akkaraman flocks in Kars, North-Eastern Anatolia, Turkey.

MATERIAL and METHODS

Animals, Data Collection and Farm Management

This study was carried out on two sheep farms located in the central of Kars province in North-Eastern Anatolia, Turkey in 2009. All ewes and lambs were kept under identical feeding and management conditions. Management was typical of North-Eastern Anatolian flocks with lambs being born in winter (December to February) or spring (March to May), and being raised intensively. Other than deworming agents and routine vaccines against clostridial diseases, no drugs and other compounds administered during gestation or parturition. Only lambs resulting from observed parturitions were included in the study. Plastic ear tags were attached to both ears of study lambs soon after their birth and the following data were recorded for each lamb: an individual identification number, gender, date of birth, age of dam, ear tag number of dam and type of birth. The lambs were weighed at birth (before colostrum intake) using a scale [CASIA DB2-150 kg (±30 g)]. After this procedure, lambs were allowed to naturally suckle their dams. The newborn lambs were kept with their dams during their first week of life. After this period, the lambs were transferred to a separate pen and allowed to suckle twice a day (in the morning and evening) for 3 months. Lambs had access to hay after the first week of neonatal life, and to straw and commercial growth feed (Bayramoglu AS, Turkey) from the third week of life. This feeding regime lasted for three months. Subsequently, lambs were turned on pasture and supplemented with hay and commercial feed when they were brought in for the night. No vaccines, drugs or other compounds were administered to lambs during the neonatal period. Deworming agents and routine vaccines against clostridial diseases were administered to the lambs after the neonatal period. The lambs were also weighed on day 28 and day 84 using the same scale.

Blood Sampling and IgG Measurement

Blood samples were collected from lambs by jugular venipuncture at 24±1 h. Serum was harvested by centrifugation and stored at –20°C until analysis. SIgG-24 was measured using a commercial ELISA kit (Bio-X Competitive ELISA Kit For Ovine Blood Serum IgG Assay-BIO K 350, Bio-X Diagnostics, Belgium).

Clinical Examination

Clinical examination was performed as previously defined by the authors [14]. The health status of the lambs was monitored by visiting farms daily during the neonatal period and every two days in the period covering 5 to 12 weeks of life referred as post-neonatal period in the text.

Statistical Analysis

The present study was conducted on 301 Akkaraman crossbreeds and 347 lambs born to these ewes. Those lambs whose birth weight, body weight on 28 and 84 days of life, gender, type of birth, health status, serum IgG concentration, birth date and dam's age were recorded and those survived neonatal or post-neonatal period were included in statistical analysis. Therefore, only 298 and 280 lambs were used to evaluate growth performance during neonatal and post-neonatal periods, respectively. Mean ± SD (Range) values for SIgG-24, birth weight, neonatal (day 28) and post-neonatal (day 28 to day 84) weight, neonatal (birth to day 28) and post-neonatal (from 28 to 84 days) Average Dailiy Gain (ADG) were calculated. The lambs were categorized as healthy or ill based on their clinical examination results. Birth weights were categorized as low (≤ 3 kg), medium (>3 to ≤4 kg) or high (>4 kg). Dam's age was categorized as ≤2, 3, 4 or ≥5 years. SIgG-24 was also categorized as <1000 mg/dL, 1001-2000 mg/dL or >2000 mg/dL. The Tukey HSD test was used to identify differences in growth performance in lambs grouped according to SIgG-24, birth weight and dam's age. Independent Samples T test was used to identify variations in growth performance according to gender, type of birth, birth date and health of the lambs. Multivariable stepwise linear regression analysis (MSRA) was used to evaluate the association between SIgG-24, birth weight, age of dams (considered as continuous independent variables), type of birth, gender and health status of lambs, lambing season (considered as categorical independent variables) and the growth performance outcomes, including ADG and body weight. The linear regression model with the all potential independent variables considered in the study defined as follows: $Y = \alpha + \beta_1X_1 + \beta_2X_2, \beta_3X_3 + \beta_4X_4 + \beta_5X_5 + \beta_6X_6 + \beta_7X_{7+\varepsilon}$. Where Y denotes the weight or ADG, X_1 is the birth weight, X_2 is the type of birth (twin=1 and single=0), X_3 is the health of the lambs (healthy=0 and ill=1), X_4 is the passive immune status (SIgG-24), X_5 is gender (male=0 and female=1), X_6 is age of the dam and X_7 is the lambing season [Spring (March, April, May)=0 and Winter (December, January, February)=1], α is the y-intercept, and β_1, β_2 β_3, β_4 β_5, β_6, β_7 are the regression coefficients and Ɛ, indicates the random part of the theoretical model. To identify the best models in the stepwise technique, the coefficients of determination (R^2) were used. The coefficient of determination was multiplied by 100 and expressed as a percentage to indicate the total variation in Y explained by the selected independent variables. The relationship between SIgG-24 and growth performance (weight or ADG) was explored by simple regression analysis. The methods of multivariable and simple regression have been described previously in detail [10,11]. The computer program SPSS was used for all analyses and values of P<0.05 were considered to be significant. The program Origin 6 was used to obtain scatter diagram illustrations (Origin 6 Copyright© 1991-1999 Microcol TM, Software, Inc) in regression analysis.

RESULTS

The study evaluated 298 lambs and mean SIgG-24 was 22.1±10.74 (2.71-53.02) mg/mL. Birth weight ranged from 2.260 to 5.900 g (mean ± SD, 4.075±631 g). Changes in growth performance based on birth weight, gender, type of birth, SIgG-24 and health status in lambs, age of dam and lambing season have been given in *Table 1*. In addition, the effects of these factors on neonatal and post-neonatal growth performance (ADG or body weight) have been presented in *Table 2* and *Table 3*, respectively.

Effect of Passive Immunity and Other Some Variables on Neonatal Growth Performance

Body weight at 28 days of life ranged from 4.364 to 14.015 g (8.852±1.930 g). Average daily gain from birth to day 28 ranged from 16 to 339 g/d (mean ± SD, 170±53 g/day). Lambs with SIgG-24 levels below 1000 mg/dL had significantly lower neonatal growth performance (ADG or body weight) than lambs with SIgG-24 in the range of 1001 to 2000 (P<0.001) and above 2000 mg/dL (P<0.001). Lambs having SIgG-24 in the range of 1001 to 2000 mg/dl had significantly lower growth performance when compared to lambs with SIgG-24 above 2000 mg/dL. With regard to birth weight, the neonatal growth performance in the low weight category was significantly lower than those of medium (P<0.01) and high birth weight category (P<0.001). Similarly, lambs with medium birth weight had significantly (P<0.001) lower weight or ADG than those with high birth weight. Neonatal growth performance was significantly lower in twins (P<0.001) and diseased (P<0.001) lambs. Body weight of day 28 was significantly (P<0.05) higher in male lambs when compare to female lambs. However, ADG from birth to 28 day was not significantly different between male and female lambs (P>0.05). There was no significant difference in growth performance between lambs born in spring and winter seasons (P>0.05). Dam's age did not significantly effect on neonatal growth performance.

The simple regression models disclosed that SIgG-24 was significantly (P<0.001) associated with both weight determined on day 28 (R^2 = 0.148; *Fig. 1A*) and ADG determined for the period of birth to 28 days (R^2 = 0.151; *Fig. 1B*). Each 1 mg/mL increase in SIgG-24 was associated with a 1.92 g/d increase in ADG and a 69.2-g increase in body weight of day 28.

On multiple regressions analysis, type of birth, SIgG-

Table 1. *Changes in growth performance of lambs according to various factors*
Tablo 1. *Kuzularda bazı faktörlere bağlı büyüme performansındaki değişiklikler*

Factor	Group	Period					
		Neonatal Growth Performance (g) (up to 4 Weeks)			Post-Neonatal Growth Performance (g) (5 to 12 Weeks)		
		n	WG1	ADG1	n	WG2	ADG2
SIgG-24 (mg/dL)	≤1000	25	7029±19513^{a}	120±54^{a}	22	8793±2066^{a}	156±37^{a}
	1001-2000	142	8631±2026^{b}	165±55^{b}	135	11077±2844^{b}	197±51^{b}
	>2000	131	9439±1530^{c}	186±43^{c}	123	12700±2313^{c}	226±41^{c}
Type of Birth	Twin	80	6794±1178***	118±34***	74	9639±2486***	172±44***
	Single	218	9606±1569	190±45	206	12320±2561	220±46
Gender	Male	160	9109±2011*	175±57	150	12069±2849**	215±51**
	Female	138	8554±1794	165±48	130	11084±2656	197±47
Dam Age (Years)	≤2	45	8545±1923^{a}	168±58^{a}	41	10696±2958^{a}	190±53^{a}
	3	134	8766±1880^{a}	169±49^{a}	126	11232±2787^{ac}	200±50^{ac}
	4	82	9087±1795^{a}	176±51^{a}	78	12109±2588^{bc}	216±46^{bc}
	≥5	37	9017±2370^{a}	168±65^{a}	35	12946±2520^{b}	231±45^{b}
Health Status	Ill	40	7411±2125***	129±60***	81	10313±2426***	184±43***
	Healthy	258	9075±1802	177±49	199	12141±2774	216±50
Lambing Season	Winter	91	8980±1835	176±52	87	10671±2615***	190±47***
	Spring	207	8796±1792	168±54	193	12036±2783	214±50
Birth Weight (g)	Low	17	6266±1175^{a}	124±40^{a}	16	8723±3074^{a}	155±55^{a}
	Medium	117	7622±1606^{b}	143±51^{b}	109	10512±2595^{b}	187±44^{b}
	High	164	9997±1308^{c}	195±41^{b}	155	12684±2799^{c}	226±43^{c}

*** P<0.001, ** P<0.01, * P<0.05, ***WG1*** *= weight on day 28,* ***ADG1*** *= [(day 28 weight-birth weight)/28x1000],* ***WG2*** *= (weight on day 84-weight at day 28),* ***ADG2*** *= [(WG2/56)x1000]*

Table 2. *Regression models between independent variables and neonatal growth performance in lambs*
Tablo 2. *Kuzularda neonatal büyüme performansı ve bağımsız değişkenler arasındaki regresyon modelleri*

	Healthy Status	Formulas	R^2	P
Simple Regression Analysis *	General (n=298)	W=7328.9 + 69.2 x IgG	0.148	<0.001
		ADG=128.2 + 1.92 x IgG	0.151	<0.001
	Healthy (n=258)	W=7805.8 + 55.3 x IgG	0.105	<0.001
		ADG =141.8 + 1.53 x IgG	0.109	<0.001
	Ill (n=40)	W=5837.9 + 98.2 x IgG	0.215	<0.01
		ADG=86.27 + 2.67 x IgG	0.197	<0.01
	Model	**Formulas**	**R^2**	**P**
Multiple Regression Analysis **	Onset	W=2.4 x BW - 949.7	0.622	<0.001
	Final	W=1602 + 1.7 x BW - 1186 x TB - 796 x HS + IgG x 21.1	0.721	<0.001
	Onset	ADG=190.3-72*TB	0.364	<0.001
	Final	ADG=57.4 - 42 x TB + 0.28 x BW - 28.48 x HS + 0.75 x IgG	0.527	<0.001

W *= Weight (g),* ***ADG*** *= Average daily gain (g),* ***BW*** *= Birth weight (g),* ***TB*** *= Type of birth,* ***HS*** *= Health status of lambs,* ***IgG*** *= Serum IgG concentration at 24 h after birth,* ***AD*** *= Age of dam,* ***LS*** *= Lambing season, * Indepedent variable: IgG and dependent variable: (W or ADG), ** Independent variables: BW, TB, HS, IgG, Gender, AD and dependent variable: (W or ADG)*

24, birth weight and health of lambs were significantly associated with body weight at day 28 and ADG from birth to 28 days. The variation in weight at day 28 and ADG from birth to day 28 was 72% (R^2=0.721) and 53% (R^2=0.527) respectively *(Table 2)*.

Effect of Passive Immunity and Other Some Variables on Post-neonatal Growth Performance

Total body weight from day 28 to day 84 (post neonatal period weight) ranged from 3.400 to 18.650 g (mean ± SD:

Table 3. *Regression models between independent variables and post-neonatal growth performance in lambs*
Tablo 3. *Kuzularda post-neonatal büyüme performansı ve bağımsız değişkenler arasındaki regresyon modelleri*

Simple Regression Analysis*	**Healthy Status**	**Formulas**	**R^2**	**P**
	General (n=280)	W = 9010.9 + 118.5 x IgG	0.198	<0.001
		ADG = 160.4 + 2.1 x IgG	0.198	<0.001
	Healthy (n=199)	W = 9642.2 + 105.9 x IgG	0.167	<0.001
		ADG = 171.7 + 1.89 x IgG	0.168	<0.001
	Ill (n=81)	W = 8452.2 + 103.9 x IgG	0.145	<0.001
		ADG = 150.3 + 1.86 x IgG	0.146	<0.001
Multiple Regression Analysis**	**Model**	**Formulas**	**R^2**	**P**
	Onset	W = 2165.7 + 2.32 x BW	0.273	<0.001
	Final	W = 4918.5 + 1.2 x BW + 68.3 x IgG - 956.9 x LS - 1097.2 x HS - 1088.4 x TB + 440.2 x DA - 639.9 x Gender	0.471	<0.001
	Onset	ADG = 38.2 + 0.041 x BW	0.273	<0.001
	Final	ADG = 87.29 + 0.021 x BW + 1.2 x IgG - 17.1 x LS - 19.5 x HS - 19.4 x TB + 7.89 x AD - 11.5 x Gender	0.472	<0.001

***W** = Weight (g), **ADG** = Average daily gain (g), **BW** = Birth weight (g), **TB** = Type of birth, **HS** = Health status of lambs, **IgG** = Serum IgG concentration at 24 h after birth, **AD** = Age of dam, **LS** = Lambing season, * Indepedent variable: IgG and dependent variable: (W or ADG), ** Independent variables: BW, TB, HS, IgG, Gender, AD and dependent variable: (W or ADG)*

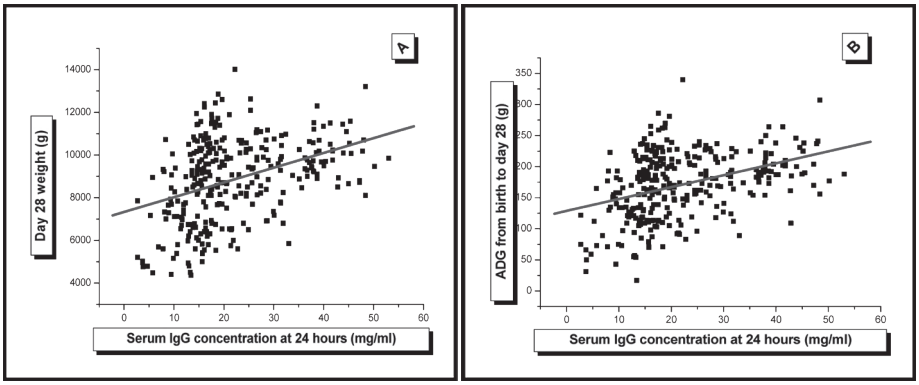

Fig 1. Scatterplots of serum IgG concentration (mg/mL) 24 h after parturition in 298 lambs versus neonatal growth performance [body weight (g) at day 28 (A), ADG (g/d) from birth to day 28 (B)]. In each graph, the solid line represents the best fit for the data, as determined by means of simple linear regression

Şekil 1. 298 kuzuda doğumdan sonra 24. saat serum IgG konsantrasyonu ve neonatal büyüme performansı [28. gün vücut ağırlığı (g) (A), doğumdan 28. güne kadar olan ADG (g/d) (B)] arasındaki ilişkiyi gösteren dağılım grafiğinin görünümü. Basit doğrusal regresyon ile belirlenen grafikte, koyu çizgi, iki değişken arasındaki en iyi uyum noktasını göstermektedir

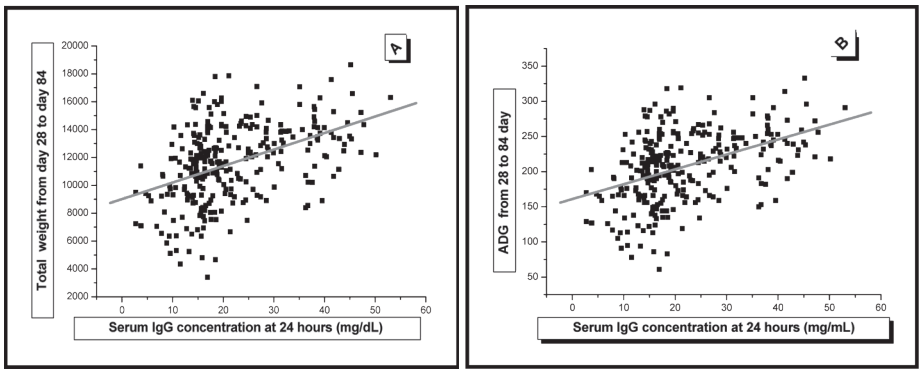

Fig 2. Scatterplots of serum IgG concentration 24 h after parturition in 298 lambs versus post-neonatal growth performance [total gain (g) (C) and ADG (g/d) (D) between day 28 and day 84)]. In each graph, the solid line represents the best fit for the data, as determined by means of simple linear regression

Şekil 2. 280 kuzuda doğumdan sonra 24. saat serum IgG konsantrasyonu ve post-neonatal büyüme performansı [28 ve 84. günler arasındaki gün vücut ağırlığı (g) (A), doğumdan 28. güne kadar olan ADG (g/d) (B)] arasındaki ilişkiyi gösteren dağılım grafiğinin görünümü. Basit doğrusal regresyon ile belirlenen grafikte, koyu çizgi, iki değişken arasındaki en iyi uyum noktasını göstermektedir

11.612±2.799 g). ADG during this period ranged from 60 to 333 g/d (mean ± SD: 207±50 g/d). Lambs with SIgG-24 levels below 1000 mg/dL had significantly lower post-neonatal growth performance than lambs with SIgG-24 in the range of 1001 to 2000 (P<0.001) and SIgG-24 above 2000 mg/dL (P<0.001). As the SIgG-24 increased the growth performance was enhanced *(Table 2)*. Lambs in low birth weight had significantly lower growth performance when compare to those born with medium (P<0.001) and high birth weight (P<0.001) in post-neonatal period. Lambs born from dams aged 4 (P<0.05) and ≥5 years old (P<0.01) had significantly better growth performance during the post-neonatal period when compare to other categories. Lambs born in the winter season, twin and female lambs and those with exposure to any disease had significantly lower growth performance from day 28 to day 84 than their contemporaries born in the spring season (P<0.001), singleton (P<0.01), male lambs (P<0.001) and those that remained healthy (P<0.001).

The simple regression models showed that SIgG-24 was significantly (P<0.001) associated with body weight gain (R^2 = 0.198; *Fig. 1C*) and ADG from day 28 to day 84 (R^2=0.198 *Fig. 1D*). Each 1 mg/mL increase in SIgG-24 was associated with a 2.1 g/d increase in ADG and a 118.5 g increase in weight during the post neonatal period.

Birth weight, gender, type of birth, SIgG-24 and health of lambs, age of dam and lambing season had significant relationship with body weight and ADG from day 28 to day 84 on multiple stepwise regression analysis. The final model showed that 47% (R^2=0.471) of the variance in total weight gain and 47% (R^2=0.472) in ADG from day 28 to day 84 was explained by the independent variables.

DISCUSSION

Recent studies have shown that passive immunity in newborn ruminants [17-22] not only helps to prevent disease but also enhances growth performance. However, the relationship between growth performance and passive immunity in lambs was studied for only healthy and small number of lambs (generally <40) and the period generally restricted to neonatal period (first 28 days of life) [10,15,20]. Therefore this study explained the relationship between passive immunity and other parameters and growth performance beyond the neonatal period for the first time.

Passive Immunity and Growth Performance

The results of our study indicated that passive transfer, was a significant source of variation in both neonatal and post-neonatal periods with regard to growth performance in lambs that were allowed to suckle naturally. Similar results were reported for dairy heifer calves [18,23,25], dairy healthy goats [21,22], beef calves [17,24] and healthy buffalo calves [19]. Our study determined a significant positive linear correlation between SIgG-24 and body weight at day 28 and ADG from birth to day 28 as reported by a single previous study [10] but this study was conducted only 20 healthy lambs. However, no study has yet investigated the relationship between growth performance and SIgG-24 in lambs during the post-neonatal period. The present study also revealed a significant linear relationship between SIgG-24 and post-neonatal growth performance as SIgG-24 increased, the growth performance also improved significantly in both periods of life.

The present study did not explicitly disclose the biological basis for relationship between SIgG-24 and growth performance. However, this relationship can be explained by a number of theories in the light of earlier studies [10,19,21]. First of all, there may be a direct cause-effect relationship between SIgG-24 and growth performance as passive transfer of immunity might have enhanced efficient metabolic systems and rendered disease occurrence which in turn contributes to better growth performance [10,12,21,26,27]. The positive linear relationship among SIgG-24, health status and growth performance in this study added credence to above assumptions as low serum IgG concentrations at 24 to 72 h associated with reduced performance of surviving lambs through increased susceptibility to diseases [10,12,21,27]. Secondly, Massimini et al.[10], suggested that lambs receives and absorbs not only IgG but also nonimmunoglobulin factors present in colostrums which may contribute to growth performance as it is well known that colostrum also contains nutrients, minerals, trace elements, vitamins, enzymes, hormones, growth factors, antibodies and other immunologically active component such as cells, cytokines and acute-phase proteins which are necessary for normal development [10-13,28-31]. Neonatal ruminants are pseudomonogastrics and maturation of their gastrointestinal tract is modulated by growth factors through absorption of colostrums [30,31]. In recent years, considerable number of researchers examined the relationships between the development of neonatal calves and various growth factors, such as insulin-like growth factor-I and the growth hormone [30,31] and yet this aspects remains to be explored in lambs. Studies also revealed the effect of non-immunoglubulin components such as neutrophils, serum opsonic activity, colostral cytokines, complement proteins and lactoferrin on improvement of weight gain by protecting against diseases [10,21,28-32] through boosting the immune and metabolic system. However, much is required to be performed with regard to the effect of non-immunoglobulin colostral content on neonatal and post-neonatal health and growth [10].

In contrast, many researchers also reported no correlation between passive immunity and growth performance in ruminants [15,20,26,33,34]. However, these studies had many different aspects to the present study such as small number of subjects (n<40) [15,20] and species of ruminant (goat, calves) studied [21,26,33,34], method of colostrum feeding (heat

treated colostrums, bottle fed) [26,34], and measurement of Ig concentration (zinc sulfate turbidity test) [33,34], and length of study [20,21].

Other Factors that Affect Growth Performance

The present study revealed that the variation attributable to SIgG-24 accounted on average for 15% of the total variation in ADG and weight from birth to day 28 and 20% of the total variation in weight and ADG from day 28 to day 84. Thus, a large proportion of variation in growth performance was not explained by SIgG-24. Multivariable stepwise linear regression analysis determined a considerable variation in growth performance by health status, gender, type of birth, birth weight, dam's age and lambing season. Of these factors, birth weight, type of birth and health status were considerable source of variation for growth performance in neonatal period, while post-neonatal growth performance was influenced by all variables.

Health Status

Health of the lambs was positively associated with growth performance and in both periods as healthy lambs had a better growth rate than lambs exposed to disease and disease caused 796 g and 1097 g decrease in body weight for each lamb that became ill in neonatal and post-neonatal period, respectively. It is well known that the severity and duration of the disease results in decreased growth performance [26,27]. This is attributed to the fact that diseased animal cannot fully take advantage of the nutrients for growth and development, since a portion of the ingested nutrients must be committed to attacking and eliminating pathogens as well as repairing any damage caused [18,35].

Birth Weight

A positive and strong linear relationship between birth weight and growth performance in both periods was evident in our study. As birth weight increased, growth performance also increased considerably. Similar results were reported by previous studies [2,4,36,37]. Several explanations have been put forward. Of these, lambs born with acceptable birth weight (generally >3 kg) are able to ingest sufficient amounts of colostrum earlier because they are physically strong and viable. This in turn not only increases their resistance to diseases, it also ensures that they ingest many components of the colostrum that affect growth performance [10,38]. On the other hand, lambs with lower birth weight are physically weak, to stand at birth and to suckle sufficient amount of colostrum and this consequently results frequent exposure to disease as was the case in our study where lambs with low birth weight were more prone to disease development and poor growth performance [35,38-40]. It is already known that lambs with a birth weight of ≤3 kg usually suffer failure of passive transfer and that most of them die during the neonatal period, or even if they survive, they do not exhibit good growth performance [5,12,35,39-41]. Other explanation may be that lambs with low birth weights are less mature than those with high birth weights with regard to the development of their metabolic and endocrine systems that improve lambs ability to generate energy from amino acids and sustain gluconeogenesis immediately after parturition [42,43].

Type of Birth

Type of birth had a significant effect on the weight of the lamb as single-born lambs were heavier and had higher ADG in the neonatal and post-neonatal periods as compared to twins in this study. Twin lambs were approximately 1088 g lighter than singleton lambs in the neonatal period and 1186 g in the post-neonatal period and ADG of twin lambs was 42 g lower than single lambs during the neonatal period and 19 g lower during the post-neonatal period. Single lambs have already been reported to attain better growth performance in comparison to twin lambs [2,4,7,44-48]. This has been attributed to competition between the siblings for dam's milk supply, inadequate milk to rear two lambs, lower birth weight of twins, mismothering, hypothermia, or the inability to obtain sufficient milk or colostrum due to physical weakness [1,6,8,12,35,41].

Gender

This study revealed no significant association between gender and growth performance in the first month of life on multiple regression analysis as reported by others [2,4,47]. On the contrary, studies reported that gender had a significant effect on ADG and weight in this period [36,45,48]. On the other hand, male lambs recorded a higher ADG and were heavier than female lambs from day 28 to day 84, and gender was a significant source of variation in post-neonatal growth performance in this study. Similar results have previously been reported [4,6,7,8,45]. The faster growth of male lambs could be due to hormonal differences in their endocrinological and physiological functions which play role in enhancement of growth and their superior weight at birth compared to females [4,6,44]. On the contrary studies also reported no significant effect of gender on ADG [1,2,36,44,47] and body weight [1,2,48] from birth to three months of life.

Age of Dam

Dam's age had no significant effect on growth performance in the neonatal period as earlier studies reported [2,4,44,45] but some studies found the effect of parity or dam's age on weight at day 28 or on ADG from birth to day 28 [36,47]. On the other hand multiple regression analysis revealed a significant positive effect of dam's age on growth performance in post-neonatal period. This effect might be explained by the difference in milk production and maternal care since maiden or first parity ewes are less

mature physiologically and consequently produce less milk, and are less experienced in taking care of their lamb [2,4,6,9,44,47]. Additionally, younger ewes produce lighter lambs due to competition between fetus growth and maternal growth, which has a negative effect on birth weight and subsequent growth performance [1,38,41,49-51]. However, contradicting results suggesting no effect of parity on growth performance from birth to three months of life [1,2,4,44,45].

Lambing Season

Lambing season had no effect on growth performance in the neonatal period in this study as reported by Taye et al.[4]. In contrast, earlier studies reported that lambing season had a significant effect on neonatal growth performance [2,36,47]. However, a significant relationship between lambing season and growth performance was determined in the post-neonatal period on multiple stepwise regression analysis. Other studies have also reported positive association between lambing season and growth performance during the first three months of its life [6,9,36,47,51]. Studies suggested that lambing season could cause changes in growth performance due to temperature during the birth season, morbidity rates, birth weight due to the dam's nutrition in late pregnancy and milk yield due to feeding regime of dams in post-parturient period [6,8,35,44,46,51].

This study revealed association between growth performance and birth weight, type of birth, health status and SIgG-24 in neonatal period and additionally lambing season, dam's age and gender in post-neonatal period. Statistical models used in this study also suggested that variation in growth performance remained to be effected by some other factors noted previously [5,10,38,43,50]. These factors include, managemental interventions such as feeding regime and other animal-related factors such as body condition score, breed, placental development and function, dam and lamb behavior at birth, maternal ability, gastrointestinal absorptive ability of the lambs [2,6,7,38,43,50]. The amount of variation attributable to farm management procedures, the production system and the ewe's prenatal nutrition and breed was minimized in this study as all lambs were taken from two farms that had similar management practices and reared Akkaraman crossbreeds.

In conclusion, this study indicated that passive transfer causes a significant amount of variation in growth performance for lambs from birth to 12 weeks of life, a conclusion that was noted for the first time. It is possible for passive transfer monitoring programs to identify lambs suffering from FPT, but there are many factors that cause FPT [10,11,21,41]. That is to say that programs designed only to identify affected lambs will not be as effective as comprehensive flock management programs as our study has found that aside from passive immunity, growth performance is affected by birth weight, type of birth, health status of lambs, dam's age and lambing season. These factors may be improved by managemental intervention in order to increase growth performance and thus farm income and welfare.

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Kafkas Univ Vet Fak Derg
19 (4): 629-633, 2013
DOI: 10.9775/kvfd.2013.8568

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

Effects of FGA Sponge and Ovsynch Based Protocols on Reproductive Performance of Fat-tailed Ewes During the Breeding Season

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Makale Kodu (Article Code): KVFD-2013-8568

Summary

The effects of different synchronisation prototocols on the reproductive parameters of fat-tailed ewes during the breeding season were studied herein. Seventy-one multiparous fat-tailed ewes were randomly divided into four treatment groups, as follows; Group 1 (long-term FGA): for a total of 20 ewes, 20 mg FGA sponges inserted intravaginally for 14 d, plus an i.m. injection of 400 IU PMSG upon the sponge withdrawal, Group 2 (short-term FGA): for 18 ewes, the insertion of sponges for 8 d, plus the PMSG injection on day 8 upon the sponge withdrawal, Group 3 (Ovsynch): for 17 ewes, an i.m. injection of 0.004 mg GnRH on day zero, followed by the injection of 125 µg $PGF_{2\alpha}$ on day 7 and the second GnRH injection on day 9, and Group 4 (short-term FGA plus Ovsynch): for 16 ewes, the FGA sponge inserted along with the first GnRH injection (as day zero), $PGF_{2\alpha}$ injection on day 7 upon the sponge withdrawal, and the second GnRH injection on day 9. The results showed that; time to onset of oestrus ($P<0.001$), duration of oestrus ($P<0.05$), the rates of pregnancy ($P<0.01$) and lambing ($P\leq0.001$) differed between the synchronisation groups. Overall, findings of onset/duration of oestrus and pregnancy/lambing rates suggest that; the standard Ovsynch synchronisation programme was markedly inferior to the long- and short-term FGA sponge protocols in fat-tailed ewes during the breeding season.

Keywords: *Fat-tailed ewe, Oestrus synchronisation, FGA, Ovsynch*

Aşım Mevsimindeki Yağlı Kuyruklı Koyunlarda FGA ve Ovsynch Temelli Senkronizasyon Protokollerinin Üreme Performansı Üzerine Etkileri

Özet

Sunulan çalışmada, aşım sezonundaki yağlı-kuyruklu koyunlarda farklı senkronizasyon protokollerinin reprodüktif parametreler üzerine etkileri araştırıldı. Bu amaçla, toplam 71 baş birden fazla doğum yapmış yağlı-kuyruklu koyun rasgele 4 deneme grubuna ayrıldı: Birinci gruptaki koyunlara (n=20, uzun süreli FGA), 20 mg FGA içeren süngerler intravaginal olarak yerleştirildikten sonraki 14. günde uzaklaştırılarak, 400 IU PMSG intramuskuler olarak enjekte edildi. İkinci gruptaki koyunlara (n=18, kısa-süreli FGA), 20 mg FGA içeren süngerler intravaginal olarak yerleştirildikten sonraki 8. günde uzaklaştırılarak, 400 IU PMSG intramuskuler olarak enjekte edildi. Üçüncü gruptaki koyunlara (n=17, Ovsynch grubu), Ovsynch protokolü (0. günde 0.004 mg GnRH, 7. günde 125 µg $PGF_{2\alpha}$ ve 9. günde 0.004 mg GnRH) uygulandı. Dördüncü gruptaki koyunlara (n=16; kısa-süreli FGA + Ovsynch) ise, 20 mg FGA içeren süngerlerin intravaginal olarak yerleştirilmesiyle birlikte 0.004 mg GnRH enjekte edildi. Süngerlerin 7 gün sonra uzaklaştırılmasını takiben 125 µg $PGF_{2\alpha}$ ve 9. günde 0.004 mg GnRH uygulandı. Senkronizasyon grupları arasında, son uygulama-östrus aralığı ($P<0.001$), östrus süresi ($P<0.05$), gebelik ($P<0.01$) ve doğum oranları ($P\leq0.001$) açısından önemli farklar olduğu gözlendi. Sonuç olarak; standart Ovsynch protokolünün, üreme sezonundaki yağlı-kuyruklu koyunlarda klasik kısa ve uzun süreli FGA içeren vajinal sünger uygulamalarına göre gebelik ve doğum oranlarında önemli ölçüde azalmaya yol açtığı kanısına varıldı.

Anahtar sözcükler: *Yağlı kuyruklu koyun, Östrus senkronizasyonu, FGA, Ovsynch*

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INTRODUCTION

Numerous methods have been used to for improving the reproductive performance in sheep. For this, some researchers have focused on animal husbandry practices (e.g. flushing or the ram effect), while others adopted the administration of certain agents [1-3].

Oestrus synchronisation has been practised in sheep industry for almost a half century [4]. The methods are based on the control of the lifespan of corpus luteum (by using prostaglandin or progestagen). The most common techniques are progestagen devices, inserted for 12-14 d, followed by an administration of eCG [5]. Administrations of intravaginal progestagen (FGA or CIDR), for 10-16 d, followed by intramuscular (i.m.) injection of eCG, have been successfully used [6-8]. Progestagens appear to be the most practical hormones of choice. However, a prolonged time of administration could result in low conception rates [9-11], as likely to be attributable to the impaired sperm transport *in vivo* [12]. Meanwhile, short-term protocols possibly allow for facilitating the managerial tasks, minimising the vaginal discharge and infection risks, and thus increasing the fertility rates. Indeed, short periods of sponge administration, for as short as 5-7 d, have been successful in sheep regardless of breeding season [10,13].

Alternatively, a GnRH-based $PGF_{2\alpha}$ protocol has also been reported to be effective in ewes during the breeding season [13,14]. Furthermore, their combination with the Ovsynch protocol has also been used in cattle [15]. Likewise, this protocol was also used recently in ewes [16]. Physiological aspects of Ovsynch protocol may be described as follows: on day zero, the ovulation occurs followed by the progression of corpus luteum or, alternatively, intrafollicular luteinisation takes place after the first GnRH injection. On day 5, the injection of $PGF_{2\alpha}$ induces luteolysis, while the injection of second GnRH on day 7 evokes the ovulation. Indeed, Deligiannis et al.[16] reported that the majority of animals eventually became pregnant following the artificial insemination performed 36-62 h after the second GnRH. Furthermore, Holtz et al.[17] comparing progesterone sponge and Ovsynch protocols in goats concluded that the latter protocol could also be an alternative choice with reasonable outcome.

In the literature, there exist only a little information on the effect of Ovsynch protocol or its combination with FGA-impregnated intravaginal sponges in fat-tailed ewes. Therefore, the present study was performed to investigate the reproductive efficiency of Ovsynch and or FGA protocols for oestrus synchronisation during the natural breeding season in fat-tailed Akkaraman and Awassi ewes.

MATERIAL and METHODS

Locations and Animals Used

This study was conducted during the breeding season at the Experimental Research and Practice Farm, Faculty of Veterinary Science, Ankara University, Ankara, Turkey. Ewes were in dry period (not lactating) and clinically healthy.

A total number of 71 fat-tailed ewes (40 Akkaraman and 31 Awassi breed) were used. The animals were 2-5 years old with body weight between 45-55 kg and body condition score of 2.5-3.0 based on the method described by Ucar et al.[18]. The ewes were kept in open pen (2.5 meter square per ewe) with adequate watering, and offered alfalfa hay *ad libitum* together with a commercial concentrate supplement (400-500 g/head).

Oestrus Synchronisation Protocols

The ewes were randomly divided into four treatment groups, as follows:

Group 1 (Long-term FGA sponge plus PMSG injection): For a total of 20 ewes (11 Akkaraman and 9 Awassi), 20 mg FGA, Fluorogestone acetate, sponges (Chronogest® CR, Intervet, Istanbul, Turkey), inserted intravaginally for 14 d, followed by i.m. injection of 400 IU PMSG, pregnant mare serum gonadotropin (Chronogest/PMSG, Intervet) upon the sponge withdrawal.

Group 2 (short-term FGA sponge plus PMSG injection): For a total of 18 ewes (10 Akkaraman and 8 Awassi), 20 mg FGA sponges inserted intravaginally for 8 d, followed by i.m. injection of 400 IU PMSG upon the sponge withdrawal.

Group 3 (Ovsynch): For a total of 17 ewes (10 Akkaraman and 7 Awassi), i.m. injection of 0.004 mg GnRH analogue, Buserelin (Receptal®, Intervet) on day zero followed by an i.m. injection of 125 µg $PGF_{2\alpha}$ analogue, Cloprostenol (Estrumate®, DIF; Istanbul, Turkey) 7 d later, and finally a second injection of 0.004 mg Buserelin on day 9.

Group 4 (short-term FGA plus Ovsynch): For a total of 16 ewes (9 Akkaraman and 7 Awassi), we implemented the FGA sponge inserted along with the injection of 0.004 mg Buserelin (as day zero), followed by an injection of 125 µg Cloprostenol on day 7 upon the sponge withdrawal and, finally the second injection of Buserelin on day 9.

Oestrus Detection, Mating and Fertility

Oestrus signs were monitored four times a day for 15 min each time as described by Ucar et al.[8]. The signs were observed following each protocol for 18-72 h with intervals of 6 h. Teaser rams were used for detection of ewes on heat.

Once the ewes were detected in oestrus, they were then separated from the rest of flock and hand-mated with fertility-proven rams (n=4, for each breed) used rotationally. Duration of oestrus was recorded as the time starting from the onset of oestrus signs until the rejection of mounting ram, as the end of receptivity. The pregnancy rates as well as the rates of lambing and twinning were recorded after parturition. Ongoing pregnancies were determined by considering those ewes that have previously showed oestrus, mated, and non-returned (no oestrus/no mating again) within the subsequent oestrus cycle (between day 14-21 post-mating/synchronisation).

Statistical Analyses

The present data (mean ±SEM) from the onset of oestrus signs and duration of oestrus were analysed by one-way analysis of variance (ANOVA). The analyses of oestrus responses, pregnancy rate as well as lambing and twinning rates were performed using Chi square test. A 95% confidence interval was used. Differences between the synchronisation groups were considered statistically different, using the least significant difference ($P<0.05$). Minitab statistical software programme (MINITAB, Version 11.2; Minitab Inc., Pennsylvania, USA) was used for all statistical analyses.

RESULTS

According to our preliminary statistical analyses (ANOVA), considering the breed as co-factor (Akkaraman *vs.* Awassi), there were no significant differences in any of the reproductive parameters studied. Therefore, all the animals from the two breeds were considered as 'fait-tailed ewes'.

Considering the effect of different oestrus synchronisation groups *(Table 1)*, the onset of oestrus signs, duration of oestrus, pregnancy and lambing rate of ewes were differed significantly between the groups.

The onset of oestrus signs was significantly shorter in the long-term FGA-PMSG group (Group 1) as compared to those in other groups. Also, the duration of oestrus was significantly shorter in the Ovsynch group (Group 3), as compared to those in others.

Additionally, the rates of pregnancy and lambing were significantly lower in the Ovsynch group as compared to those in the long-term FGA-PMSG (Group 1) and short-term FGA-PMSG groups (Group 2). The concerned rates of Ovsynch and short-term FGA plus Ovsynch groups (Group 4) both were also significantly lower than those in the long-term and short-term FGA groups

Finally, for the twinning rates, the values in the Ovsynch protocol were numerically lower as compared to those in others.

DISCUSSION

Our findings indicated that the standard Ovsynch programme was inferior to the conventional FGA-PMSG based on the classical reproductive traits in ewes during the breeding season.

The oestrus rate was relatively higher (87.5%) in the short FGA plus Ovsynch-treated ewes than those (75-82.4%) in other groups. So far, a high oestrus response (75-100%) has been recorded with the FGA-PMSG [19-21]. Indeed, using the FGA-PMSG (control) herein, the oestrus rate was 75%, similar to those (73-77%) in Menze and Dorset ewes [19,22]. A higher (up to 100%) rate was also reported in other breeds [21,23]. Different results might be related to differences in the PMSG dose, the source of progesterone, breed type, etc. The oestrus rate with the short FGA-PMSG was 77.8%, similar to 83.3-88% in Awassi [24] and Ossimi ewes [25]. However, Martemucci and D'Alessandro [26], using the Ovsynch in cross-bred Altamurana ewes in non-breeding season reported a rather lower oestrus rate

Table 1. *The effects of different oestrus synchronisation protocols upon the reproductive traits in fat-tailed ewes*
Tablo 1.*Yağlı kuyruklu koyunlarda farklı senkronizasyon protokollerinin reprodüktif parametreler üzerine etkileri*

Parameters Studied	Synchronisation Groups≠				Statistics		
	Group 1 (n=20)	Group 2 (n=18)	Group 3 (n=17)	Group 4 (n=16)	F-Ratio	P Value	Significance
Oestrus, %	75.0±9.9	77.7±10.1	82.3±9.5	87.5±8.5	0.32	0.812	N.S.
Interval to onset of oestrus*, h	37.2±1.7 [a]	49.7±1.3 [b]	50.5±1.3 [b]	51.0±0.8 [b]	23.77	0.000	P<0.001
Duration of oestrus*, h	27.2±0.8 [b]	27.0±0.8 [b]	24.0±1.2 [a]	27.8±0.8 [b]	3.30	0. 027	P<0.05
Pregnancy rate*, %	93.3±6.6 [b]	85.7±9.7 [b]	42.8±13.7 [a]	57.1±13.7 [a]	4.51	0. 007	P<0.01
Lambing rate*, %	93.3±6.6 [b]	85.7±9.7 [b]	35.7±13.3 [a]	50.0±13.9 [a]	6.24	0.001	P≤0.001
Twinning rate*, %	33.3±12.6	35.7±13.3	7.1±7.1	21.4±11.4	1.31	0.281	N.S.

≠ ***Group 1:*** *" long-term FGA-PMSG";* ***Group 2:*** *"Short-term FGA-PMSG";* ***Group 3:*** *"Ovsynch";* ***Group 4:*** *"Short-term FGA + Ovsynch"; * The number of treated ewes and those in oestrus were 57 only; a total of 14 ewes (of 5, 4, 3 and 2 ewes from Group I-V, respectively) with no signs of oestrus were excluded from the analyses; [a,b] Means (± SEM) having different superscripts within the same row are significantly different from each other (P<0.05);* ***F-ratio:*** *Frequency ratio;* ***P value:*** *Probability value;* ***Significance:*** *Statistical significance;* ***N.S.:*** *not significant (P>0.05)*

(33%), as compared to that in our study (80%). The low rates of oestrus reported might be due mainly to the physiological status and breed type of ewes used therein.

Interval to onset of oestrus upon the sponge withdrawal was markedly shorter in the long-term treatment as compared to others. Herein, the oestrus interval with the long-term FGA-PMSG was 37.2 h, similar to 38.1-44.7 h in Awassi and Dorper ewes [27,28]. Small differences between the durations might be related to a smaller number of animals, shorter duration of sponge administration and a higher dose of PMSG used in the latter [29]. Herein, the interval was 49.7 h after the short-term FGA, similar to 46.2 h in a previous study [30]. However, higher times of 69-70 h [24,25] were also reported elsewhere. Martemucci and D'Alessandro [26], using the Ovsynch reported that the interval was 59 h, while it was 50 h herein. Small differences of oestrus intervals might be due to the individual ewes in different breeds and to different management conditions used.

The duration of oestrus was shorter (24 h) in ewes treated with the Ovsynch, as compared to the other protocols. The durations of oestrus with both the FGA protocols were similar to those in previous studies [27,29]. However, the durations of oestrus observed (24-27.9 h) were shorter than the 34.9 h in Awassi ewes [24], but longer than the 18.7 h in Dorper ewes [28]. Differences might be related mainly to the season as well as to other co-factors (e.g. protocol, feeding, etc.).

The present results of long- or short-term FGA were not markedly different based on the pregnancy, lambing and twinning rates. Likewise, Ustuner et al.[24] reported that both the long- and short-term resulted in similar fertility (22% vs. 20%, resp.) in Awassi ewes during the season. By contrast, the short-term (6 d) treatment resulted in a higher pregnancy rate due likely to the ovulation of newly recruited growing follicles [10]. Therein, however, the rates of pregnancy and lambing with both durations did not differ widely. Meanwhile, pregnancy and lambing rates of Ovsynch, either alone or its combination with short-term FGA, were markedly lower than the FGA-sponge administrations, regardless of its duration. In this respect, we simply consider that, the conventional Ovsynch protocol originally developed for cattle species [15] may be inappropriate for sheep. This could simply be attributable to abrupt species differences in the seasonality and durations of oestrus cycles (21 d *vs.* 16-17 d) for cattle and sheep, respectively [2]. Apparently, the standard Ovsynch protocol should be modified for the ewe, as having different follicular and luteal dynamics. In a broader sense, the variations might also be due to different synchronisation protocols, the type of hormones and breed used [3,8]. In this respect, we considered that progesterone-primed treatment would mimic the luteal phase likely to be followed by the propagation of follicular development upon the sponge withdrawal. Indeed, the FGA-PMSG presumably ensure both an increase in the number of females in oestrus [2,8] and the reduction of short cycles especially in late breeders, as leading them to enter into the breeding season more efficiently. In another study, however, similar lambing rates (47-50%) were reported with either the Ovsynch alone or its combination with the short-term FGA [16,31]. This was also the case herein.

The higher pregnancy/lambing rates observed in the FGA-PMSG based groups may be due to the PMSG action. In fact, the Ovsynch protocol mainly acts as the source of exogenous doubling GnRH (mainly provoking the endogenous LH release), while the PMSG acts mainly as the source of exogenous FSH that seems to be more advantageous over the LH *per se*. It was thought that the standard Ovsynch (injections on day zero-7-9) used in ewes might be inappropriate, as reported elsewhere [16]. Hence, it would be logical that both different days of the injections and the administrative sequence of GnRH (before or after the FGA) should be studied further [16]. However, progesterone plus PMSG might be well enough for stimulating the ovarian cycle, eventually leading to a successful lambing at early, mid- or late in the breeding season [3].

Overall, the findings showed that different synchronisation protocols could markedly alter the onset/interval (shortest with the FGA-PMSG) and duration of oestrus as well as the rates of pregnancy and lambing (lowest with the Ovsynch) in fat-tailed ewes during the breeding season. In that, however, there was only a numerical (but not significant) improvement in reproductive parameters (e.g. lambing) as compared to the standard Ovsynch protocol studied herein. Hence, not only the protocol itself but also the follicular/luteal dynamics should be studied further for a better understanding of its *in vivo* regulatory mechanisms of exogenous hormones in sheep. Nevertheless, our study would provide some clues for the future synchronisation trials in sheep. In this respect, we might recommend some modifications (such as studying the durations between GnRH and $PGF_{2\alpha}$ administrations) of present synchronisation protocols to achieve further improvements in the ultimate fertility. Additionally, future investigations of synchronisation with a higher number of animals from other breeds should also comprise an early determination of the plasma progesterone levels in- and outside the breeding seasons.

Acknowledgements

The authors gratefully acknowledge INTERVET Company for provision of hormones used. We further thank Vet. Med. Taskin Dalci of the farm and Intern Vet students.

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Kafkas Univ Vet Fak Derg
19 (4): 635-639, 2013
DOI: 10.9775/kvfd.2013.8587

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

The Axon Number of the Rat Sciatic Nerve: A Stereological Study

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Makale Kodu (Article Code): KVFD-2013-8587

Summary

This study was designed to determine with stereological methods the number of axons in the sciatic nerve as a result of peripheral nerve blockage following injection of lidocaine and prilocaine into hind limb muscle. Nine adult female Sprague-Dawley rats weighted 150-200 g were used in the study. Ketamine was employed intraperitoneal to the rats as 50 mg/kg. The prilocaine and lidocaine were equally (5 mg/ml) injected to the left and right limbs of the 4 rats. As a control group, isotonic sodium chloride (0.9%) was performed as 0.2 ml into the extremities of the remaining 5 rats. When the application completed, surrounding muscles and soft tissues as well as sciatic nerve were dissected as 0.5x1.0 cm length. In order to determine neurotoxic effects of lidocaine and prilocaine on sciatic nerve, the number of axons was computed by unbiased stereological method. Following of the square root transformation, Mann-Whitney U test was performed to compare groups. When the unbiased comparisons were executed to both groups, the effects of lidocaine and prilocaine on sciatic nerve were found statistically non-significant.

Keywords: *Axons number, Sterology, Rat, Sciatic nerve*

Sıçan Siyatik Sinir Akson Sayısı: Stereolojik Bir Çalışma

Özet

Bu çalışma, Lidokain ve prilokain'in arka ekstremite kası enjeksiyonunu takiben oluşan periferik sinir blokajı sonucunda siyatik sinirde akson sayısını stereolojik metotlarla belirlemek için planlandı. Bu çalışmada 150-200 g ağırlığında dokuz adet Sprague-Dawley tipi erişkin dişi sıçan kullanıldı. Sıçanlara Ketalar 50 mg/kg intraperitoneal uygulandı. Dört adet dişi sıçanın sağ ekstremitelerine eşit miktarda prilokain 5 mg/ml (0.2 ml) ve sol ekstremitelerine lidokain 5 mg/ml (0.2 ml) verildi. Kontrol grubu olarak %0.9 izotonik sodyum klorür kalan 5 adet sıçan ekstremitelerine 0.2 ml uygulandı. Uygulama sona erince çevre kas ve yumuşak dokular siyatik sinir ile beraber 0.5x1.0 cm uzunlukta çıkarıldı. Lidokain ve prilocainin siatik sinir üzerine nörotoksik etkilerini ortaya koymak için tarafsız stereolojik metod ile akson sayısı hesaplandı. Bu özellik bakımından denek ve kontrol gruplarını karşılaştırmada karekök transformasyonu yapıldıktan sonra Mann-Whitney U testi kullanıldı. Her iki grup arasında yapılan karşılaştırma tarafsız stereolojik hesaplamalarla yapıldığında sıçanlara uygulanan Lidokain ve prilokain akut olarak siyatik sinir akson sayılarına etkileri istatistiksel olarak anlamlı bulunmadı.

Anahtar sözcükler: *Akson sayısı, Stereoloji, Rat, Siyatik sinir*

INTRODUCTION

Regional anesthesia with a nerve -blocking is a useful technique for day-stay surgery to the hand [1]. Regional anesthetic techniques provide efficient postoperative analgesia [2,3]. The most often used local anesthetics for regional anesthesia in Europe is prilocaine and lidocaine. They have a relatively short duration of action and are the least toxic of the amino-amide local anesthetics [4,5]. Peripheral nerve injury is a rare but sometimes has the devastating complication of regional anesthesia.

Neurotoxicity of local anesthetics *per se* is not new although it is largely ignored by clinicians [6]. Laboratory

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evidence suggests that local anesthetic neurotoxicity is at least partially dose and concentration dependent [7]. Studies of local anesthetic-induced nerve injury have eliminated several possible mechanisms of toxicity. For example glucose included in the local anesthetic solution is not neurotoxic which suggests that toxicity results from an action of the local anesthetic itself [8]. Therefore we investigated stereological the acute neurotoxic effects of lidocaine and prilocaine on sciatic nerve using injection technique of continuous peripheral nerve blockade.

MATERIAL and METHODS

Animals

This study was approved by the Animal Use Ethics Commission from Yüzüncü Yıl University (2003/03-01). Nine female rats (150-200 g) were kept in a standard plastic cage on sawdust bedding in an air-conditioned room (22°C) under a 12/12 h light/dark cycle and fed *ad-libitum*.

Chemicals

The animals were anesthetized by ketamine hydrochloric (50 mg/kg) intraperitoneally without muscle relaxation and placed in prone position. The rats were separated as a control (n=5) and experiment (n=4) groups. Under the general anesthesia by using peripheric nerve-stimulator (2 Hz and 0.3 ms B/Braun Stimuplex) and a nerve stimulator needle (22 G x 2-0.7 x 50 mm) skin was incised. Proximal hindlimb muscle and sciatic nerve were exposed. The nerve catheters (20 gauges) were inserted into the sciatic nerve sheaths and fixed bilaterally into two extremities along the sciatic nerve trace. After this procedure skin and subcutaneous tissue were closed by single stitches.

Four animals after placing the catheters were administered lidocaine (5 mg/ml) in left side and prilocaine (5 mg/ml) in right side with a rate of 0.2 ml for a total period of 6 h by opening 1 mA. In control group (n=3) the animals were treated with normal saline by same rate and doses on both sides. After six hour from this procedure, the sciatic nerve with 0.5×1.0 cm volume was cut out with surrounding tissues by removing the stitches and withdrawing the catheters. The animals were sacrificed.

Histological Analysis

The entire sciatic nerves were removed bilaterally en bloc from all rats. The nerves were stretched to in situ length by pinning onto a card and then fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate (pH 7.4) for 4-6 h in 4°C. After fixation nerve samples about 0.5 cm long that were taken from the nerves were rinsed in 0.1 M sodium cacodylate (pH 7.4) twice. Specimens were post fixed in 1% osmium tetroxide for 1 h dehydrated in an ascending alcohol series and propylene oxide took into for a 50:50 mixture propylene oxide and Epon 812 for 24 h. These procedures were completed by embedding the tissues 48 h in Epon Embedding Kit (Fluka Chemie Gmbt, Swithzerland). Semi-thin and ultra-thin sections (of 1 mm and 90 nm thickness. respectively) were cut by an ultramicrotome (Super Nova Reichert-Yung, Austria) and stained with 1% toluidine blue.

Stereology

Stereological analyses of sciatic nerves were done according to principles described previously [9]. A manual stereological workstation composed of a digital camera (Nikon COOLPX5400, Tokyo, Japan) a manual dial indicator controlled specimen stage and a light microscope (Nikon Microphot-FX Tokyo. Japan) was used for axon number counting [10]. To obtain an estimation of total myelinated axon number in an unbiased manner the axon profiles in the nerve cross-section are sampled with equal probability regardless of shape size orientation and location that means each sampled item was selected with a systematic random manner [11]. For this aim we used an area fraction approach in the application area of unbiased counting frame was 900 mm^2. A counting frame was placed on to a monitor and the sampled area was chosen by a systematic uniform random manner via dial indicator controlled specimen stage. Meander sampling of each sectioned nerve profiles was done in 70 µm-70 µm step size in a systematic-random manner. This ensures that all locations within a nerve cross-section were equally represented and that all axon profiles were sampled with an equal probability regardless of shape size orientation and location [12,13].

Descriptive statistics were presented as Mean, Median SD, Minimum and Maximum values for axon count. After square-root transformation Mann-Whitney U test was used to compare treatment and control groups for this trait 5% values was considered to statistical significant; MINITAB statistical pocket programmed was used for all statistical.

RESULTS

In our study axon number of sciatic nerve was observed on histological sections from the region applied isotonic injection in both treated and control groups. The neurotoxicity was not observed in both lidocain and prilocain group statistically (P>0.05) *(Table 1, Fig 1)*.

DISCUSSION

Peripheral nerve blockade with a local anesthetic provides excellent pain relief but its clinical utility for the treatment of acute or postoperative pain is sometimes limited by a short duration of effect [14,15]. After placing the nerve catheter it is important to provide fast onset of the block to improve the workflow within the operating theatre. This aim can be reached by using a drug with fast onset [16].

Table 1. *Descriptive statistics of the axon number of the groups treated and control (P>0.05)*
Tablo 1. *Denek ve kontrol gruplarının akson sayılarına ait tanımlayıcı istatistik (P>0.05)*

Groups	N	Mean	SE Mean	StDev	Minimum	Median	Maximum	CV
Lidokain	4	59.74	3.67	7.34	52.70	58.10	70.07	0.1228
Control Left	5	69.53	5.38	12.03	55.46	76.93	80.32	0.1730
Control Right	5	70.09	4.12	9.20	58.75	71.42	83.19	0.1313
Prilokain	4	54.20	3.59	7.17	47.82	52.25	83.19	0.1323

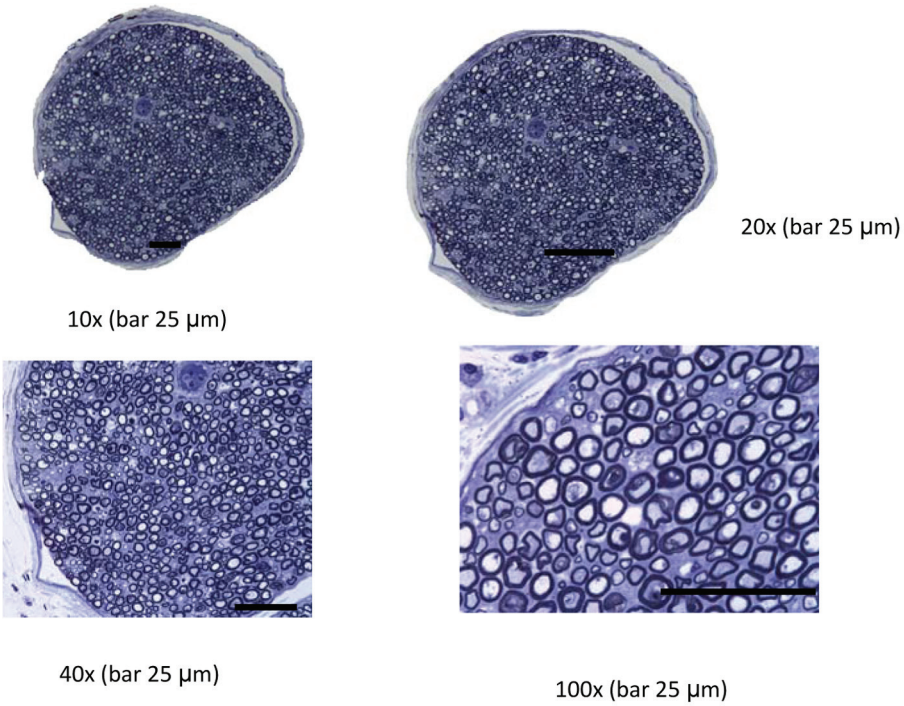

Fig 1. Cross-section of sciatic nerve

Şekil 1. Siyatik sinirin enine kesiti

Local anesthetics are a group of drugs defined by their ability to prevent sodium entry into axons thereby preventing the generation of propagated action potentials in axons. However they have other actions such as prevention of axonal sprouting and effects on G-protein-coupled receptors and on conductance of ions in addition to sodium that might be important in the management of pain [17]. The transport of the amide local anesthetics such as lidocaine and prilocaine is by passive diffusion this transport is dependent on the pH across biological membranes in the case of the amide local anesthetics. Higher concentrations of the anesthetic will be found in the tissue or compartment with lower pH [18].

Prilocaine is medium-long-acting local anesthetics with a fast onset of action [16]. Prilocaine stabilizes the neuronal membrane by inhibiting the ionic fluxes required for the initiation and conduction of impulses thereby effecting local anesthetic action. Local anesthetics bind selectively to the intracellular surface of sodium channels to block influx of sodium into the axon [19].

Studies of clinical adverse effects of intrathecal local anesthetics suggest cauda equina syndrome results from local anesthetic neurotoxicity [20]. Lidocaine toxicity is not the result of an immediate and irreversible breakdown in the integrity of the plasma membrane as would be reflected by a rapid and permanent loss in membrane potential. The mechanism of the lidocaine-induced depolarization currently is unknown but may reflect the simultaneous blockade of ion channels and pumps responsible for the maintenance of neuronal resting potential [21].

Peripheral nerve injury is occurs as a complication of regional anesthesia. Ischemia is one of the causative mechanisms. This may result from changes in peripheral blood flow caused by the local anesthetic itself and/or a vasopressor adjuvant. Peripheral nerves have a dual blood supply of intrinsic exchange vessels in the endoneurium and an extrinsic plexus of supply vessels in the epineurial space that crosses the perineurium to anastomose with the intrinsic circulation. The extrinsic supply is known to be responsive to adrenergic stimuli [22].

Persistent reductions in peripheral nerve blood flow (NBF) can lead to pathological changes in the structure of nerve fibers and their supporting cells [23].

The intravenous regional anesthesia (IVRA) is a technique suitable for hand and forearm surgery and

safe when performed with care by expert practitioners. The most often used local anaesthetic for IVRA in Europe is prilocaine. It has a relatively short duration of action similar to that of lidocaine [4] and is the least toxic of the amino-amide local anesthetics [5].

Although the axonal membrane repolarizes after wash of lidocaine an irreversible conduction block was observed in axons after 3 min exposure of the axon to lidocaine at concentrations as low as 40 mM [24]. Concentration dependence of lidocaine induced irreversible conduction loss. That we observed recovery of soma action potential after exposure of DRG neurons to even higher concentrations of lidocaine raises the possibility that different mechanisms underlie neuronal death and loss of conduction. Because an increase [Ca^{++}] can disrupt cytoskeleton [25,26] and disruption of cytoskeleton can decrease excitability [27]. Lidocaine-induced increase in [Ca^{++}] possibly could contribute to both cell death and long-lasting conduction failure. Regional anesthesia-induced nerve injury may in fact require a combined mechanical and chemical insult [28].

In rat sciatic nerve relative neural toxicity and relative motor nerve conduction blockade were assessed for two amide-linked local anesthetics (etidocaine and lidocaine) and two ester-linked local anesthetics (chloroprocaine and procaine). The nerve fiber injury and edema were assayed by light microscopic examination of nerve tissue sampled two days after perineural (next to the sciatic nerve) injection of various concentrations of the local anesthetics. Both nerve injury and edema increased with concentration of local anesthetics but injury was frequently present in nerve fascicles with little or no edema. In previous studies the amplitude of the electrical activity elicited from the interosseous muscles of the foot following ipsilateral electrical stimulation at the sciatic notch was monitored for up to are minutes to assess the extent of motor nerve blockade. The resulting low concentration-response curves were analyzed for differences in potency. Both for injury and for conduction block the order of decreasing potency was etidocaine, lidocaine, chloroprocaine and procaine. These results are not consistent with the proposal that ester-linked agents are more likely than other local anesthetic agents to cause nerve injury [29]. With specific regard to lidocaine neurotoxicity an irreversible block of impulse conduction was induced in the frog sciatic nerve within a clinical concentration range of 1% to 2% lidocaine [24]. In rats increasing doses of intrathecal 5% hyperbaric lidocaine resulted in persistent sacral sensory deficits associated with motor weakness [30]. All axons counts were based on the gold standard [31] and fractionators technique [12,32].

In conclusion we found that the comparison between the infusion of lidocaine and/or prilocaine and the placebo showed no significant difference in the histological axon numbers ($P>0.05$).

Acknowledgement

The researchers would like to thank sainted but casualty Prof. Dr. Atif AYDINLIOGLU for her opinion on the stereological subject.

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DOI: 10.9775/kvfd.2013.8592

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

The Impacts of Prebiotic Supplementation on Humoral and Cell-Mediated Immune Responses of Broilers under Combined Stresses Caused by Feed Restriction and *Salmonella enteritidis* Challenge

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Makale Kodu (Article Code): KVFD-2013-8592

Summary

This experiment was conducted to investigate the impacts of prebiotic on humoral and cell-mediated immune responses of broilers under combined stresses caused by feed restriction and *Salmonella enteritidis* challenge. 320 male broilers (Ross 308) at a 23 factorial arrangement in randomized complete block design were divided into 8 treatment groups with 4 replicates per each. The Impact of these factors and their interaction on cell-mediated immune response to cutaneous basophil hypersensivity (CBH) test, antibody titer response against Newcastle disease virus (NDV) and sheep red blood cell (SRBC), and the relative weight of lymphoid organs were measured. Prebiotic caused an increase and feed restriction caused a decrease in cell-mediated immune responses on days 15 and 30 respectively, and interaction among the factors was differ ($P<0.05$). Also, factors' interaction had a significant effect on antibody titer response against NDV ($P<0.05$) at day 27. *Salmonella* and feed restriction significantly reduced the relative weight of thymus at day 21 and bursa of Fabricius at day 42, respectively ($p < 0.05$). Differences among other measured parameters were not significant ($P>0.05$).

Keywords: *Prebiotic, Feed restriction, Salmonella, Humoral immune response, Cell-mediated immune response*

Prebiyotik İlavesinin Yem Kısıtlaması ve *Salmonella enteritidis* İnokulasyonu İle Kombine Stres Altındaki Broylerlerde Sıvısal ve Hücresel İmmun Yanıt Üzerine Etkileri

Özet

Bu çalışma, prebiyotik ilavesinin yem kısıtlaması *Salmonella enteritidis* inokulasyonu ile kombine stres altındaki broylerlerde sıvısal ve hücresel immun yanıt üzerine etkileri araştırmak üzere gerçekleştirildi. Rastgele tam blok tasarım içinde 23 faktoriyel düzenlemede 320 erkek boyler (Ross 308) 4 tekrar olmak üzere 8 tedavi grubuna bölündü. Bu faktörlerin ve onların etkileşiminin hücresel immun cevap üzerin etkilerini belirlemek üzere kutanöz bazofil aşırı duyarlılık testi (CBH), Newcastle hastalığı (NDV) virüsü ve koyun eritrositlerine (SRBC) karşı gelişen antikor titreleri ve lenfoid organların göreceli ağırlıkları belirlendi. Hücresel immun yanıtı prebiyotik 15. günde arttırırken yem kısıtlamasını 30.günde azalttığı ve faktörler arası etkileşimin faklı ($P<0.05$) olduğu gözlemlendi. Ayrıca, faktörlerin etkileşiminin 27. gündeki NDV'ye karşı gelişen antikor titresinin gelişimi üzerine önemli etkisinin ($P<0.05$) olduğu belirlendi. *Salmonella* ve yem kısıtlamasının, timus ve bursa fabricius'un göreceli ağırlığını, sırasıyla 42. ve 21. günde azalttığı belirlendi ($P<0.05$). Diğer ölçülen parametreler arasında farklılıklar anlamlı bulunmadı ($P>0.05$).

Anahtar sözcükler: *Prebiyotik, Yem kısıtlaması, Salmonella, Sıvısal bağışıklık cevabı, Hücresel bağışıklık cevabı*

INTRODUCTION

In recent years, feed restriction has been used for improving broilers' performance and their carcass quality. One of the common methods is restriction in the starter feeding period on the base of compensatory growth. It has been reported that decrease in broilers' primary growth reduces the rate of metabolic reactions; therefore there

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is less need to oxygen, and the metabolic disorders such as ascites and SDS (sudden death syndrome) resulted from fast growth are prevented [1]. Beside the advantages, feed restriction has some disadvantages too. Limitations in access to food make stress for the chickens, and this stress changes the microbial population of their gut. It has been shown that hunger in birds reduces the amount of *Lactobacilli* spp. in the crop [2]. Also there are reports on the suppressing effects of feed restriction on some immune system parameters in chickens [3,4].

Furthermore, broilers commonly meet several internal and external stressors like stocking density, temperature, transportation, feed contamination and microbial infections [5]. Among the microbial infections, *Salmonella* spp. is one of the main, and it is the leading cause of human food-borne infections associated with consumption of poultry products in the world [6].

The reduced immunocompetence caused by feed restriction beside the effects of bacterial enteric pathogens like *Salmonella* spp. make severe stresses in broilers. In such a condition, using additives such as prebiotics for improving the immune function and gut microflora is helpful. Prebiotics beneficially affect the host by making changes in the population of gut microflora in favor of useful bacteria [7] as well as increasing its humoral and cell-mediated immune responses [8]. In addition, prebiotics help the host resist against the bacterial infections [9].

Several studies have been done on the useful effects of prebiotics on infections [10,11] and the negative effects of feed restriction on immune system [3,4]. Gursoy et al.[12] reported that exposure to combined stresses may have synergistic, additive, or adaptive effects on the immune system parameters. In the literature, there was no report concerning the effect of dietary prebiotic on immune responses of broilers under combined stresses caused by infection and feed restriction. Therefore, our purpose in this study was to investigate the effect of prebiotic supplementation on immune system parameters of broiler chickens under feed restriction and *Salmonella* challenge.

MATERIAL and METHODS

Birds, Diets, and Management

Three hundred twenty 1-d-old male broilers (Ross 308) were obtained from a commercial hatchery. At a 2^3 factorial arrangement in randomized complete block design, they were divided into 8 treatment groups with 4 replicates and 10 broilers in each replicate, and were then put in special cages. Environmental temperature in the first week of life was 32°C and decreased to 20°C until the end of the experiment. During the first week, 22 h of light was provided with a reduction to 20 h afterward. Chickens had access to *ad libitum* water and a diet based on corn and soybean meal which provided 3.000 kcal/kg ME and 21.7% CP up to day 21, and 3075 kcal/kg ME and 19.25% CP from day 21 to day 42. This basal diet was unmedicated.

Prebiotic Supplementation

The prebiotic was gained from TechnoMOS (Biochem, Lohne, Germany). It was used at the amount of 0.1% of the diet. This product consists of mannanoligosaccharide (MOS) and β-1,3-Glucan and has been obtained from *Saccharomyces cerevisiae*.

Feed Restriction Procedure

From day 7 to day 14, the daily feed intake of the groups getting ad libitum consumption was measured, and 80% of this feed was given to feed restricted treatment groups the next day.

Salmonella Culturing, Counting and Challenge

Salmonella enteritidis (PTCC 1709) was provided freeze-dried from the Persian Type Culture Collection (IROST, Tehran, Iran) isolated from the liver of chickens. Freeze-dried inoculum was grown in nutrient broth media at 37°C for 8 h and passed to fresh nutrient broth for 3 incubation periods. Counting colony-forming units (cfu) was done with Neobar lam at day 10. After counting, chickens in the challenged groups received 2.6×10^5 cfu/chick of passaged medium through oral gavage with utilizing micropipette on the same day.

Treatment Groups

The treatment groups included 1. a control group with no supplementation and challenge, 2. a group under feed restriction (Control + R), 3. a *Salmonella* challenged group (Control + S), 4. a group under feed restrictin and Salmonella challenge (Connrol + R + S), 5. a prebiotic treated group, 6. a prebiotic treated and feed restricted group (Prebiotic + R), 7. a prebiotic treated under *Salmonella* challenge (Prebiotic + S), 8. a prebiotic treated under feed restriction and *Salmonella* challenge (Prebiotic + R + S).

Immunological Tests

Cell-mediated immune response was assessed according to the method of Corrier and DeLoach [13] by a cutaneous basophil hypersensivity (CBH) test using phytohem-agglutinin P (PHA-P) produced by Gibco company (Gibco, Invitrogen Corporation, Scotland, UK). At days 15 and 30, the toe web of the right foot (2 chickens per pen) was measured with a Caliper with an accuracy of 0.01 mm. Immediately after measurement, 100 µg of PHA-P (suspended in 0.1 mL of PBS) was injected into the toe web. The toe web swelling was measured 24 h after injection. The response was measured by subtracting the skin thickness of the first measurement from the skin thickness 24 h after dermal injection. At day 7, vaccination against Newcastle disease virus (NDV) was done by instilling

B1 strain of NDV (Intervet International BV, Boxmeer, Netherlands) in the chickens' eyes, and 2 birds per each pen were injected in breast muscle by 0.1 ml of 5% sheep red blood cell (SRBC) diluted in PBS. The chickens were bled from the wing vein at days 17 and 27, and sera were collected individually in separate sterile vials. The hemagglutination inhibition and hemagglutination tests were done to determine the antibody titer response against NDV [14] and SRBC [15], respectively.

At days 21 and 42, a chicken from each replicate pen was weighed and then killed by cervical cutting. Carcasses were dissected immediately after euthanasia. Thymus, bursa of Fabricius, and spleen were then precisely removed and weighed separately on a sensitive digital scale.

Statistical Analysis

All data were subjected to GLM procedures of SPSS version 18 software as a 2^3 factorial arrangement of treatments in randomized complete block design that included prebiotic supplementation, feed restriction and *Salmonella* challenge as the main factors and their respective interactions. The treatment means were compared by Duncan's multiple range tests. Probability values of less than 0.05 ($P<0.05$) were considered significant.

RESULTS

As it is shown in *Table 1*, there were significant differences among treatments regarding the cell-mediated immune response to CBH test ($P<0.05$). Prebiotic caused an increase at day 15, and feed restriction caused a decrease at day 30 in toe web thickness, and interaction among the factors was significant ($P<0.05$). There were no significant differences among treatments and factors' interaction regarding antibody titer responses against SRBC at days 17 and 27, and to NDV at day 17 ($P>0.05$); but there were significant differences among factors' interaction regarding antibody titer response against NDV at day 27 ($P<0.05$).

Table 2 shows the relative weight of lymphoid organs at days 21 and 42. *Salmonella* challenge had a significant effect on thymus relative weight at day 21 ($P<0.05$). Feed restriction significantly reduced the bursa of Fabricius at day 42 ($P<0.05$). There were no significant differences among treatments and factors' interaction regarding relative weight of spleen at days 21 and 42 ($P>0.05$).

DISCUSSION

In this study, prebiotic had advantageous and feed restriction had disadvantageous effects on cell-mediated immune response to PHA-P injection. Savino [3] expressed that sever feed restriction can cause significant decrease of mitogenic lymphocyte responses in vitro. Also in the study done by Hangalapura et al.[4], feed restriction reduced cell-mediated immune response which is likely because the cellular components of immune system are energy demanding. Savino and Dardenne [16] reported that infections as well as malnutrition can cause thymus atrophy leading

Table 1. *Cell-mediated immune response to PHA-P, and antibody titer response against NDV and SRBC*
Tablo 1. *PHA-'ye karşı hücresel immun yanıt ve NDV ve SRBC'ye karşı antikor yanıtı*

Treatment	CBH[1] Test (mm)		Antibody Titer (log_2) Against NDV[2]		Antibody Titer (log_2) Against SRBC[3]	
	Day 15	Day 30	Day 17	Day 27	Day 17	Day 27
Control	0.643^{bc}	0.580^{cd}	3.25	4.625^{ab}	2	2.5
Control + R[4]	0.695^{abc}	0.528^{d}	2.5	5.250^{a}	2	3
Control + S[5]	0.783^{ab}	0.888^{a}	3.75	5.625^{a}	1.25	3.5
Control + R + S	0.480^{c}	0.503^{d}	3.25	3.250^{b}	2.25	3.375
Prebiotic	0.905^{a}	0.843^{ab}	3.25	5.000^{a}	2	2.625
Prebiotic + R	0.693^{abc}	0.530^{d}	3.25	4.500^{ab}	2.5	3.5
Prebiotic + S	0.840^{ab}	0.728^{abc}	3	4.750^{a}	2	3.5
Prebiotic + R + S	0.890^{a}	0.663^{bcd}	2.75	4.875^{a}	2.5	3.625
SEM	0.052	0.043	0.412	0.458	0.330	0.592
P-value						
Prebiotic	0.002	0.135	0.672	0.775	0.122	0.607
Restriction	0.061	0.001	0.212	0.116	0.043	0.421
Salmonella	0.786	0.093	0.672	0.506	0.597	0.171
P×R×S[6]	0.007	0.003	0.672	0.011	0.296	0.941

1. *Cutaneous basophil hypersensitivity reaction to phytohemagglutinin P injection into the toe web skin, and measured as skin swelling before and after injection,* ***2.*** *Antibody response against Newcastle disease virüs,* ***3.*** *Antibody response against Sheep Red Blood Cell,* ***4.*** *Restriction in feed,* ***5.*** *Salmonella challenge,* ***6.*** *Interaction among prebiotic, feed restriction and Salmonella challenge,* ^{a,b,c,d} *Within the same column, means with different superscripts are significantly differ (P<0.05)*

Table 2. *The relative weights (% of live BW) of lymphoid organs at days 21 and 42*
Tablo 2. *21. ve 42. günlerde lenfoid organların göreceli (% canlı ağırlığa göre) ağırlığı*

Treatment	Day 21			Day 42		
	Bursa (%)	Thymus (%)	Spleen (%)	Bursa (%)	Thymus (%)	Spleen (%)
Control	0.213	0.373^{a}	0.078	0.220^{a}	0.218	0.125
Control + R[1]	0.250	0.263^{ab}	0.075	0.188^{ab}	0.193	0.110
Control + S[2]	0.268	0.288^{ab}	0.075	0.228^{a}	0.275	0.120
Control + R + S	0.208	0.258^{ab}	0.085	0.140^{bc}	0.140	0.133
Prebiotic	0.243	0.363^{ab}	0.080	0.223^{a}	0.165	0.118
Prebiotic + R	0.218	0.323^{ab}	0.075	0.180^{abc}	0.188	0.110
Prebiotic + S	0.260	0.228^{b}	0.083	0.218^{a}	0.210	0.105
Prebiotic + R + S	0.253	0.310^{ab}	0.083	0.125^{c}	0.200	0.105
SEM	0.023	0.042	0.011	0.018	0.043	0.013
P-value						
Prebiotic	0.596	0.726	0.081	0.566	0.610	0.198
Restriction	0.407	0.424	0.935	0.001	0.235	0.793
Salmonella	0.329	0.04	0.570	0.065	0.610	1.00
P×R×S[3]	0.91	0.726	0.807	0.923	0.527	0.601

1. *Restriction in feed,* ***2.*** *Salmonella challenge,* ***3.*** *Interaction among prebiotic, feed restriction and Salmonella challenge,* ^{a,b,c} *Within the same column, means with different superscripts are significantly differ (P<0.05)*

to decrease in cellular immune response mediated by T-cells. As Hooge [17] expressed, MOS advantages are more noticeable in stressor conditions which is in agreement with our findings showing that MOS increased cell-mediated immune response against the stresses caused by feed restriction and *Salmonella* challenge. The mechanism of prebiotic's function in immunity regulation is not yet exactly known. But some studies show that *Salmonella* infections stimulate inflammatory cytokine, interleukin-1 production [10,11]. Furthermore, MOS can competitively adsorbs pathogens from mannose and prevent their attack to gut epithelium [18]; this can cause increase in energy intake which is helpful in cell-mediated immunity.

Antibody titer responses against NDV had significant differences among treatment groups at day 27. The group under feed restriction and *Salmonella* challenge had the least antibody titer response against NDV and its difference with the group which was supplemented with prebiotic under the same condition, was significant. This indicates the advantageous effect of prebiotic on immunity in agreement with Alavi et al.[19], but in contrast with Silva et al.[20] findings. Also, Fanoosi and Torki [21] did not observe any decrease in antibody titer response against NDV under 10% feed restriction, but Jahanpour et al.[22]) reported that feed restriction as amount as 75% standard catalogue during days 8 to 14 decreased antibody titer response against NDV. There was no significant difference among treatments regarding antibody titer response against SRBC. Khalaji et al.[23] and Hangalapura et al.[4] found similar results using prebiotic in broilers and feed restriction in line chickens respectively; but Khajavi et al.[24] reported that antibody titer response increased against SRBC under moderate feed restriction in broilers which is in contrast with Jahanpour et al.[22] who reported that feed restriction as amount as 75% standard catalogue during days 8 to 21 decreased antibody titer response against SRBC. Riberio et al.[25] expressed that prebiotic effect on IgG against *Salmonella enteritidis* is not significant in broilers; whereas Cetin et al.[26] and Woo et al.[27] indicated that IgG level increases by MOS supplementation in turkeys and layers respectively. These contradictions might be because of differences in strain and age of the birds, the prebiotic type, feed restriction severity and intensity of exposure to *Salmonella*. However it seems that stresses and nutrient intake decrease have less suppressive effect on humoral immunity than on cell-mediated immunity.

There was a significant difference regarding the relative weight of thymus at day 21 among treatments. The treatment group with prebiotic under *Salmonella* challenge had the least relative weight of thymus. The significant difference between this treatment and the control treatment indicates that despite using prebiotic, *Salmonella* caused a decrease in the weight of thymus. Savino and Dardenne [16] expressed that infections cause thymus gland Atrophy. At day 42, the relative weight of bursa of Fabricius had a significant difference among treatments. The treatment group under prebiotic supplementation, restriction and *Salmonella* challenge had the least relative weight of bursa of Fabricius. Feed restriction and *Salmonella* infection stresses might be the main reason of decrease in the relative weight of this lymphoid organ. The plasma corticosterone level can increase because of feed

restriction [4] and microbial challenge [28] stresses. The high level of plasma corticosterone can cause a decrease in the immunpcompetance and the weight of lymphoid organs.

The results of this study show that using prebiotics during combined stresses can have some advantages in broilers. Although feed restriction has beneficial effects on broilers, its suppressive impact on immunity system especially when used severely must be considered. These suppressive impacts are more noticeable in cell-mediated immunity than humoral immunity, because the cellular immunity components are more sensitive to energy demand. Therefore, prebiotics are helpful in such a condition as well as in *Salmonella* infections.

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Kafkas Univ Vet Fak Derg
19 (4): 647-653, 2013
DOI: 10.9775/kvfd.2013.8600

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

Effects of Low Doses of Bisphenol A on Primordial Germ Cells in Zebrafish (*Danio rerio*) Embryos and Larvae [1] [2]

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[1] *This study was supported by Research Foundation of the Sakarya University, Project Number: 2012-02-20-014*
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Makale Kodu (Article Code): KVFD-2013-8600

Summary

Primordial germ cells are the precursors of gametes in sexually reproducing organisms. The migration and homing process of primordial germ cells are prone to environmental effects and the endocrine system hormones. One of the environmental pollutants which humans are exposed to is bisfenol A (BPA). BPA which is an endocrine disrupter generally used for making plastics harder. In this study we investigated the effects of low doses of BPA (4 mg/L and 8 mg/L) on primordial germ cells at the zebrafish embryos and larvae. Whole mount in situ hybridization for germ cell marker gene vasa showed that upon exposure to BPA, the primordial germ cell number increased and these cells ectopically localized outside the normal germ cell niche in zebrafish. Migration of primordial germ cells into ectopic locations and morphological changes in these cells were also proved by histological studies. Additionally, results of acridine orange staining to detect apoptotic cells showed that low doses of BPA did not increase apoptosis. Results of the present study showed that low doses of BPA exposure increased the number of primordial germ cells and induced ectopic primordial germ cell localization in the zebrafish embryos.

Keywords: *Zebrafish, Primordial germ cell, Bisphenol A*

Düşük Dozlardaki Bisfenol A'nın Zebra Balığı (*Danio rerio*) Embriyo ve Larvalarında Primordiyal Germ Hücreleri Üzerine Etkisi

Özet

Primordiyal germ hücreleri, eşeyli üreyen canlılarda gametlerin öncül hücreleridir. Endokrin sistem hormonları ve çevresel etkenler, primordiyal germ hücrelerinin göçünü etkilemektedir. İnsanların maruz kaldığı çevresel kirleticilerden biri de bisfenol A (BPA)'dır. Endokrin sistemde hasar oluşturan biri olan BPA, genellikle plastik sertleştirme işleminde kullanılmaktadır. Bu çalışmada zebra balığı embriyo ve larvalarında düşük dozlardaki (4 mg/L and 8 mg/L) BPA'nın etkileri incelenmiştir. Bir germ hücresi belirteci gen olan vasa ile yapılan whole mount in situ hibridizasyon deneyleri sonucunda BPA'nın primordiyal germ hücre sayısında artışa sebep olduğu ve bu hücrelerin göç yolu dışındaki (ektopik) bölgelerde bulunduğu tespit edilmiştir. Primordiyal germ hücrelerinin ektopik bölgelere göçü ve bu hücrelerdeki morfolojik değişiklikler histolojik çalışmalarla da doğrulanmıştır. Buna ek olarak, düşük dozlarıyla yapılan boyamalarda apoptozisin tespitinde kullanılan akridin turuncusu boyaması sonucunda BPA'nın apoptozisi artırmadığı saptanmıştır. Bu çalışma sonuçları, düşük dozlardaki BPA'nın primordiyal germ hücre sayısını artırdığını ve bu hücrelerin ektopik bölgelere göç etmesine yol açtığını ortaya koymaktadır.

Anahtar sözcükler: *Zebra Balığı, Primordiyal germ hücresi, Bisfenol A*

INTRODUCTION

Primordial germ cells (PGCs) constitute an embryonic cell type that migrate to gonadal precursors and form the gametes. Thus PGCs are the cells that provide the permanence of the species and are crucial for the development of a new organism in the next generation [1]. In many organisms PGCs are specified during early

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embryonic development and they contain germ granules [2-7].

PGC migration must be finely regulated as it follows a complex pathway through various developing tissues [8]. The ultimate location of PGCs in zebrafish is between the endodermal cells the yolk sac which are close to allantois, at early stages of the embryonic development [9]. They migrate into dorsal mesentery and reaches in the gonad primordia with amoeboid movements. The pathway which PGCs follows defined as "gonadal pathway" [10-13]. PGC migration in zebrafish takes place during the first 24 h of the embryonic development [14-16].

In zebrafish, germ line lineage can be traced throughout embryogenesis by means of transcripts of *vasa* [17] which is a gene that is first identified in *Drosophila melanogaster* as a maternal determinant of germ cell formation [18]. In zebrafish, *vasa* is a molecular marker for PGC [19,20]. Vasa protein can be detected in the germline cells in early embryos, because that it specifically located into polar granules, which are located where the germ cells are specified. PGCs are specified at four positions along the margin of the blastodisc and start migrating dorsally during gastrulation [16]. PGCs move towards the intermediate targets around somites 1-3 at 10.5 h post fertilization (hpf), and subsequently to the final target region at the level of somites 8-10 at 13 hpf. At 24 hpf, PGCs localize to the junction between the yolk ball and yolk extension in the gonadal region, forming compact clusters [16].

Several chemicals or environmental pollutants can cause cellular damage in PGCs which has ramifications in different diseases/disorders in organisms [21,22]. Therefore, investigating the migratory behaviour and regulations of PGCs is an intriguing biological question. Additionally, human are exposed to many of the environmental toxicants everyday. Information obtained from experiments on the reproductive systems of the experimental animals can provide important findings about the impact of humans.

Endocrine disrupting chemicals (EDCs) are compounds in the external environment that modulate the physiology of the endocrine system and often cause health disorders [23]. Bisphenol A (2 bis(4-hydroxiphenyl) propane, BPA) is one of the most potent endocrine disrupting chemicals which mimics estrogen *in vivo* and *in vitro* [24] and therefore is classified as xenoestrogen. BPA is widely used in industry for making plastics harder. It's found in polycarbonate plastics (baby feeding bottles, carboys) [25] and inside of epoxy coated cans [26]. With the increasing usage of these materials in daily life, humans are exposed to BPA in an escalating manner, which could cause endocrine disorders. High concentrations of estrogens stimulate PGC growth *in vitro* through the somatic cells of the gonadal ridges [27]. BPA acts by several different mechanisms to perturb the early stages of oogenesis and also affects primordial germ cells, by influencing mitotic proliferation and the timing of meiotic entry [28].

Duan and Zhu [29] investigated the toxicity of BPA on the growth of zebrafish embryos and found the median embryo lethal concentration (LC50) for 24 h as 16.36±0.40 mg/L. They also mentioned that all embryos died at 25 mg/L dose at 24 hpf. So in the present study, doses were chosen according to LC50 value of bisphenol A on zebrafish embryos. We carried out zebrafish embryos 4 mg/L and 8 mg/L BPA and investigated the effects on primordial germ cells of the zebrafish embryos. In 24hpf zebrafish embryos we determined the location of PGCs with whole mount in situ experiments. With histological analysis we observed the migration pathway of PGCs for two weeks.

MATERIAL and METHODS

This study was approved by Kocaeli University Animal Researches Ethics Committee (HADYEK- 5/3 - 2011).

Material

All animal experiments and procedures were carried out in accordance with the recommendations and official guidelines for animal handling and research. The temperature and humidity were kept at 28.5°C and 61%, respectively. Sexually matured zebrafish were fed daily with *Artemia* sp. and Tetra- Min© Hauptfutter (Tetra Werke, Germany) under standardized conditions (20-L glass aquaria, 28±1°C, light/dark cycle = 14 h/10 h) and after egg laying embryos were collected immediately. Under a stereo microscope, fertilized and unfertilized eggs were separated. Fertilized embryos were transfered into special small embryo aquariums.

Methods

BPA Exposure

BPA, 2 bis(4-hydroxiphenyl), (Sigma Aldrich, CAS No: 50-05-7) was dissolved in 1% dimethylsulfoxide (DMSO) to generate desired concentrations for treatment of the embryos. Two control and 2 experimental groups were used as: control (untreated), solvent control group (1% DMSO), 4 mg/L BPA and 8 mg/L BPA. DMSO and BPA were applied from 1 h post fertilization until sacrificing the embryos.

Whole Mount In Situ Hybridization

24-hpf-old embryos from control and BPA treatment groups were collected and fixed in 4% paraformaldehyde (PFA, Sigma) overnight at 4°C. After removing chorion, the embryos were washed in methanol series and stored in 100% MeOH at -20°C. Digoxigenin-labelled antisense RNA probes were generated for vasa gene as described Miyake et al.[30]. Whole mount *in situ hybridizations* with chromogenic substrates Nitro Blue Tetrazolium (NBT)/5-Bromo4-Chloro3-Indoyl phosphate (BCIP) were on the 24-hpf-embryos performed as previously described [31,32].

Acridine Orange Staining

After removing the chorion of the zebrafish embryos 5 µg/L acridine orange (Sigma) was added into the embryo rearing medium. Embryos were incubated in acridine orange solution for 30 min. They were anaesthetised with 0.04% of 3-aminobenzoic acid methyl ester (MESAB) and imaged under dissecting fluorescent microscope.

Histology

Embryos and larvae (0-2 weeks old) were fixed in Bouin's fixative for 8 h. After fixation, tissue was dehydrated in ascending concentrations of ethanol, equilibrated in xylene. The tissues were then embedded in paraffin wax and cut into 5-7 µm sections on a microtome. The sections were mounted on glass slides and stained with haematoxylin and eosin, toluidine blue and Best's carmine before examination under a light microscope.

Statistical Analysis

Statistical analysis were performed by using IBM SPSS Statistics 5.0 programme. Quantitative data was given as mean ± SD. T test were used for analyses and $P<0.05$ was considered as statistical significance value. For statistical analysis, 7 samples were used in each group (n=7).

RESULTS

Results of Expression of vasa Gene in Primordial Germ Cells

Vasa gene is a germ cell marker [19,20]. At embryonic stages of development, *vasa* transcripts are concentrated in an electron-dense structure, the putative zebrafish germ plasm [20,33].

The location and number of PGCs were detected at 24 h post fertilization (*Fig. 1* and *Fig. 2*). In each group, 7 embryos were tested. PGCs that migrate the correct and ectopic locations were counted. In control (untreated) and DMSO groups PGCs migrate to the target region correctly (*Fig. 1-a* and *1-b*). When compared the 4 mg/L BPA and 8 mg/L BPA groups with control ones, there was an increase in the number of PGCs *(Fig. 2)* and a significant portion of germ cells were at ectopic locations (*Fig. 1-c* and *1-d*). It was observed that 1% DMSO and BPA treatment increased the PGCs numbers in comparison to the untreated group. t test results showed that DMSO and BPA treatment increased the total PGC number *(Table 1)*. The number of PGCs increased in BPA treated group *(Table 2)*. BPA treatment also increased ectopic PGCs *(Table 3)*. Our data suggest that BPA caused an increase in the number

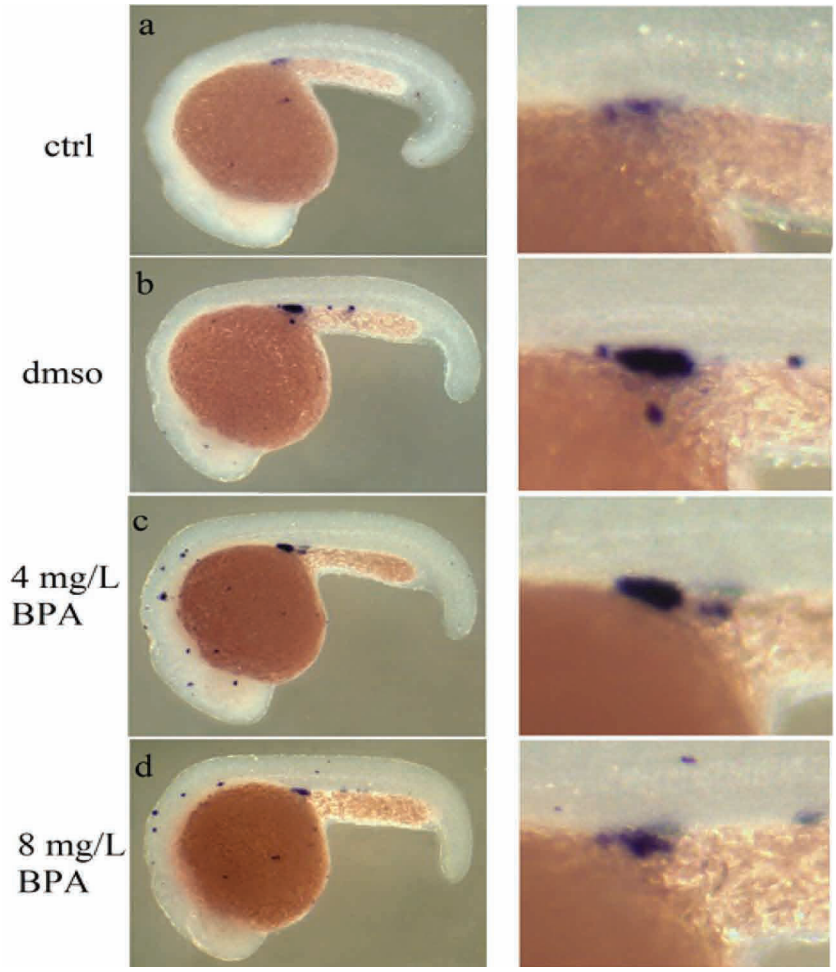

Fig 1. Whole mount in situ hybridization results of 24 hpf old zebrafish embryos. Magnifications at the right side show PGCs which locate the right direction. **a)** Untreated (ctrl) group, PGCs located to the right direction, **b)** 1% DMSO (solvent control) group, PGCs located to the right direction but there are few ectopic cells, ***c)*** 4 mg/L BPA treated group, most of PGCs are ectopic, **d)** 8 mg/L BPA treated group, most of PGCs are ectopic

Şekil 1. 24 saatlik zebra balığı embriyolarında whole mount in situ hibridizasyon sonuçları. Sağ taraftaki büyütülmüş kısımlar doğru bölgede bulunan primordiyal germ hücrelerini (PGH) göstermektedir. **a)** Kontrol grubu, PGH doğru bölgede bulunmaktadır, **b)** Çözücü kontrol (%1 DMSO) grubu, PGH doğru bölgede bulunmaktadır fakat birkaç hücre ektopik bölgede bulunmaktadır, **c)** 4 mg/L BPA uygulanmış grup, pek çok PGH ektopik bölgelerdedir, **d)** 8 mg/L BPA uygulaması yapılmış grup, pek çok PGH ektopik bölgededir

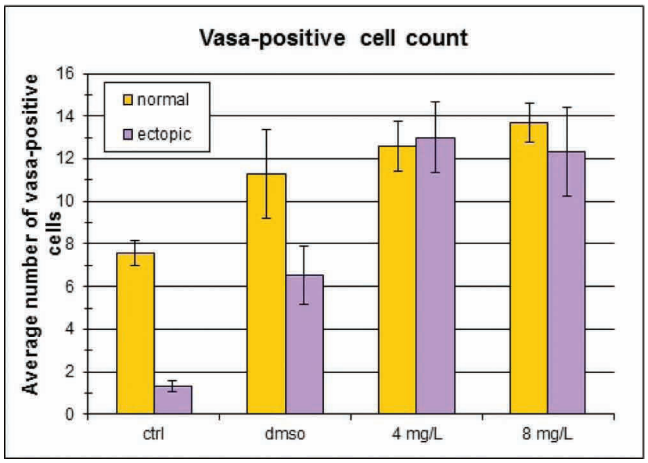

Fig 2. General comparement of control and BPA treatment groups. When compare with control group, as a result of BPA treatment more vasa-positive cells (PGCs) were detected at ectopic and right locations (n=7)

Şekil 2. Kontrol ve BPA gruplarının genel karşılaştırması. Kontrol grubu ile karşılaştırıldığında BPA uygulaması sonucunda vasa pozitif hücreler (PGH) doğru ve ektopik bölgelerde tespit edilmişlerdir (n=7)

Table 1. *Total (ectopic + gonadal region) PGC frequency at 24 hpf zebrafish embryos*

Tablo 1. *24 saatlik zebra balığı embriyolarındaki toplam (ektopik + gonadal bölgedeki) PGH sıklığı*

Group	Total PGCs Frequency			
	Average		Standart Deviation	*
Control (untreated) (n=7)	4.43	±	0.94	a
1% DMSO (n=7)	9.00	±	1.45	b
4 mg/L BPA (n=7)	13.50	±	0.81	c
8 mg/L BPA (n=7)	13.71	±	0.98	c

** There are significant difference between samples that are shown with different letters (P<0.05)*

Table 2. *PGC frequency at gonadal region at 24 hpf zebrafish embryos*

Tablo 2. *24 saatlik zebra balığı embriyolarındaki gonadal bölgedeki PGH sıklığı*

Group	PGCs Frequency at Gonadal Location			
	Average		Standart Deviation	*
Control (untreated) (n=7)	7.57	±	0.65	a
1% DMSO (n=7)	11.29	±	2.22	a,b
4 mg/L BPA (n=7)	12.57	±	1.25	b
8 mg/L BPA (n=7)	13.71	±	0.99	b

** There are significant difference between samples that are shown with different letters (P<0.05)*

of PGCs, although all of these cells cannot migrate into the prospective primordia and remained at ectopic locations.

Results of Acridine Orange Staining

In the second part of the study we investigated the effects of BPA on the apoptosis frequnecy in zebrafish

Table 3. *PGC frequency at ectopic locations at 24 hpf zebrafish embryos*

Tablo 3. *24 saatlik zebra balığı embriyolarındaki ektopik bölgelerdeki PGH sıklığı*

Group	PGCs Frequency at Ectopic Locations			
	Average		Standart Deviation	*
Control (untreated) (n=7)	1.29	±	0.29	a
1% DMSO (n=7)	6.71	±	1.57	b
4 mg/L BPA (n=7)	14.43	±	1.00	c
8 mg/L BPA (n=7)	13.71	±	1.77	c

** There are significant difference between samples that are shown with different letters (P<0.05)*

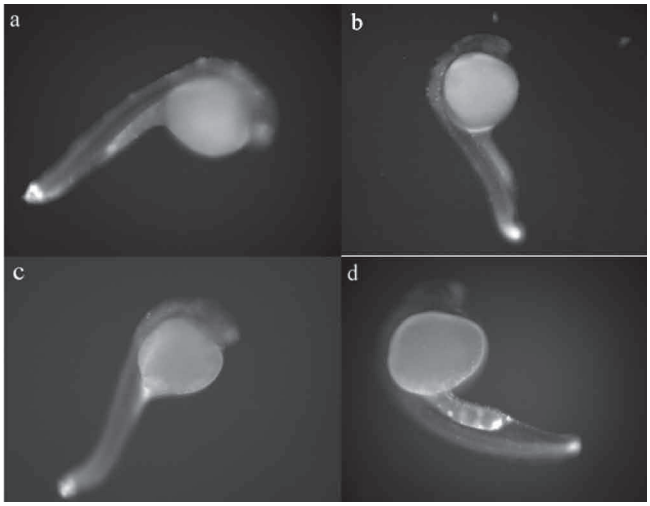

Fig 3. Acridine orange staining results **a)** Control group **b)** Solvent control group (1% DMSO) **c)** 4 mg/L BPA treated group **d)** 8 mg/L BPA treated group. When compare BPA treatment groups with control ones, it was not seen any differences between the groups

Şekil 3. Akridin turuncusu boyama sonuçları **a)** Kontrol grubu **b)** Çözücü kontrol grubu (%1 DMSO) **c)** 4 mg/L BPA uygulaması yapılmış grup **d)** 8 mg/L BPA uygulaması yapılmış grup. Kontrol grupları BPA uygulaması yapılmış gruplarla karşılaştırıldığında gruplar arasında bir fark görülmemiştir

embryos. Acridine orange is a nucleic acid fluorochrome which emits fluorescence when it intercalates into the DNA [34]. BPA treatment did not change apoptosis frequency and the frequency was in normal range that normally prevails during the embryonic development *(Fig. 3)* of zebrafish embryos.

Histological Studies

Morphology of primordial germ cells were detected with histological methods during 2 weeks. The embryos of the 8 mg/L BPA group could survived 7 days maximally. PGCs which are bigger than somatic cells and have large nucleus, nuage material and glycogen granules were detected by H&E, toluidine blue and Best's carmine stains. It was found that results of histological studies were supported by whole mount *in situ* results. In DMSO and BPA treated groups, increase in the number of PGCs which located at ectopic regions were detected.

At 3 day post fertilization (dpf) embryos, PGCs were observed at the dorsal part of yolk sac in untreated group. PGC structure was normal *(Fig. 4-a)*. Location of PGCs were shown with arrows *(Fig. 4-b,c,d)*. In BPA treated groups, decrease at glycogen granules in PGCs were observed *(Fig. 4-c,d)*.

At 7 dpf prelarvae, PGCs were observed near muscle tissue *(Fig. 5-a,b,c,d)*. In untreated and DMSO group, the shapes of migrating PGCs were ameboid (shown with star) *(Fig. 5-a,b)*. In BPA treated groups, most of PGCs did not have ameboid shape *(Fig. 5-c,d)*.

At 14 dpf larvae, in untreated and DMSO groups, PGCs were observed at gonadal region *(Fig. 6-a,b)*. PGCs could not migrate gonadal region at 4 mg/L BPA treated group. In this group, PGCs were observed near somites *(Fig. 6-c)*. Decrease at nuage material of PGCs were also detected in this group *(Fig. 6-c,d)*.

DISCUSSION

We conducted this study to evaluate the effects of BPA on primordial germ cells of zebrafish (*Danio rerio*) embryos because very little is known about the effects of BPA on primordial germ cells in vertebrates. There are not any study the effects of BPA on primordial germ cells. so, our study is pioneer about this subject.

Avcı et al.[35] determined the effects of nonylphenol on growth parameters and antioxidant defense system

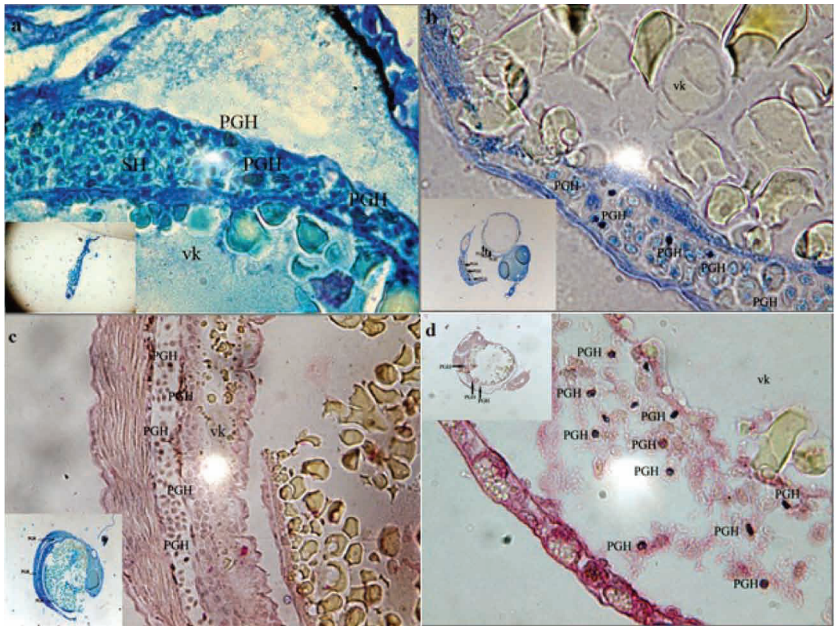

Fig 4. Histologic sections of 3 dpf embryos. Location of PGCs were shown with arrows. **PGH:** primordial germ cell, **SH:** somatic cell, **vk:** yolk sac, **a)** Untreated group, Toluidine blue staining (x4), (x100), **b)** Solvent control group (1% DMSO), Toluidine blue staining (x10), (x100), **c)** 4 mg/L BPA treated group, Toluidine blue and Best carmine staining (x10), (x100), **d)** 8 mg/L BPA treated group, Best carmine staining (x10), (x100)

Şekil 4. 3 günlük embriyoların histolojik kesiti. PGH bulunduğu yerler oklar ile gösterilmiştir. **PGH:** Primordiyal germ hücresi, **SH:** somatik hücre, **vk:** vitellüs kesesi, **a)** Kontrol grubu, Toluidine mavisi boyaması (x4), (x100), **b)** Çözücü kontrol grubu (%1 DMSO), Toluidine mavisi boyaması (x10), (x100), **c)** 4 mg/L BPA uygulaması yapılmış grup, Toluidine mavisi ve Best Carmine boyaması (x10), (x100), **d)** 8 mg/L BPA uygulaması yapılmış grup, Best carmine boyaması (x10), (x100)

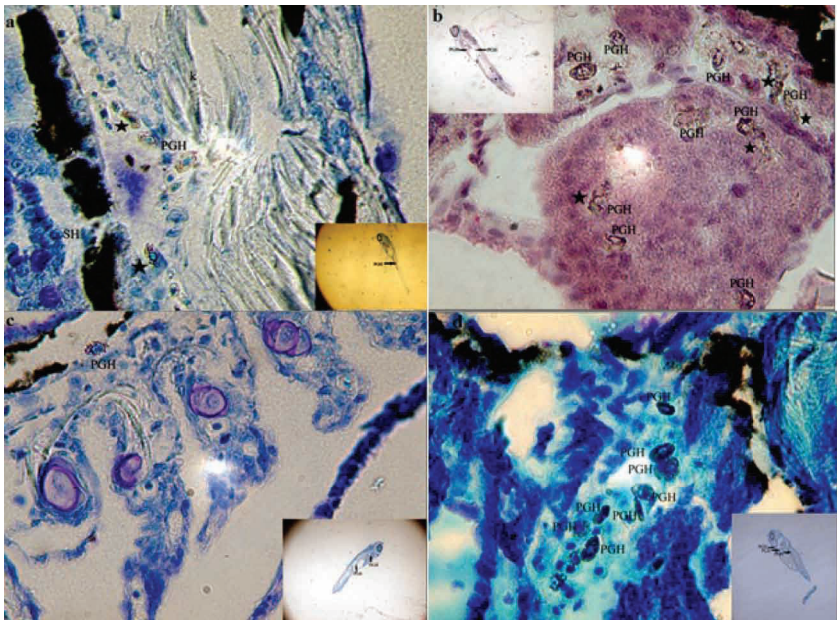

Fig 5. Histologic sections of 7 dpf prelarvae. Location of PGCs were shown with arrows. Migrating PGCs were mentioned with star. **PGH:** primordial germ cell, **SH:** somatic cell, **k:** muscle tissue, **a)** Untreated group, Toluidine blue staining (x10), (x100), **b)** Solvent control group (1% DMSO), Best carmine staining (x10), (x100), **c)** 4 mg/L BPA treated group, PGC at ectopic region, Toluidine blue staining (x10), (x100), **d)** 8 mg/L BPA treated group, Toluidine blue staining (x10), (x100)

Şekil 5. 7 günlük prelarvaların histolojik kesiti. PGH bulunduğu yerler oklar ile gösterilmiştir. Göç eden PGH yıldız ile gösterilmiştir. **PGH:** Primordiyal germ hücresi, **SH:** somatik hücre, **k:** kas dokusu. **a)** Kontrol grubu, Toluidine mavisi boyaması (x10), (x100), **b)** Çözücü kontrol grubu (%1 DMSO), Best carmine boyaması (x10), (x100), **c)** 4 mg/L BPA uygulaması yapılmış grup, ektopik bölgedeki PGH, Toluidine mavisi boyaması (x10), (x100) **d)** 8 mg/L BPA uygulaması yapılmış grup, Toluidine mavisi boyaması (x10), (x100)

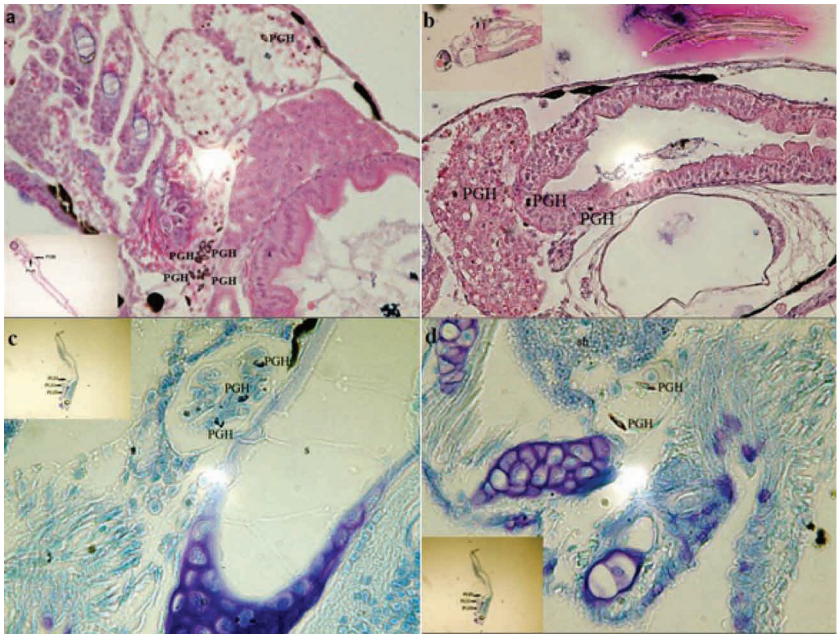

Fig 6. Histologic sections of 14 dpf larvae. Location of PGCs were shown with arrows. **PGH:** primordial germ cell, **sh:** somatic cell, **s:** somites, **a)** Untreated group H&E staining (x10), (x40), **b)** Solvent control group (1% DMSO), H&E staining (x20), (x40), **c)** 4 mg/L BPA treated group, Toluidine blue staining (x10), (x100), **d)** 4 mg/L BPA treated group, PGC at ectopic region, Toluidine blue staining (x10), (x100)

Şekil 6. 14 günlük larvaların histolojik kesiti. PGH bulunduğu yerler oklar ile gösterilmiştir. **PGH:** Primordiyal germ hücresi, **sh:** somatik hücre, **s:** somitler, **a)** Kontrol grubu, H&E boyaması (x10), (x100), **b)** Çözücü kontrol grubu (%1 DMSO), H&E boyaması (x20), (x40), **c)** 4 mg/L BPA uygulaması yapılmış grup, Toluidine mavisi boyaması (x10), (x100), **d)** 4 mg/L BPA uygulaması yapılmış grup, ektopik bölgedeki PGH, Toluidine mavisi boyaması (x10), (x100)

Japanese quails *(Coturnix japonica)* and they found that nonylphenol has dramatic effects on egg production rate and anti-oxidant system in Japanese quails *(Coturnix japonica)*. In addition, Willey and Krone [21] investigated effects of endosulfan and nonyphenol on the primordial germ cell population in pre-larval zebrafish embryos during the first day of development. In this study, vasa antisense RNA probe was used for whole mount in situ hybridization. As a result of the study, they proved that endosulfan treatment cause decrease the number of PGCs in 5th and 6th somites, increase the number of PGCS in 7th and 8th somites. Between 9th and 13th somites any change in the number of PGCs didn't observed. In nonylphenol treated group they found decrease in the number of PGCs between 6th and 7th somites. As a result, these chemicals cause alternations in the distrubition of PGCs along the posterior and anterior axis. In our study, we proved that BPA treatment cause ectopic localization of PGCs. Besides, it cause increase in the number of total PGCs.

Lawson et al.[28] studied the effects of low doses of BPA on gene expression in the fetal mouse ovary and they suggested that BPA acts to down-regulate mitotic cell-cycle genes, raising the possibility that fetal BPA exposure may act to limit expansion of the primordial germ cell population. On contrary, we found an increase in the number of PGC population after BPA exposure in zebrafish embryos.

Doitsidou et al.[36] found that SDF-1a chemokine is the guidance for primordial germ cell attraction. In our study, it's obvious that BPA treatment cause an increment at the number of ectopic primordial germ cells. This result can be interpreted with 2 ways. First, BPA can inhibit SDF-1a chemokine signal mechanism. Second, BPA treatment increses primordial germ cell production and the ectopic cells that can be seen from figures are migrating cells to the targets.

In some references it's mentioned that BPA induces apoptosis at rodents. Li et al.[6] gave high doses of BPA to mice and they used TUNNEL assay, immunohistochemistry and western blotting techniques. As a result they found that high doses of BPA exposure induces apoptosis and upregulation of fas/fasl and caspase-3 expression in testes of mice. Similarly, Wang et al.[37] found that BPA induced germ cell apoptosis in testes. These studies were done at tissues of adult individuals. On contrary in our study we studied with embryos and we could not see any apoptotic effects of low doses of BPA on zebrafish embryos. Similarly to our results, Oka et al.[38] studied the effects of BPA on apoptosis in central neural cells during early development of Xenopus and they also couldn't observe apoptotic effects in early stages.

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Kafkas Univ Vet Fak Derg
19 (4): 655-658, 2013
DOI: 10.9775/kvfd.2013.8605

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

Importance of Vaccination Against Atrophic Rhinitis in Pigs on Average Daily Gain and Mortality Rate

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Makale Kodu (Article Code): KVFD-2013-8605

Summary

The aim of this study was to investigate the effect of pigs, sows and piglets vaccination against atrophic rhinitis (AR) on the production results, the average daily gain (ADG) and mortality rate. Unvaccinated piglets, originating from vaccinated sows had a slightly higher ADG and only slightly less mortality than the control piglets (unvaccinated piglets and their mothers). Vaccinated piglets had a significantly higher ($P<0.01$) ADG and almost twice lower mortality rate than piglets unvaccinated, whether originating from vaccinated or unvaccinated mothers. Based on the observed results it is concluded that vaccination of pigs against AR significantly increases ADG and reduces mortality, and vaccination of sows against AR only slightly improves production performance and it is not economically justified for the studied farm.

Keywords: *Atrophic rhinitis, Vaccination, Average daily gain, Mortality rate, Pig*

Domuzlarda Atropik Rinite Karşı Aşılamanın Ortalama Günlük Kazanç ve Mortalite Oranı Üzerindeki Önemi

Özet

Bu araştırmanın amacı, domuzların, dişi domuzların ve domuz yavrularının atropik rinite (AR) karşı aşılamalarının üretim sonuçları, günlük kazanç oranı (ADG) ve mortalite oranı üzerine etkisini araştırmaktır. Aşılanmış domuz, yavruları aşılanmış ya da aşılanmamış annelerden olup olmadığına bakılmaksızın aşılanmamış yavrulara göre yüksek ADG ($P<0.01$) ve neredyse iki kat düşük mortalite oranı gösterdiler. Bu sonuçlara dayanarak, domuzlarda AR'e karşı aşılamanın önemli derecede ADG'nı arttırdığı ve mortaliteyi düşürdüğü, ve dişi domuzların AR'e karşı aşılanmasının sadece az miktarda üretim performansını arttırdığı ve bunun çalışılan çiftliklerde ekonomik olarak anlamlı olmadığı sonucuna varılmıştır.

Anahtar sözcükler: *Atropik rinit, Aşılama, Ortalama Günlük Kazanç, Mortalite Oranı, Domuz*

INTRODUCTION

Gram-negative bacteria *Pasteurella multocida* and *Bordetella bronchiseptica* are the primary infectious etiologic factors of atrophic rhinitis (AR). It is characterized by the formation of thick dry crusts in a roomy nasal cavity, which are result of progressive wasting away or decrease in size (atrophy) of the mucous nasal lining (mucosa) and underlying bone [1]. The various symptoms include foetor (strong offensive smell), crusting/nasal obstruction, nosebleeds, anosmia (loss of smell) or cacosmia (hallucination of disagreeable odour), secondary infection, maggot infestation, nasal deformity, pharyngitis, otitis media and even, rarely, extension into the brain and its membranes [2]. Atrophic rhinitis can be classed as primary or, where it is a consequence of another condition or event, secondary. Infections of pigs with *P. multocida* and *B. bronchiseptica* can also result in bronchopneumonia [3,4]. Moreover, AR causes significant global economic loss in swine production due to growth retardation [5].

Vaccines to coritrol AR have exploited knowledge of

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the pathogenesis of AR. Vaccines have been assessed for efficacy in reducing clinical signs and lesions of AR following both natural challenge in field trials and artificial challenge in controlled experiments [5]. Researchers have also evaluated the efficacy of AR vaccines in improving the growth rate of pigs, eliminating infection by toxigenic *P. multocida* and *B. bronchiseptica* from a farm, and inducing immune responses in sows and piglets to these bacteria [6,7].

On the basis of this, the objective of this investigation was to evaluate the effect of commercial vaccines against AR on the average daily weight gain and mortality rate.

MATERIAL and METHODS

Experimental Animals

The experiment was performed on a pig farm, with a capacity of 2500 sows, with an intensive way of keeping the pigs infected with AR enclosed. Infection status was assessed by serologic testing and detection of *Pasteurellae* and *Bordatellae* genom in serum. The experiment was performed on 4 groups of sows and their piglets (A, B, C and D) of the breeds Landras x Large Yorkshire hybrid (F_1), Large Yorkshire, and Landrace. Group A piglets originated from group A sows, group B piglets originated from group B sows, group C piglets originated from group C sows, and group D piglets originated from group D sows. All the animals had tags on their ears and a tattooed number.

Vaccinations of Animals

Animals were vaccinated with commercially vaccine against AR to the manufacturer's instructions (IDT Biologika GmbH). The vaccine had inactivated bacteria *Bordetella bronchiseptica* and inactivated bacteria *Pasteurella multocida* (A type and D type) and toxoid (dermonecrotoxin).

Vaccination of Sows

A total of 103 pregnant sows were used in this study. The health of the animals before vaccination was clinically normal. Two programs were performed: in groups A and B 50 sows were vaccinated twice at 5 and 2 weeks before parturition, and in groups C and D, 53 sows were unvaccinated. The vaccine was applied in 4 ml dosages, subcutaneous, behind the ear.

Vaccinations of Piglets

A total of 627 piglets were used in this study. Two programs were performed: in groups A and C 304 piglets were vaccinated twice at 35 and 56 days of age (the vaccine was applied in 1 ml dosages, subcutaneous, behind the ear), and in groups B and D 323 piglets were unvaccinated.

Average Daily Gain and Mortality

Each of the groups A and B contained 25 sows and their 152 piglets, group C contained 26 sows and their 152 piglets and group D (control group) contained 27 sows and their 198 piglets. The piglets were selected by random sampling method. After weaning, all piglets were housed in one building and were divided by 10 piglets per box. Average daily gain and mortality were monitored from 28th to 175th day of life.

Statistical Analyses

The data were processed by ANOVA and Post Hoc Test was used for comparison of the means of treatments. Statistical significance of differences between means was determined at the level of $P<0.01$.

RESULTS

Influence of Vaccination on Average Daily Gain of Pigs (ADG)

Table 1 presents the results of the ADG in tested piglets. In group A piglets (sows and piglets vaccinated) ADG values were significantly higher ($P<0.01$) than in group B piglets (sows vaccinated only). Between group A and group C piglets (vaccinated pigs only) there was no significant difference ($P>0.01$) in ADG. Group A piglets had significantly higher ($P<0.01$) ADG than group D piglets (control group). Group B piglets had significantly lower ($P<0.01$) ADG than group C piglets. At the same time, group B piglets had higher ($P<0.01$) ADG compared to control piglets (group D). Vaccinated piglets (group C) had significantly higher ($P<0.01$) ADG compared to the control group.

Influence of Vaccination on Mortality of Piglets

To determine the effect of vaccination against AR on mortality of piglets, mortality was calculated using the

Table 1. *Average daily gain*
Tablo 1. *Ortalama günlük kazanç*

Group	Frequency	Percent	Mean	SD	MIN	MAX
GROUP A	152	23.2	682	33.36	620	740
GROUP B	152	23.2	632	45.10	570	730
GROUP C	152	23.2	678	49.90	570	765
GROUP D	198	30,3	615	64.64	480	740
Total	654	100.0				

initial number of the piglets in each observed group on the 28th day of life. Group C piglets had the lowest number of deaths (5.3%), which is almost twice lower value compared to the control piglets (group D). Unvaccinated piglets whose mothers were vaccinated (group B) had a very high mortality (9.2%), almost as in control group piglets. Vaccinated piglets, whose mothers were also vaccinated, had a mortality of 6.6% *(Table 2)*.

Table 2. *Mortality rate (%)*
Tablo 2. *Mortalite oranı (%)*

Group			
A	**B**	**C**	**D**
6.6	9.2	5.3	10.1

DISCUSSION

The results of this study clearly indicate that vaccination of pigs against AR on studied farms significantly influenced the increase of the ADG and reduced mortality of piglets. Vaccinated piglets, originating from vaccinated and non-vaccinated sows had about 10% higher daily gain, reducing the feeding duration for 10 days as determined in their studies by Liao et al.[5]. In vaccinated piglets there were almost half the deaths than in non-vaccinated pigs [8]. Unvaccinated piglets, originating from vaccinated sows had a slightly higher ADG and only slightly less mortality than the control piglets. This is a consequence of the termination of colostral immunity and the occurrence of infection in the same piglets [9,10]. Evaluation of the safety and efficacy vaccines in this study suggest that they have potential in reducing the cost of AR in commercial farms. However, based on the experience of others [8,11], these vaccines are unlikely to eliminate the causative agents of AR, *P. multocida* and *B. bronchiseptica* from a farm. By vaccinating the piglets better production results were achieved. This justifies the vaccination economically and at the same time on the studied farm there is no need for sows vaccination. The vaccinated piglets originating from both vaccinated and unvaccinated sows show no significant difference in ADG and mortality rate is almost identical. Nielsen [12] found that there is no difference in ADG between piglets originating from vaccinated and unvaccinated mothers during lactation period. Considered that, as well as the results obtained in this study, where there is almost no significant difference in production results between unvaccinated piglets originating from vaccinated and unvaccinated sows, economic justification was not confirmed vaccination of sows on farm examined. In the previous researches performed by other authors [13,14] it has been established that vaccination of older pigs, has little benefit against clinical AR and is generally less effective than sow vaccination. However recent results in researches show that there is no difference between ADG and mortality rate with pigs derived from vaccinated and non-vaccinated sows. Distinction in ADG and mortality rate shall be shown in at a later stage of life, aged 90 days and over, so pigs vaccination has been recommended in herds in which AR causes have been established [15].

Vaccination of piglets has unquestionable beneficial effect but current vaccines are not able to eliminate the bacteria. Optimal results can only be obtained when vaccination is combined with husbandry and management measures such as all-in/all-out production, correct vaccination, hygiene, reduction of stocking density and avoiding of stress [16].

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DOI: 10.9775/kvfd.2013.8609

Journal Home-Page: http://vetdergi.kafkas.edu.tr
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RESEARCH ARTICLE

Kontrol Günü Süt Verimlerinin Zaman Serisi Yöntemi ile Modellenmesi [1][2]

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Makale Kodu (Article Code): KVFD-2013-8609

Özet

Bu çalışmanın amacı kontrol günü süt verimlerinin zaman serisi yöntemi ile modellenmesi ve en isabetli öngörüleri sağlayan kontrol günü sayısının belirlenmesidir. Bu amaçla 1070 süt sığırına ait 10700 kontrol günü kaydı kullanılmıştır. Veri seti her birinde 5350 kayıt bulunan iki guruba ayrılmıştır. Bu gruplardan biri model parametrelerini tahmin amacı ile, diğeri ise modelin öngörü başarısını değerlendirmek ve en isabetli öngörüleri sağlayan kontrol günü sayısını belirlemek amacı ile kullanılmıştır. Çalışma sonucunda ARIMA(2,0,0)(1,1,1)$_{10}$ modeli uygun model olarak belirlenmiş ve öngörü değerleri bu model kullanılarak elde edilmiştir. Söz konusu modelin tahmin edilen parametre değerleri ile gösterimi $(1-B^{10})^1y_t = [(1-0.99129B^{10})/(1-0.36889B-0.06934B^2)(1-0.08352B^{10})]a_t$ şeklindedir. Gerçek değerler ile öngörü değerleri arasında yüksek ve istatistiksel olarak anlamlı korelasyonlar saptanmıştır. Sonuçlar zaman serisi yaklaşımının süt veriminin öngörüsünde kullanışlı olabileceğini ortaya koymaktadır.

Anahtar sözcükler: *Kontrol günü, Süt verimi, Zaman serisi, ARIMA, Öngörü*

Modeling the Test Day Milk Yields via Time Series Method

Summary

The aim of this study is to model the test day milk yields via time series methodology and to determine the number of test days which provide the most accurate forecasts. For this purpose, 10700 test day records belonging to 1070 dairy cattle were used. Data were divided into two groups of 5350 records in each. One set of observations was used to model parameters, while the remaining was used for evaluating the forecast power of the model and for determining the number of test day records which provide the most accurate forecasts. ARIMA(2,0,0)(1,1,1)$_{10}$ model was determined to be suitable and it was used to obtain the forecast values. The expression of the model using estimated parameter values is $(1-B^{10})^1y_t = [(1-0.99129B^{10})/(1-0.36889B-0.06934B^2)(1-0.08352B^{10})]a_t$. Statistically significant and high correlations were determined between the actual and forecast values. The results indicated that the time series approach can be useful for prediction of milk yields.

Keywords: *Test day, Milk yield, Time series, ARIMA, Forecast*

GİRİŞ

Süt verimini modellemeye yönelik çalışmaların 1900'lü yılların başlarından itibaren yürütüldüğü bilinmektedir [1]. Sığırların süt veriminin modellenmesi için yapılan çalışmalar kısmi ya da tamamlanmış laktasyon kayıtlarından elde edilen günlük, haftalık ya da aylık süt miktarları üzerinden doğrusal ya da doğrusal olmayan deterministik modeller tahminlemek üzerine kurulmuştur [2]. t zamanındaki süt verimi y_t; $y_t = f(t)+\varepsilon_t$ veya $y_t = f(t)\times\varepsilon_t$ gibi iki mümkün şekilde modellenebilir [3]. Burada, f(t) sürekli ve laktasyonun uzunluğuna bağlı olarak tüm zaman aralıklarında tanımlı bir fonksiyon iken ε_t, şansa bağlı hata terimlerini simgelemektedir [4]. Wood, Gamma, Ali-Schaeffer, Glasbey, Logaritmik, Wilmink, Goodall modelleri laktasyon eğrisinin modellenmesinde yaygın olarak kullanılmaktadır [5-8].

Farklı sağım günlerinde ölçülen kontrol günü süt verimlerinin genetik olarak farklı değişkenler olarak adlandırılması ve çok değişkenli yöntemlerle analiz edilmesi

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mümkündür [9]. Bununla birlikte, yaygın olan tek değişkenli yaklaşıma göre her bir laktasyonda farklı zamanlarda alınan süt verimleri aynı deneme ünitesindeki tekrarlanan ölçümler olarak adlandırılabilir [10]. Laktasyon dönemi süresince belirli zaman aralıklarıyla yapılan ölçümler zamana bağlı olduğu için kurulan regresyon modellerindeki artıklar arasında bir ilişkinin olabileceği düşünülebilir. Aynı şekilde her kontrolde elde edilen verimin bir önceki verimlerle ilişkisi de söz konusu olmaktadır [3].

Süt veriminin modellenmesinde kullanılan farklı bir yaklaşım ise Deluyker ve ark.[2], tarafından yapılan çalışmada ortaya konmuştur. İlgili çalışmada Holstein ırkı ineklere ait süt verim kayıtları zaman serisi yöntemi ile modellenmiştir. Macciotta ve ark.[9,11], Sarda koyunu ve Simmental ırkı ineklere ait kontrol günü süt verimlerini zaman serisi yöntemi ile modelledikleri çalışmalarını yayınlamışlardır. Söz konusu çalışmalar, zaman serisi yönteminin, laktasyon eğrisinin şeklini tanımlamada ve belirli sayıda kontrol günü kaydı mevcut olduğunda hayvanların devam eden laktasyondaki süt verimlerinin öngörüsünde kullanışlı olduğunu ortaya koymuştur.

Bu çalışmanın amacı daha çok ekonomi alanında kullanılan ve bir seriye ait değerlerin kendi geçmiş değerleri, güncel ve geçmiş dönem rassal artıklarının ağırlıklı toplamı yardımıyla modellenmesine olanak sağlayan tek değişkenli zaman serisi analizi ile süt sığırlarının kontrol günü süt verimlerinin modellenmesidir. Ayrıca, zaman serisi modellerinin öngörü başarısından yararlanarak en isabetli öngörüleri sağlayan kontrol günü sayısının belirlenmesi de amaçlanmıştır.

MATERYAL VE METOT

Hayvan Materyali

Bu çalışmanın hayvan materyalini İngiltere Ulusal Süt Kayıtları Birliği'nde kaydı tutulan ve ilk kontrolleri Kasım 1988 ile Ekim 1989 tarihleri arasında yapılmış olup 10 adet verim kaydı bulunan 1.070 İngiliz Siyah Alaca düvesi oluşturmaktadır. Verim kayıtları yaklaşık olarak birer aylık aralıklarla alınmıştır. Her bir kontrol günü süt verimi 24 saatlik süre içerisinde alınan bireysel günlük süt verimlerinin toplamıdır ve kg cinsinden kayıt edilmiştir.

Zaman Serisi Analizi

Bir zaman serisi, zaman içerisinde sıralı olarak toplanan gözlem değerlerinin dizisi olarak tanımlanabilir [12]. Zaman vasfının gün, hafta, ay, üç ay, yıl vb. periyotlarını simgeleyen şıkları t=1,2,..,T olarak ifade edilirse, Y değişkenine ait bu dönemlerde elde edilen gözlem değerleri de y_1, y_2,…y_T şeklinde gösterilirler [13].

Zaman serisi ile iyi uyum gösteren bir modelin belirlenme aşamasında ilk olarak serinin durağan olması veya olmaması, mevsimsel özelliklere sahip olup olmaması gibi özellikler ön plana çıkmaktadır [14]. Durağan olmayan Y serisinin ortalamada durağan hale getirilmesi için d-kez farkının alınması gerekiyorsa, durağan w_t serisi B olarak ifade edilen geri kaydırma işlemcisi yardımıyla $w_t = (1\text{-}B)^d y_t$ şeklinde gösterilmektedir [12]. Mevsimsel seyri olan serinin mevsimselliğini ortadan kaldırmak amacı ile D-kez fark alma işlemi uygulandığında durağan w_t serisi ise $w_t = (1\text{-}B^s)^D y_t$ şeklinde gösterilmektedir [14].

Zaman serisinin belirli bir noktada aldığı değer, y_t, serinin p adet geçmiş değeri ve bir şok ile tanımlanan bir fonksiyona sahip ise, serinin altında yatan veri yaratma süreci p'inci mertebeden otoregresif süreç, AR(p), olarak tanımlanmaktadır [15]. Bir zaman serisinin bugünkü değeri, mevcut ve q adet geçmiş dönemdeki rassal şokun ağırlıklı toplamı ise süreç q mertebesinde hareketli ortalama, MA(q), sürecidir [4]. Gerçek hayatta karşılaşılan pek çok durumda ise her iki süreci de içeren karma otoregresif hareketli ortalama modeli, ARMA(p,q), ile çalışmak söz konusu olmaktadır.

Zaman serilerinde gözlenen periyodik ya da mevsimsel hareketler, birbirini izleyen ölçüm dönemlerinde aynı ölçüm zamanları arasında gözlenen benzer yükselme ya da düşme hareketleri olarak tanımlanmaktadır. Bu tür serilerde mevsimsel ilişkinin yanında ardışık gözlemler arasında da ilişki ortaya çıkabilmektedir. ARIMA(p,d,q)(P,D,Q)$_s$ olarak gösterilen çarpımsal mevsimsel ARIMA modelleri ile mevsim içerisindeki ve mevsimler arasındaki bağımlılık yapısı birlikte modellenmektedir [4]:

$$(1-B)^d (1-B^s)^D y_t = \frac{(1+\theta_1 B+\ldots+\theta_q B^q)(1+\Theta_1 B^s+\ldots+\Theta_Q B^{Qs})}{(1-\varphi_1 B-\ldots-\theta_p B^p)(1-\Phi_1 B^s-\ldots-\Phi_p B^{Ps})} a_t$$

Burada, θ mevsimsel olmayan MA parametrelerini, q mevsimsel olmayan MA parametre sayısını, φ mevsimsel olmayan AR parametrelerini, p mevsimsel olmayan AR parametre sayısını, Φ mevsimsel AR parametrelerini, P mevsimsel AR parametre sayısını, Θ mevsimsel MA parametrelerini ve Q mevsimsel MA parametre sayısını simgelemektedir.

Zaman serisini oluşturan sürecin olasılık dağılımını belirlemek ve kesin bir tanımlamasını yapmak için otokorelasyon fonksiyonu (OKF) ve kısmi otokorelasyon fonksiyonu (KOKF) kullanılmaktadır [14].

Veri Setinin Hazırlanması ve İstatistiksel Analizler

Klasik zaman serisi tertibinden farklı olarak bu çalışmada kontrol günü süt verimlerini içeren veri seti Macciotta ve ark.[9,11] tarafından uygulanan bir yaklaşımla hazırlanmıştır *(Tablo 1)*. Macciotta ve ark.[9], indeks değişkeninin, içerdiği anlam ne olursa olsun çıktıyı, yani Y değerlerini, sıralamada kullanılan bir araç olduğunu ve bu nedenle herhangi bir zaman kavramına bağlı olmak zorunda olmadığını belirtmişlerdir. Veri setinin izlenecek yönteme uygun olarak düzenlenmesi amacıyla öncelikle

hayvanlar rastgele sıralanmış, ardından laktasyon kayıtları her bir hayvan içerisinde sıraya konmuştur. Toplamda 10.700 laktasyon kaydına karşılık gelen gözlemlere 1'den 10.700'e kadar bir indeks değeri atanmıştır. Örneğin 26 numaralı indeks değeri 3. sıradaki hayvanın 6. laktasyon kaydını belirtmektedir.

Veri seti 535 hayvanın 5350 kontrol günü süt verimi kaydını içeren iki ayrı kısma ayrılmıştır. Model parametreleri SAS programının ARIMA prosedüründe ilk 535 hayvana ait kayıtlar için en çok olabilirlik yöntemi ile tahmin edilmiştir [16]. Modelin öngörü başarısı model tahmin basamağında elde edilen parametre değerleri ve ikinci gruptaki 535 hayvanın kayıtları kullanılarak değerlendirilmiştir. En isabetli öngörüleri sağlayan minimum kayıt sayısını belirlemek amacı ile, her bir hayvanın bilindiği varsayılan 2, 3, 4, 5, 6, 7 ve 8 kaydı kullanılarak geriye kalan, sırasıyla 8, 7, 6, 5, 4, 3 ve 2 kaydını öngörmek şeklinde bir algoritma işletilmiştir. Gerçek değerler ile öngörü değerleri arasındaki korelasyonlar SAS programının CORR prosedürü kullanılarak elde edilmiştir.

Zaman serisi analizi daha çok ekonomi alanında uygulama olanağı bulduğundan kullanılan terminoloji de bu alana özgüdür. Çalışmada, ele alınan veri seti dikkate alındığında; zaman-indeks, yıl-her bir hayvana ait laktasyon dönemi, ay-kontrol günü ve mevsim-laktasyonun pik verim dönemi ya da artış veya azalış seyri izlediği dönemler vb. kavramlarının karşılığı olarak yorumlanmalıdır.

Tablo 1. *Çalışmada kullanılan veri setinin yapısı*
Table 1. *Structure of the data used in the study*

Sığır	Kontrol Günü	Verim	İndeks
1	1	*	1
1	2	*	2
1	3	*	3
1	4	*	4
1	5	*	5
.			.
.			.
1070	7	*	10697
1070	8	*	10698
1070	9	*	10699
1070	10	*	10700

** hayvanın ilgili kontrol günündeki toplam süt verimi (kg)*

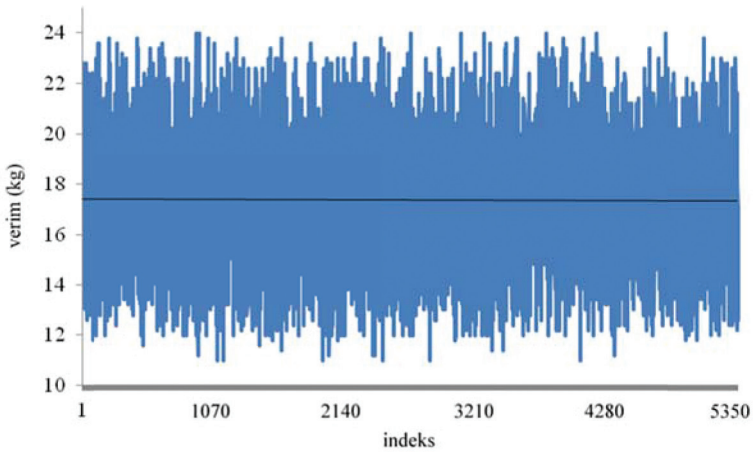
BULGULAR

Tablo 2'de model kurma basamağında kullanılan veri setine ilişkin bazı tanımlayıcı istatistikler sunulmuştur. Beklendiği gibi en yüksek ortalama süt verimi 2. kontrol gününde saptanmış olup ortalamalar tipik laktasyon eğrisine benzer bir seyir izlemektedir. *Şekil 1* kontrol günü süt verimlerinin zaman serisi grafiğini göstermektedir. Şekilden de görüleceği gibi süt verimi belirli bir bandın dışına çıkmamakta ve alçalma ya da yükselme yönünde herhangi bir eğilim göstermemektedir.

Şekil 2'de sunulan otokorelasyon katsayılarının 0,10, 20,…, gecikmelerinde zirve yapması periyodik bileşenin varlığına işaret etmektedir. OKF(1), OKF(10) ve OKF(20) değerleri yüksek derecede bir ilişkinin varlığını ortaya koymaktadır. Benzer şekilde yüksek korelasyonlar OKF(5), OKF(15) ve OKF(25)'te de saptanmıştır. *Şekil 2*'den korelasyonların güven sınırları dışına çıktığı ve bu nedenle de

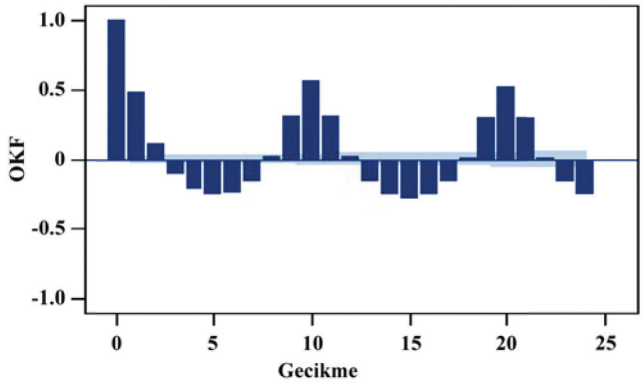
Tablo 2. *Veri setine ilişkin bazı tanımlayıcı istatistikler*
Table 2. *Some descriptive statistics of the data*

Kontrol Günü	N	Ortalama	Std. Sapma
1	535	19.18	2.118
2	535	20.30	1.878
3	535	19.56	1.840
4	535	18.52	1.731
5	535	17.63	1.787
6	535	16.89	1.790
7	535	16.51	1.902
8	535	15.94	1.916
9	535	15.15	1.809
10	535	14.06	1.384



Şekil 1. Süt verimlerinin zaman serisi grafiği

Fig 1. Time sequence plot of milk yield data



Şekil 2. Orjinal verilere ait OKF grafiği
Fig 2. ACF plot of original data

Tablo 3. *Parametre tahminleri*
Table 3. *Parameter estimates*

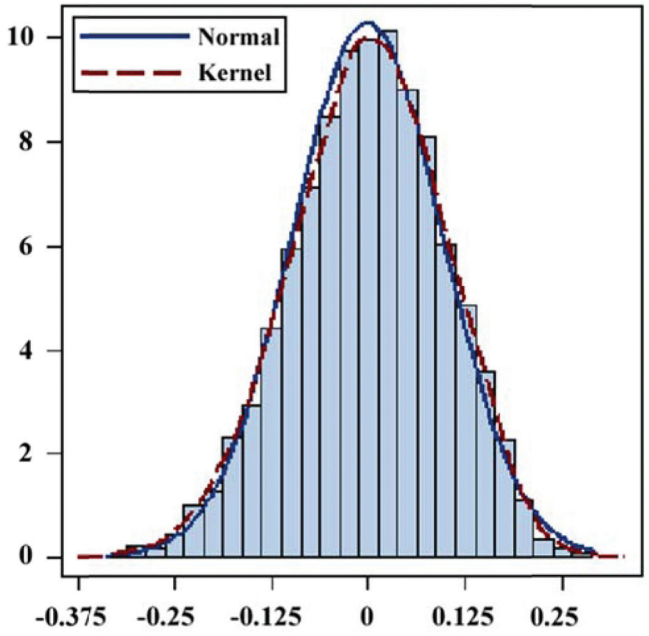
Parametre	Tahmin	Standart Hata	t-Değeri	P	Gecikme
SMA	0.99129	0.0022861	433.61	<.0001	10
AR1	0.36889	0.01362	27.08	<.0001	1
AR2	0.06934	0.01361	5.09	<.0001	2
SAR	0.08352	0.01377	6.07	<.0001	10

SMA *(Seasonal MA): Mevsimsel MA, AR1-2 (Autoregressive): Otoregresif parametreler,* ***SAR*** *(Seasonal AR): Mevsimsel AR parametresi*

istatistiksel olarak önemli oldukları görülmektedir. Bu durum veri setinin oluşturulma şekli düşünüldüğünde beklenen bir sonuçtur. Her ne kadar çalışmanın materyalini oluşturan kontrol günü süt verim kayıtları rastgele sıralanmış olsa da teorik olarak her bir laktasyonda aynı dönemde pik verimine ulaşıldığı bilinmektedir. Periyodik gecikmelerde ortaya çıkan bu yüksek otokorelasyonlar ve bu otokorelasyonların aynı seviyede varlığını devam ettirmesi, güçlü bir mevsimsellik göstergesi olup veri setine mevsimsel fark işlemi uygulanması gerektiğini göstermektedir.

Mevsimsel farkı alınmış seriye ait OKF incelenmiş ve gecikme uzunluğu arttıkça OKF'nun hızla azalıp sıfırı kestiği, bununla birlikte orjinal seriye ilişkin OKF'nda 10 ve 10'un katlarında gözlenen periyodik davranışın giderildiği belirlenmiştir. Buna karşın 1. ve 10. gecikmelerdeki otokorelasyonların model oluşturulurken dikkate alınması gerektiği saptanmıştır. Bu nedenle MA sürecinin mevsimsel olmayan ve mevsimsel olan kısımlarını simgeleyen, q ve Q mertebeleri, 1 olarak belirlenmiştir. Mevsimsel farkı alınmış seriye ait KOKF'nun sıfırı ikinci gecikmede kestiği görüldüğünden mevsimsel olmayan AR mertebesinin 2 olduğu düşünülmüştür. Ayrıca ilk iki mevsimsel gecikmede önemli olan kısmi otokorelasyonların da dikkate alınması gerektiği saptanmıştır.

Analizin sonraki aşamasında, aday model olarak belirlenen çarpımsal-mevsimsel ARIMA modeline ait parametreler tahmin edilmiş ve mevsimsel AR mertebesinin 2 olması durumunda ilgili parametrenin anlamsız olduğu (P>0.05) görüldüğünden analiz dışında tutulmasına karar



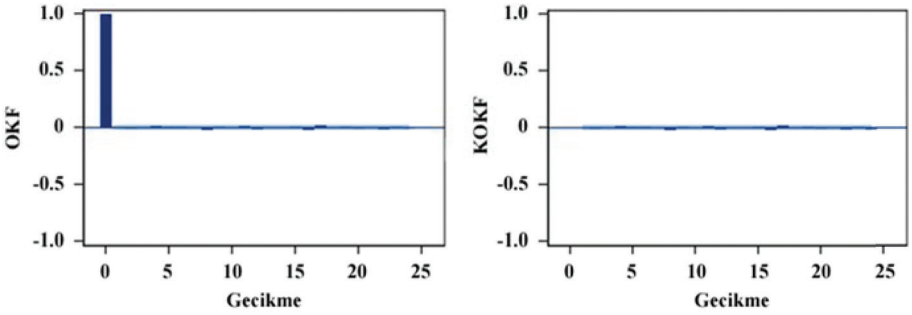
Şekil 3. Artıkların histogramı ile normal ve kernel yoğunlukları
Fig 3. Histogram with normal and kernel density plots of the residuals

verilmiştir. Serinin D=1 olmak üzere 1 kez mevsimsel farkı alındığından nihai model ARIMA(2,0,0)(1,1,1)$_{10}$ modeli olarak belirlenmiş ve bu modelin en çok benzerlik yöntemi ile tahmin edilen parametre değerleri *Tablo 3*'te sunulmuştur. Söz konusu model parametrelerinin tümünün istatistiksel olarak önemli olduğu belirlenmiştir (P<0.05). Bununla birlikte, model artıklarına ilişkin ilk 48 gecikme için hesaplanmış olan ki-kare değerleri için P>0.05 olduğu saptanmış ve modele herhangi bir AR ya da MA terimi eklenmesine gerek olmadığı sonucuna ulaşılmıştır.

Model artıklarının *Şekil 3*'te yer alan dağılımı incelendiğinde artıkların yaklaşık sıfır ortalama etrafında normal dağılışa sahip olduğu görülmektedir. Artıklara ilişkin OKF ve KOKF'karı da modelin veri setini tanımlamada uygun olduğunu göstermektedir *(Şekil 4)*. Model artıklarına ilişkin otokorelasyon ve kısmi otokorelasyonların tümü güven sınırları içerisinde kaldığından, bir başka ifade ile model artıkları arasında istatistiksel açıdan önemli korelasyonlar olmadığından modelin doğru bir şekilde tanımlandığı sonucuna ulaşılmıştır. Model, tahmin edilen parametre değerleri ile aşağıdaki gibi gösterilmektedir:

$$\left(1-B^{10}\right)^{1} y_t = \frac{\left(1-0{,}99129B^{10}\right)}{\left(1-0{,}36889B-0{,}06934B^{2}\right)\left(1-0{,}08352B^{10}\right)} a_t$$

Çalışmada gerçek değerler ile modelden tahmin edilen değerler arasındaki korelasyon 0.90 olarak bulunmuştur. Mevcut gözlem sayısı arttıkça model yardımı ile öngörüsü yapılan değerler ile serinin gerçek değerleri arasındaki korelasyonların artma eğiliminde olduğu görülmektedir *(Tablo 4)*. Bununla birlikte, öngörüsü yapılacak kontrol günü (ay) son kontrol kaydının alındığı tarihten uzaklaştıkça sözü



Şekil 4. Artıklara ait OKF ve KOKF grafikleri

Fig 4. ACF and PACF plots of the residuals

Tablo 4. *Mevcut gözlem değerleri ile öngörü değerleri arasındaki korelasyonlar*
Table 4. *Correlations among the actual and forecasted values*

Öngörüsü Yapılan Kontrol Günü (ay)	Mevcut Gözlem Sayısı						
	2	3	4	5	6	7	8
3	0.74						
4	0.74	0.82					
5	0.74	0.79	0.81				
6	0.75	0.78	0.80	0.85			
7	0.76	0.78	0.80	0.85	0.87		
8	0.76	0.78	0.79	0.83	0.87	0.87	
9	0.75	0.77	0.78	0.81	0.83	0.84	0.87
10	0.74	0.75	0.76	0.79	0.80	0.80	0.80

Tüm korelasyonlar için P<0.001'dir

edilen değerler arasındaki korelasyonlar azalmaktadır. Çalışmanın bulguları 6 kaydın mevcut olduğu durumun serinin gelecekteki değerlerinin öngörüsü için yeterli seviyede bilgi içerdiğini göstermektedir.

TARTIŞMA ve SONUÇ

Macciotta ve ark.[9], Sarda koyunlarına ait mevsimsellikten arındırılmış kontrol günü süt verimlerini modelledikleri çalışmalarında çarpımsal mevsimsel ARIMA(2,0,0)$(1,0,1)_7$ modelini uygun model olarak belirlemişlerdir. Bu model çalışmamızda belirlenen model ile örtüşmektedir. Bunun yanında Macciotta ve ark.[11], Simmental ırkı inekler üzerinde yürüttükleri çalışmalarında çarpımsal mevsimsel ARIMA$(1,0,1)(1,0,1)_8$ modelini, Deluyker ve ark.[2] ise Holstein ırkı ineklere ait kontrol günü süt verim kayıtlarını modelledikleri çalışmalarında ARIMA(0,1,1) modelini uygun model olarak belirlemişlerdir.

Macciotta ve ark.[11], çalışmalarında süt verimi için 2 adet mevcut kontrol günü kaydını 3. kayıt gününe ait verimleri öngörmede kullanmışlar ve gerçek değerler ile öngörü değerleri arasındaki korelasyonları 0.85 olarak saptamışlardır. Aynı şekilde 3, 4, 5 ve 6 kaydın olduğu durumlar için de bir sonraki kayda ilişkin öngörüler ile gerçek değerler arasında tüm durumlar için 0.85'lik korelasyonlar saptamışlardır. Buna karşın çalışmamızda 6 gözlemin mevcut olduğu durumda 7 ve 8. gözlem değerlerine ilişkin gerçek değerler ve öngörü değerleri arasında her iki kontrol günü için 0.87'lik korelasyonlar saptanmıştır. Sözü edilen çalışmada korelasyonlar 0.85-0.44 arasında değişim göstermekte iken çalışmamızda tüm durumlar için korelasyonlar 0.87-0.74 aralığındadır.

Serilerin değerleri arasında az da olsa gözlenen farklılıkların daha homojen hayvan grupları ile çalışılması durumunda daha da azalması beklenmektedir. Ayrıca model tahmini yapılan hayvan gurubu ile modelin öngörü başarısının değerlendirildiği hayvan gurubunun da benzer koşullarda yetiştirilen ve genetik potansiyel olarak da birbirinin benzeri hayvanlar olmaları daha isabetli öngörüler yapılmasına olanak sağlayacaktır. Bu açıdan bakıldığında çalışmada ele alınan yöntemin sürü ya da işletme bazında kullanılması uygun olacaktır. Özellikle otomatik kontrol sistemlerinin kullanıldığı işletmelerde bireyler için tahmin edilen değerlerden büyük sapmalar görülmesi durumunda bu hataların değerlendirilmesi gerekmektedir.

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19 (4): 665-668, 2013
DOI: 10.9775/kvfd.2013.8629

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

Molecular Characteristics of *Staphylococcus aureus* Isolates from Buffaloes Milk [1]

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Makale Kodu (Article Code): KVFD-2013-8629

Summary

The aim of the present study was to analyse the *aroA* and X region of *spa* genes on *Staphylococcus aureus* isolates collected from milks of native buffaloes in north-west of Iran. For this purpose, seventy five *S. aureus* isolates were examined by PCR-RFLP. Amplification of *AroA* gene revealed a single amplicon with a size of approximately 1,153 bp, but restriction profile analysis of this amplicon yielded three different genotypes including genotype A (9.3 %), B (88%) and N (2.7%). Amplification of X region of the *spa* gene showed an amplicon of 1110; 1130; 1160; 1190 bp in 19, 15, 12 and 29 isolates, respectively. *Hhal* digestion of this amplicons demonstrated that the isolates having the same PCR band size were in the same PCR-RFLP patterns and there was not variation into isolates belonged to the same PCR amplicon.

Keywords: *Staphylococcus aureus, aroA gene, spa gene, PCR-RFLP*

Manda Sütünden Izole Edilen *Staphylococcus aureus* Türlerinin Moleküler Karakterizasyonu

Özet

Bu çalışmanın amacı, İran'ın Kuzey Batısı'nda yerli mandaların sütlerinden izole edilen *Staphylococcus aureus* izolatlarında *spa* genlerinin *aroA* ve X bölgelerini analiz etmektir. Bu amaçla, yetmiş beş *S. aureus* izolatı PCR-RFLP yöntemi ile incelendi. *AroA* gen amplifikasyonu, yaklaşık olarak 1,153 bp büyüklüğünde olan tek bir amplikon oluşturmasına rağmen bu amplikon restriksiyon profil analizi ile genotip A (9.3%), B (88%) ve N (2.7%) olmak üzere üç farklı genotipi oluşturduğu belirlendi. *spa* geninin X bölgesinin amplifikasyonu 19, 15, 12 ve 29 izolatta sırasıyla 1110, 1130, 1160 ve 1190 bp büyüklüğünde fragman ürettiği gözlemlendi. Bu amplikonun *Hhal* enzimi ile kesimi, aynı PCR bant büyüklüğüne sahip izolatların aynı PCR-RFLP profillerinde olduğunu ve aynı PCR amplikonuna ait izolatlar içinde varyasyon olmadığını gösterdi.

Anahtar sözcükler: *Staphylococcus aureus, aroA gen, spa geni, PCR-RFLP*

INTRODUCTION

Buffaloes account for about 5 percent of the milk production in the world but this rate is greater in the Near East and Asia. Buffaloe milk is also considered as an important milk source in Iran [1]. Clinical and subclinical mastitis are associated in decrease of milk production [2].

Among pathogenic bacteria wich have been isolated as etiological agents of mastitis in ruminants, *Staphylococcus aureus* has been mentioned as the main one. The prevalence of subclinical and clinical bovine, caprine and ovine mastitis caused by *S. aureus* ranges from 5 to 50%, 5.6 to 17%, 0.22 to 11% in different countries, respectively [3]. However, only a few studies on staphylococcal mastitis concerning buffalo infections have been published. Staphylococcal mastitis is important in ruminants of Iran and mastitis caused by this organism is a major cause of economical losses in the Iranian dairy industry [4]. *S. aureus* is also one of the main pathogens isolated from buffaloes in this country [5]. Some studies confirm this subject in other countries in Asia [6,7].

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Different molecular methods such as PCR - restriction fragment length polymorphism (PCR-RFLP) analysis of the virulence genes [8-10], multilocus sequence typing [11], pulsed -field gel electrophoresis [12-14], multilocus enzyme electrophoresis [15,16] have been used molecular characterization of the *S. aureus* isolates. Molecular characterization of *S. aureus* isolated from bovine milk samples was studied by many researchers [17]. However, a few papers studied molecular profile of *S. aureus* isolated from buffaloes in worldwide [3,6].

In contrast of some molecular studies on bovine milk isolates of *S. aureus* in Iran [4,18], there are not similar studies in buffalo isolates. The aim of the present study was to characterize analysis of the *aroA* and *spa* gene of *S. aureus* isolates collected from native buffaloes in villages around of Tabriz city, Capital of East Azerbaijan province in Iran.

MATERIAL and METHODS

Samples

Seventy five *S. aureus* isolates were isolated from milk of buffaloes in Tabriz, Northwest of Iran from the microbiology laboratory of Islamic Azad University, Tabriz branch. All isolates had been confirmed by standard biochemical tests [4] and stored in brain heart infusion (BHI) with 30% glycerol at -70°C, previously.

DNA Extraction

A colony of Brain heart infusion (BHI) agar was transferred to a clean microtube and it was added 500 µl lysis buffer (pH 8) including (containing 5 mol NaCl, 1 mol Tris-base, 0.5 mol EDTA and CTAB 2%). Then incubated in 60-65°C for 2 h and centrifuged at 12.000 x g for 5 min. The pellet was resuspended in $CHCl_3$-isoamyl alcohol (24:1), and centrifuged for five min at 12.000 x g. Pellet was incubate with RNAase in 37°C for 30 min, then was resuspended in cold isopropanol and transfer to -20°C for 15 min. In the next step, ethanol 70% was added to supernatant and centrifuged for one min at 12.000 x g. Finally, 50 µl TE-buffer was add to pellet and stored as DNA template.

Confirmation Isolates by PCR

All isolates were confirmed as *S. aureus* by the detection of *nuc* gene by PCR described by Brakstad et al.[19].

Molecular Typing by aroA Gene Restriction Profile Analysis

The *aroA* gene was amplified by PCR using the special primers [8]. PCR products were digested with 50 U *Taq*I (Fermentas) at 65°C for 3 h. All digested fragments were assessed by electrophoretic separation in 2% (w/v) agarose gels.

Molecular Typing by X Region of the spa Gene Restriction Profile Analysis

Amplification of the repeat region of the *S. aureus* protein A gene (*spa*) was performed by using a specific primer set as described previously [18,19]. PCR products were digested with 50 U *Hha*I (Fermentas) at 37°C for 3 h. All digested fragments were assessed by electrophoretic separation in 2% (w/v) agarose gels.

RESULTS

According to amplification of the *nuc* gene, all 75 isolates confirmed as *S. aureus*.

AroA Gene Restriction Profile Analysis

AroA gene amplification revealed a single amplicon with an expected size of approximately 1.153 bp, but restriction profile analysis of mentioned gene by U *Taq*I enzyme indicated three differentiated profile. Sixty-six of the 75 isolates (88%) shows a profile known as genotype B and 7 isolates (9.3%) as genotype A. Two isolates (2.7%) have 4 detectable bands and were considered a different genotype showed in this study *(Fig. 1)*.

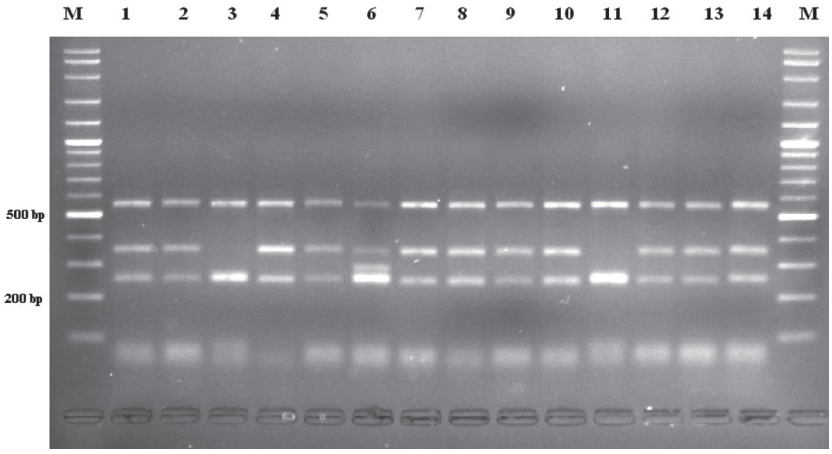

Fig 1. PCR-amplified *aro*A gene digested with the *Taq*I. **Lane M:** DNA marker, **Lanes 1,2,4,5,7** to **10,12** to**14** Genotype B, **Lanes 3,11** Genotype A, **Lane 6** new genotype in this study (N)

Şekil 1. PCR ile amplifiye edilen *aro*A gen amplikonunun *Taq*I ile kesimi. **Sıra M:** DNA marker, **Sıralar 1,2,4,5,7** 'den **10,12**'den**14**'e Genotip B, **Sıralar 3,11** Genotip A, **Sıra 6** bu çalışmadaki yeni genotip (N)

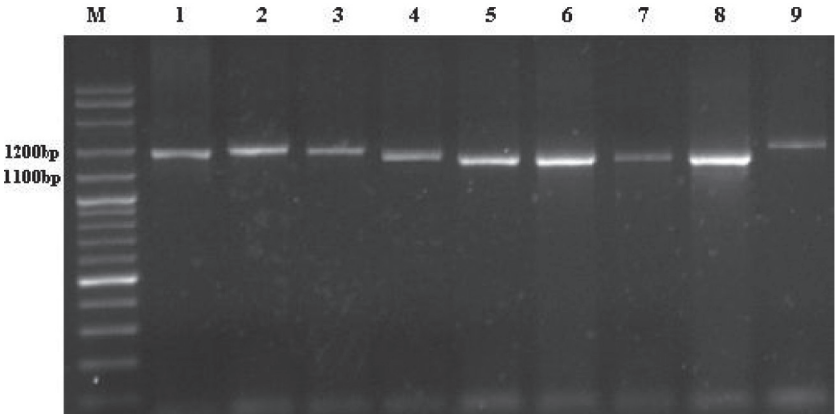

Fig 2. Agarose gel electrophoresis of PCR products obtained from amplification of the *spa* gene. **Lane M:** DNA marker, **Lanes 1,2,3:** 1160, **Lanes 4,7:** 1130, **Lanes 5,6,8:** 1110 and **Lane 9:** 1190 bp

Şekil 2. *spa* geninin amplifikasyonu sonuzu elde edilen PCR ürünlerinin agaroz jel elektroforezi. **Sıra M:** DNA marker, **Sıralar 1,2,3:** 1160, **Sıralar 4,7:** 1130, **Sıralar 5,6,8:** 1110 ve **Sıra 9:** 1190 bp

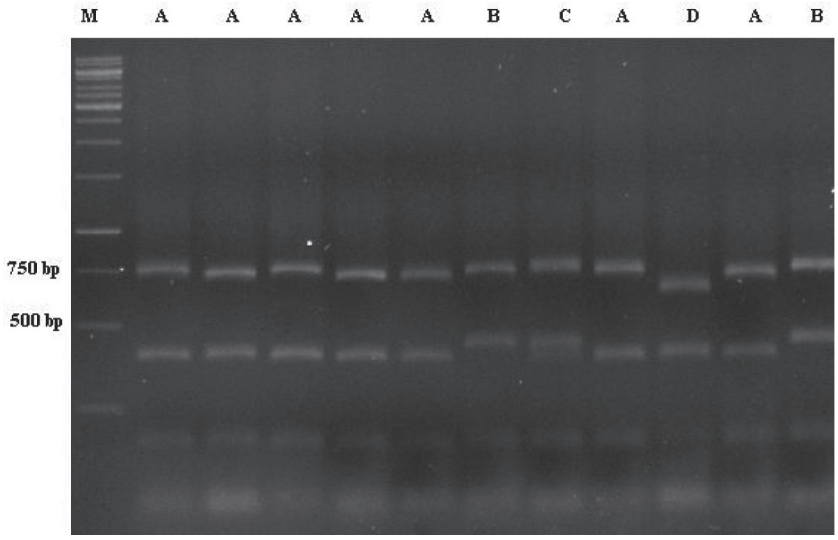

Fig 3. PCR-amplified *spa* gene digested with the *Hha*I. **Lane M:** DNA marker, **Lane A:** pattern produces by 1190 bp band size, **Lane B:** pattern produces by 1130 bp band size, **Lane C:** pattern produces by 1160 bp band size and **Lane D:** pattern produces by 1110 bp band size

Şekil 3. PCR ile amplifiye edilen *spa* gen amplikonunun *Hha*I ile kesimi. **Sıra M:** DNA marker, **Sıra A:** 1190 bp bant büyüklüğü üreten profil, **Sıra B:** 1130 bp bant büyüklüğü üreten profil, **Sıra C:** 1160 bp bant büyüklüğü üreten profil ve **Sıra D:** 1110 bp bant büyüklüğü üreten profil

Analysis of x Region of the spa Gene

All of the 75 *S. aureus* isolates were PCR positive for X region of spa gene. Amplification of the spa gene showed an amplicon of 1110; 1130; 1160; 1190 bp in 19, 15, 12 and 29 isolates, respectively *(Fig. 2)*.

PCR-RFLP of X region of the spa gene demonstrated 4 profiles. Isolates with same PCR band size were same into the PCR-RFLP patterns and there was not variation into isolates belonged to same PCR amplicon *(Fig. 3)*.

DISCUSSION

This paper is the first report describing molecular characteristic of *S. aureus* isolated from buffalo's milk. Studies of the molecular epidemiology of *S. aureus* collected from cows have been already published in the literature worldwide [17] and in Iran [4,18], but there is a few published studies of *S. aureus* isolated from buffalo [3,22].

Similar to *S. aureus* isolated from other source amplicon size of *aroA* gene is a 1,153-bp fragment and this confirmed *aroA* gene is specific for *S. aureus*, hence it could be used as a powerful tool for the identification of *S. aureus* isolates in buffaloes, too.

AroA gene restriction profile studies showed none of *S. aureus* isolated from cattle were genotype C or D in previous [4,8,23]. Our study confirms this again in buffaloes once. It may suggest that genotype C or D have link to isolates from human origins. Marcos et al.[8] reported these two genotype especially D (44%) from human isolates. In animal, instead of genotype C or D, there are different RFLP patterns with various bands named N reported up to 50% in *S. aureus* with bovine origin [4,23]. In this study, we reported 2.7% these ones in buffalo's isolates. High prevalence of genotype B in animal origin isolates in the some studies, included present study, showed this genotype may has been link to animal source [4,8]. In contrast, Marcos et al.[8] reported low prevalence of genotype B in human isolates.

Koreen, et al.[24] suggested a new typing method for *S. aureus* based on sequencing of X region of staphylococcal protein A *(spa)* gene instead of expensive, time-consuming methods such as pulsed-field gel electrophoresis (PFGE), multilocus enzyme electrophoresis (MLEE), multilocus sequence typing (MLST). The X region of *spa* gene is very highly polymorphism that reflex epidemiological variation

among different *S. aureus* isolates. As a substitution for Koreen, et al.[24] method, Wichelhaus et al.[20] designed X region of *spa* gene based on PCR-RFLP, described previously. We used the same method in this study.

The ampilcon size of X region of *spa* gene in different *S. aureus* has varieties because of polymorphism of this region of *spa* gene. The ampilcon size of X region of *spa* gene in this study has not report in previous papers and it may be belonged to new *spa* type. However, the PCR-RFLP of the mentioned region of each different amplicon size have not produced variation in internal of each groups and this suggest sequence based *spa* typing for determination of new spa type of *S. aureus* from buffaloes origin. On the other hand, *spa* gene variation may be related with the geographical and host factors similar to that of *aroA* gene.

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19 (4): 669-672, 2013
DOI: 10.9775/kvfd.2013.8635

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

Comparison of Growth Curves of Broiler under Different Stocking Densities by Gompertz Model

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Makale Kodu (Article Code): KVFD-2013-8635

Summary

The knowledge of the growth curve in poultry science is very useful for describing growth kinetics and setting commercial management procedures. The objective of this research was to fit the Gompertz growth curve from hatching weights to 42 d-old weights of broilers in 3 stocking density groups. A total of 284 Ross 308 broilers randomly divided into 3 stocking density groups (9, 13 and 17 birds/m^2) in this experiment. All birds were weighted weekly. The asymptotic weight (A) of broiler chickens for 9, 13 and 17 bird/m^2 stocking density groups were 4198.46, 3807.45 and 3999.92 g, respectively (P<0.05). The growth rates (K) of broiler chickens for 9, 13 and 17 bird/m^2 stocking density groups were 0.055, 0.058 and 0.052, respectively (P<0.01). The coefficient of determination for all stocking density groups were 0.998, 0.997 and 0.996, respectively (P<0.05). Moreover, the mean square error (MSE) value was lowest for 9 bird/m^2 group (P<0.05). The current study suggested that stocking density of 9 bird/m^2 was better for the broiler growth of the different stocking densities on the base of mature live weight (A), coefficient of determination (R^2) and mean square error (MSE).

Keywords: *Growth Curves, Gompertz model, Stocking Densities, Broiler*

Farklı Yerleşim Sıklıklarındaki Etlik Piliçlerin Büyüme Eğrilerinin Gompertz Modeli İle Karşılaştırılması

Özet

Kanatlı biliminde büyüme eğrisinin bilinmesi büyümenin tarifinde ve yetiştirme tekniklerinin uygulanmasında çok önemlidir. Bu araştırmanın amacı kuluçkadan 42. gün ağırlığına kadar üç yerleşim sıklığında yetiştirilen etlik piliçlerden elde edilen canlı ağırlıkları, Gompertz büyüme eğrisi modeli ile tahmin etmektir. Denemede 3 yerleşim sıklığında (9, 13 ve 17 etlik piliç/m^2) tesadüfen dağıtılan toplam 284 adet Ross 308 etlik piliç kullanılmıştır. Bütün hayvanlar haftalık olarak tartılmıştır. 9, 13 ve 17 etlik piliç/m^2 yerleşim sıklığında etlik piliçlerin, asimptotik ağırlıkları (A) sırasıyla; 4198.46, 3807.45 ve 3999.92 g olarak (P<0.05); büyüme oranı (K) sırasıyla 0.055, 0.058 ve 0.052 olarak (P<0.01) tahmin edilmiştir. Tüm yerleşim sıklıklarında belirtme katsayısı değeri (R^2) sırasıyla 0.998, 997 ve 0.996 olarak bulunmuştur (P<0.05). Ek olarak hata kareler ortalaması (MSE) yerleşim sıklığı 9/m^2 olan grupta en düşüktür (P<0.05). Bu çalışmanın sonucunda tahmini ergin canlı ağırlığa (A), belirtme katsayısı değeri (R2) ve hata kareler ortalamasına (MSE) göre 9 etlik piliç/m^2 olan yerleşim sıklığında etlik piliçlerin büyümelerinin daha iyi olduğu söylenebilir.

Anahtar sözcükler: *Büyüme eğrisi, Gompertz model, Yerleşim sıklığı, Etlik piliç*

INTRODUCTION

Poultry industries face various decisions in the production cycle that include nutrient and mineral supply to birds, cost and type of feed, status of bird health, welfare and environmental issues that affect the profitability of operation [1]. The stocking densities in broilers vary widely by countries, husbandry systems and final body weights [2]. Although the use of high stocking densities can diminish individual growth [3-6], increase in total production meat

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per unit of floor surface, which results in higher profit. Thus, because of the economic benefits, producers have reluctant to decrease stocking densities [7,8].

Although there have been various definitions of growth given by different biologists, for the purposes of quantitative analysis, growth was defined as the process of an animal gaining weight with time until it reaches maturity [9]. Growth functions have been shown to be valuable tools for analyzing growth responses to genetic selection, environmental change [10] and estimation of daily nutrient requirements for growth [11]. An appropriate growth functions provide a good way of summarizing the information contained in such data into a few parameters that can be interpreted biologically and physically [11,12]. Thus, the aim of the present study was to investigate the effect of stocking density on the growth curve of broiler chickens according to Gompertz growth model.

MATERIAL and METHODS

A total of 284 chicks (Ross 308) were selected from a commercial flock. Chickens were wing-tagged at 1 d of age and individual body weights were recorded weekly at the end of each one-week period. The chicks were raised in deep litter system and were subjected to the same management, hygienic and climatic conditions. Chickens were randomly placed into the floor systems in 3 stocking density groups with 3 repetitions for each group. The three stocking densities were 9 (density 1, 66 birds), 13 (density 2, 97 birds), and 17 (density 3, 121 birds), birds per m^2 and were raised to 42 d of age. The space for density 1, density 2 and density 3 were 2.77 m^2; 2.76 m^2 and 2.82 m^2 respectively for per replicate.

Chickens were fed a 4-phase feeding program with starter fed to first 11 d (23% CP and 3.050 kcal/kg of ME), grower fed from 12 to 21 d (22% CP and 3.100 kcal/kg of ME), finisher fed from 22 to 35 d (20% CP and 3.200 kcal/kg of ME) and a withdrawal feed from 36 to 42 d (18% CP and 3.200 kcal/kg of ME). Diets were provided *ad libitum* and the birds had free access to water. Birds in all 3 groups were allocated with equal space of feeders and drinkers.

The birds were raised according to a typical commercial management program. The photoperiod was 24 h/day. Temperature started at 32.0°C and was gradually reduced 1°C degrees every day until 22.0°C was attained, after which temperature remained constant.

The widely used nonlinear growth model, Gompertz function was applied to estimate the mean age-live weight relationship [13]. The mathematical relation of this model was as follows:

$$W = A.\exp(-B.\exp^{(-Kt)})$$

Where;

(W) is the body weight (g) at age (t); (A) is the asymptotic weight or maximum growth response (g); (B) is the initial weight; (K) is the growth rate; (t) is the age in days.

Statistical Analysis

The Gompertz nonlinear regression model [14] was employed using the SAS statistical package program [15]. The results obtained with Gompertz models was evaluated in an Excel spreadsheet. Kolmogorov-Smirnov test was performed to check the assumption of normality for Gompertz models coefficients. However, the estimated coefficients were non homogeneously distributed ($P<0.05$). Therefore, the coefficient differences were determined by the Kruskal Wallis H test. Dunn's multiple comparison tests was also used to compare groups.

RESULTS

The estimated parameter values by using the Gompertz growth function for live weight of broiler chickens for different stocking densities are given in *Table 1* and growth curves in *Fig 1*.

Stocking density significantly affected the asymptotic weight ($P<0.05$), initial weight ($P<0.01$), growth rate ($P<0.01$), determination of coefficients ($P<0.05$) and the mean square error ($P<0.01$).

DISCUSSION

In the current study, predicted asymptotic weights (A) were 4198.455, 3807.447 and 3999.922 g for stocking densities of 9 bird/m^2, 13 bird/m^2 and 17 bird/m^2,

Table 1. *Effects of stocking density on growth parameters estimated by Gompertz model*
Tablo 1. *Gompertz modelle tahmin edilen büyüme parametreleri üzerine yerleşim sıklığının etkisi*

Stocking Density (Bird/m^2)	A	B	K	R^2	MSE
9	4198.46±442.73^a	4.83±0.342^a	0.055±0.006^b	0.998^a	2110^a
13	3807.45±436.49^b	4.78±0.387^a	0.058±0.007^a	0.997^{ab}	2846^b
17	3999.92±585.65^{ab}	4.56±0.387^b	0.052±0.008^c	0.996^b	2950^b
P	<0.019	<0.001	<0.001	<0.011	<0.012

(A) *The asymptotic or mature weight (g);* ***(B)*** *the initial weight;* ***(K)*** *the growth rate;* ***(R^2)*** *determination of coefficients;* ***(MSE)*** *the mean square error*

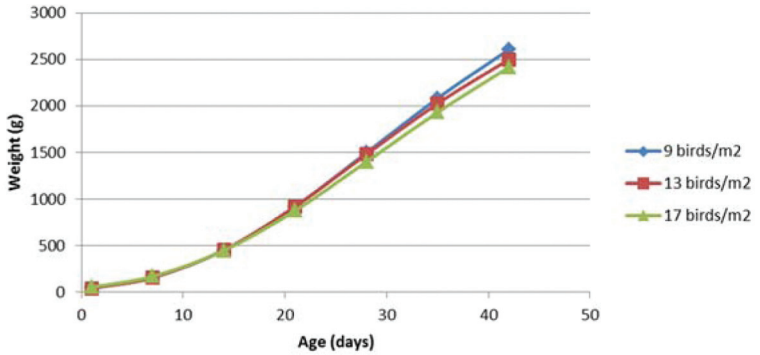

Fig 1. Estimated growth curves of Ross 308 chickens in different stocking density groups (1 to 42 d of age)

Şekil 1. Farklı yerleşim sıklığında 1-42. gün yaşa kadar Ross 308 etlik piliçlerin tahmin edilen büyüme eğrisi

respectively. Differences between stocking densities were observed in the Gompertz function parameters (P<0.05). Based on calculated A value, asymptotic weight for stocking densities is arranged in descending order as 9 bird/m^2, 17 bird/m^2 and 13 bird/m^2 . Although Santos et al.[16] found similar asymptotic weight in their study (4136-4320 g). However, other studies usually reported lower asymptotic weight (Roush et al.[17], 2936 g; Mignon-Grasteau et al.[18]; 3472 g, and Norris et al.[19]; 2691-2819 g). Overall 9 bird/m^2 group had higher estimates for asymptotic weight. Lower stocking density may contribute to animal welfare, reduce stress and consequently resulted in better performance.

The estimation of the initial weight (B) was lower in the stocking density of 17 bird/m^2, while stocking density of 9 bird/m^2 showed higher values. The growth rates (K) were ranged from 0.052 to 0.058, showing the early growth rates for chicks in stocking density of 13 bird/m^2 group compared with others. Different ranges for (K) values had been previously reported. Lower values were reported by Yakupoglu and Atil [20], Mignon- Grasteau et al.[18], and Norris et al.[19]. Similar values for the growth rate K were estimated by Marcato et al.[21]. However, Santos et al.[16] and Goliomytis et al.[22] reported higher (K) values when compared with the present study. The higher estimated growth rate values (K) suggested that broiler chickens in stocking density of 13 bird/m^2 group mature earlier than other chickens. It can be expected that individuals with lower K values would reach to the asymptotic weight (A) later than individuals with higher K values [23,24].

The determination of coefficients (R^2) of stocking density groups were quite high indicating excellent fit of the data. Differences were observed for R^2 values among the various density groups. All densities have considerably high R^2 values. R^2 values were highest for birds in 9 bird/m^2 stocking density group (R^2=0.998), intermediate for birds in 13 bird/m^2 stocking density group (R^2=0.997) and lowest for birds in 17 bird/m^2 stocking density group (R^2=0.996) (P<0.05). These findings are in agreement with the previous reports [11,16,17]. Grasteau et al.[18] have calculated the R^2 values of 0.980 through Gompertz models for broilers. In the current study, the mean square error (MSE) values ranged from 2110 to 2950. The stocking density 9 bird/m2 group ranked the highest due to the lowest MSE value. The MSE values in the current study suggested the stocking density 9 bird/m^2 depict better assessment for the growth of the stocking densities. Many authors [11,16,17] found similar results using the models outlined in this study.

Body weight and growth rates are economically important features for broiler productions. The present study provides information about the effect of stocking density on some growth function parameters in broilers. In this study, the stocking density of 9 bird/m^2 seemed to be appropriate for describing the broiler growth of the stocking densities according to the asymptotic or mature weight, determination of coefficients and the mean square error values.

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Kafkas Univ Vet Fak Derg
19 (4): 673-679, 2013
DOI: 10.9775/kvfd.2013.8644

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

Effects of Dietary Inclusion of Plant Extract Mixture and Copper into Layer Diets on Egg Yield and Quality, Yolk Cholesterol and Fatty Acid Composition [1]

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Makale Kodu (Article Code): KVFD-2013-8644

Summary

This study was conducted to determine the effects of plant extract mixture (PEM) inclusion at different levels and copper into diets of hens on performance, egg quality traits, yolk and serum cholesterol content and yolk fatty acid composition. A total of 192 Lohmann white layers, 38 wks of age, were used in this study. The experiment was carried out on a control and 7 treatments groups (basal diet plus 200 mg/kg copper ($CuSO_4.5H_2O$), 500 mg/kg PEM, 500 mg/kg PEM + 200 mg/kg copper, 750 mg/kg PEM, 750 mg/kg PEM + 200 mg/kg copper, 1000 mg/kg PEM + 200 mg/kg copper). PEM, copper and PEM plus copper combinations did not affect performance parameters. Increasing level of PEM increased quadratically shell stiffness and linearly shell thickness, but did not affect other egg traits. Treatments had no a significant effect on cholesterol and trigliserid concentrations of egg and serum. While supplemented PEM and copper did not affect fatty acid level of yolk, PEM plus copper combination increased oleic acid and total MUFA levels. Results obtained from present study showed that supplementation of a mixed herbal product containing *Origanum vulgare* (dried leaf), *Thymus vulgaris* (dried leaf), thyme oil, origanum oil, garlic oil, anise oil and fennel oil to diets of laying hens can be beneficial to improve egg quality traits especially such as shell stiffness and thickness.

Keywords: *Plant extract mixture, Copper, Performance, Egg quality, Cholesterol, Fatty acid composition of yolk*

Yumurtacı Tavuk Rasyonlarına Farklı Seviyelerde Bitki Ekstraktı ve Bakır İlavesinin Yumurta Verimi ve Kalitesi, Yumurta Sarısı Kolesterolu ve Yağ Asidi Kompozisyonuna Etkileri

Özet

Bu çalışma, yumurtacı tavuk rasyonlarına ilave edilen farklı seviyelerde bitki ekstrakt karışımının ve bakırın performans, yumurta kalitesi, yumurta sarısı ve serum kolesterol içeriği ile yumurta sarısı yağ asidi kompozisyonu üzerine etkisini belirlemek amacıyla yürütülmüştür. Araştırmada; 38 haftalık yaşta 192 adet Lohmann beyaz yumurtacı tavuk kullanılmıştır. Deneme, bir kontrol ve 7 muamele (200 mg/kg bakır, 500 mg/kg bitki ekstrakt karışımı, 500 mg/kg bitki ekstrakt karışımı + 200 mg/kg bakır, 750 mg/kg bitki ekstrakt karışımı, 750 mg/kg bitki ekstrakt karışımı + 200 mg/kg bakır, 1000 mg/kg bitki ekstrakt karışımı, 1000 mg/kg bitki ekstrakt karışımı + 200 mg/kg bakır) grubundan oluşmuştur. Bitki ekstrakt karışımı, bakır ve bakır ile bitki ekstrakt karışımının performans parametrelerini etkilemediği tespit edilmiştir. Bitki ekstrakt karışımının artan seviyesi kırılma mukavemetini ve kabuk kalınlığını artırmasına rağmen diğer yumurta kalite kriterlerin de önemli bir etkiye sahip olmadığı saptanmıştır. Muamele grupları arasında yumurta sarısı kolesterol ve trigliserid oranı ile serum kolesterol ve trigliserid oranları bakımından önemli bir farklılığın olmadığı tespit edilmiştir. Bitki ekstrakt karışımı ile bakır kombinasyonu yumurta sarısında oleik asit ve toplam MUFA seviyesini artırırken diğer uygulamaların yumurta sarısı yağ asidi oranı üzerine etkisinin olmadığı belirlenmiştir. Sonuç olarak; yumurtacı tavuk rasyonlarına kekik, kekik otu, kekik yağı, sarımsak yağı, anason ve rezene yağından oluşan bitki ekstrakt karışımı ilavesinin yumurta kalite kriterlerini özellikle kabuk kalınlığı ve kırılma mukavemetini artırmada faydalı olabileceği söylenebilir.

Anahtar sözcükler: *Bitki ekstrakt karışımı, Bakır, Performans, Yumurta kalitesi, Kolesterol, Yumurta sarısı yağ asit kompozisyonu*

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INTRODUCTION

It is known that a raised concentration of serum LDL-cholesterol increases the risk of coronary heart disease [1]. It is also known that the intake of egg cholesterol contributes to an elevation of the level of serum is considered relevant to find ways to produce eggs low cholesterol [2,3]. Nutritionist have been able to alter the diet of laying hens so that their eggs contain less cholesterol than the standard eggs. Results obtained from different studies have successfully demonstrated that natural products [4] and copper [5] could offer solutions to the health problem and cholesterol reduction. Natural products, essential extracts or oil derived from herbs and spices, exhibit hypocholesterolemic effect [6]. The hypocholesterolemic effect of essential oils has been reported for chickens [6-9]. It was reported that the pure components of essential oils inhibit hepatic 3-,hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity which is a key regulatory enzyme in cholesterol synthesis [10].

Copper (Cu) is an essential mineral which serves as a cofactor in many enzyme system[21]. Research evidences demonstrated that pharmacological levels of Cu (>250 mg/kg diet) caused changes in 17 beta-estradiol and enzymes involved in carbohydrate, lipid and amino acid metabolism in mature laying hens and suggested that copper supplements can affect reproductive physiology and lipid metabolism [11-13]. Kim et al.[14] indicated that reduced glutathione by addition of copper may play a major role in cholesterol homeostasis. Also, Iduwu et al.[15] reported that copper regulates cholesterol biosynthesis by reducing hepatic glutathione concentrations which is known to regulate cholesterol biosynthesis through the stimulation of the enzyme 3- hydroxy-3-methyl glutaryl coenzyme A (HMG-Co A) reductase, a key regulatory enzyme in cholesterol synthesis. Pesti and Bakalli [13] obtained a reduction in egg yolk cholesterol concentration and an increase in egg production when 125 and 250 mg/kg dietary levels of Cu were fed on White Leghorn hens. Skrivan et al.[16] suggested that copper supplementation at 200 mg /kg level in broiler diet had beneficial effects on growth performance. The maximum dietary tolerable level of copper for poultry was set at 300 mg kg^{-1} according to NRC [17]. Chiou et al.[18] reported that Layer diets containing 200 mg Cu per kg gave a positive response on the performance, liver function and enzyme activities but high dietary copper dosage (400, 600 or 800 mg kg^{-1}) gived a progressively negative response.

The objective of this study was to examine the dietary effect of the single or combination feeding of plant extracts mixture including *Origanum vulgare* (dried leaf), *Thymus vulgaris* (dried leaf), thyme oil, origanum oil, garlic oil, anise oil and fennel oil at different levels and copper on laying performance, egg quality, yolk fatty acid composition, yolk and serum cholesterol and trigliserid content.

MATERIAL and METHODS

This experiment was conducted by the researchers based on protocols by Atatürk University Ethical Commission Report (No: 24.04.2009/5/54).

A total of 192 Lohmann white layers, 38 weeks of age, were randomly allocated eight groups, each formed 6 replicate cages as subgroups, comprising of four hens. Experimental diets were (1) a standard commercial layer diet (Control), (2) a diet including 200 mg/kg copper ($CuSO_4.5H_2O$), (3) a diet including 500 mg/kg plant extracts mixture, (4) a diet including 500 mg/kg plant extracts mixture + 200 mg/kg copper, (5) a diet including 750 mg/kg plant extracts, (6) a diet including 750 mg/kg plant extracts mixture + 200 mg/kg copper, (7) a diet including 1.000 mg/kg plant extracts and (8) a diet including 1.000 mg/kg plant extracts mixture + 200 mg/kg copper for 14 weeks including adaptation period of 2 weeks. Diets were formulated according to NRC [17] and chemically analyzed by the AOAC [19] *(Table 1)*. A commercial blend of PEM Fitococci ® contained 463 g/kg active ingredients *, basically including *Origanum vulgare* (dried leaf), *Thymus vulgaris* (dried leaf), thyme oil, origanum oil, garlic oil, anise oil and fennel oil. PEM and Cupric sulfate ($CuSO_4.5H_2O$) as a copper source were provided from Farmavet International Inc., Kocaeli, 41400, Turkey. During the experimental period, hens were fed ad libitum once daily at 08:30 h and water was available all the times. Hens were subjected to a 16L:7D cycle.

Feed intake and egg production were recorded daily, egg weight was measured bi-weekly, and body weight was measured at the end of experimental period. Feed conversion ratio (FCR) was expressed as kilogram of feed consumed per kilogram of egg produced. To evaluate egg quality parameters 12 egg samples were randomly picked up from each experimental group every month. Before determination of egg quality parameters such as shape index, shell strength, shell thickness, albumen index, yolk index, yolk color (Yolk Colour Fan, the CIE standard colorimetric system, F. Hoffman-La Roche Ltd., Basel, Switzerland), and Haugh unit, samples were stored for 24 h in room temperature. They were assessed according to the method of Kaya et al.[20].

At the end of the experiment period, blood and egg samples were taken from the each replicate in order to determine the ratio of triglyceride and cholesterol. Triglyceride and cholesterol rations of blood and egg samples were assessed according to the method of Kaya and Macit [21].

Sample of 5 eggs was randomly collected from each

** Active ingridients given by Farmavet International Inc: 1,8-Cineole (0.24%), Allicine (0.24%), Alliine (0.12%), Alpha-Pinene (0.12%), Alpha Terpineol (0.70%), Borneol (0.18%), Caffeic-Acid (2.28%), Camphene (0.08%), Carvacrol (4.48%), Eugenol (0.12%), Geraniol (1.04%), Limonene (0.56%), Linalool (0.96%), Myrcene (0.18%), P-Cymene (2.38%), Phenol (0.86%), Polyphenol (6.00%), Tannin (12.9%), Rosmarinic-Acid (7.60%), Terpinen-4-Ol (0.06%), Ursolic Acid (1.92%), Tymol (3.26%)*

experimental group for fatty acid (FA) analysis at the end of the trial. The FA profile was determined with gas chromatograph (HP Agilent Technologies 6890N, Inc. Headquarters Santa Clara, US). Method identified by Yüksel et al.[22] was used to determine FA levels of yolk samples.

The experimental data were sitatistically analysed to the general linear models procedure of SPSS [23]. Polynomial contrast was constructed to determine the effect of the feeding level of PEM in the diets. The effects of the dietary treatments on response variables were declared to be significant at P<.05.

Table 1. *Ingredients and chemical composition of the experimental basal diet*
Tablo 1. *Bazal rasyonun bileşimi ve kimyasal komposizyonu*

Ingredients	**(%)**	**Chemical Composition (Analyzed on Dry Matter Basis)**	
Corn	52.81	Dry matter (%)	89.47
Soybean meal	18.13	Crude protein (%)	16.50
Wheat	6.00	Crude fiber (%)	4.49
Full fat Soybean oil	1.65	Crude ash (%)	11.70
Sunflower meal	7.50	Ether extract (%)	4.88
Corn gluten meal	2.04	Lysine (%)	0.7
Soybean oil	1.60	Methionine (%)	0.33
Ground limestone	6.82	Calcium (%)	3.4
Salt	0.30	Total phosphorus (%)	0.7
Di-calcium phosphate 18	2.65	ME* kcal/kg	2720
DL-Methionine 99%	0.15		
L-Lysine	0.10		
Vit-Min	0.25		

Each two kilogram of vitamin- mineral premix contained 12.000 IU of retinyl palmitate, 2.500 IU of cholecalciferol, 30 IU of DL-a-tocopheryl acetate, 34 mg of phylloquinone, 3 mg of thiamin, 6 mg of riboflavin, 30 mg of nicotin amid, 10 mg of Ca-D-pantothenate, 5 mg of pyridoxine, 15 mg of cobalamin, 1 mg of folic acid, 50 mg of D-Biotin, 300 mg of choline chloride, 50 mg of ascorbic acid, 80 mg of manganese, 60 mg of iron, 60 mg of zinc, 5 mg of copper, 2 mg of iodine, 5 mg of cobalt, 150 mg of selenium
Metabolizable Energy Calculated

RESULTS

The effects of single feeding of PEM or Cu and combination of copper and PEM on the performance traits are presented in *Table 2*. Dietary additives did not have significant effect on final body weight and body weight change of hens. Similarly, feeding as the single or combination of plant extract mixture at different levels and copper did not affect the egg weight, egg production, feed intake, feed conversion ratio and broken egg rate.

The egg quality traits of hens fed a diet supplemented with PEM and Cu or combination of copper and PEM are shown in *Table 3*. Shape index, shell weight, yolk index, albumen index and haugh unit were not affected by adddition of PEM (P>.05). But PEM supplementation affected shell stiffnes and shell thickness (P<.05). Shell stiffnes was quadratically increased by increasing level of PEM. On the other hand, a significant linear increase was found in shell thickness when PEM at 500, 750 and 1000 mg/kg levels was added to layer diets. The supplementation of copper had no significant effect on egg quality traits except for yolk color. Egg quality traits were not influenced by feeding of combination of PEM and copper.

The effect of inclusion of PEM at 500, 750 and 1000 mg/kg levels into diet on ratios of cholesterol and triglyceride as a percentage of total lipids in serum and egg yolk is presented in *Table 4*. Cholesterol and trigliserid concentrations of yolk and serum were not affected by dietary treatments.

Table 5 shows the dietary effects of PEM, 200 ppm

Table 2. *Effects of plant extract mixture and copper on laying performance parameters*
Tablo 2. *Bitki ekstrakt karışımı ve bakırın performans parametreleri üzerine etkisi*

PEM mg/kg	**Cu ppm**	**IBW (g)**	**FBW (g)**	**BWC (g)**	**EW (g)**	**EP (%)**	**FI (g/d)**	**FCR[1]**	**DER (%)**
0	0	1627.27	1760.00	132.83	67.54	89.02	131.85	2.20	0.03
	200	1560.83	1705.83	145.00	66.72	85.68	123.35	2.17	0.27
500	0	1525.63	1650.63	125.00	65.96	90.29	128.20	2.16	0.13
	200	1593.96	1668.96	75.00	65.89	90.03	130.12	2.20	0.17
750	0	1622.08	1723.33	101.25	67.64	87.54	128.44	2.19	0.20
	200	1576.04	1681.67	105.63	64.56	90.39	128.40	2.18	0.23
1000	0	1597.92	1698.13	100.21	66.22	90.78	127.87	2.14	0.07
	200	1587.71	1670.00	82.29	64.12	85.75	125.24	2.32	0.40
SEM		29.95	30.55	21.93	1.13	2.27	2.41	0.24	0.11
PEM		0.55	0.12	0.16	0.39	0.65	0.73	0.54	0.83
Cu		0.53	0.23	0.41	0.06	0.37	0.18	0.25	0.06
PEM*Cu		0.14	0.66	0.50	0.56	0.33	0.17	0.59	0.47

IBW*= Initial body weight,* ***FBW****= Final body weight,* ***BWC****= Body weight change,* ***EW****= Egg weight,* ***EP****= Egg production rate,* ***FI****= Feed intake,* ***FCR****= Feed conversion ratio (kg feed:kg egg),* ***DER****= Damage egg rate,* ***PEM****= plant extracts mixture,* ***SEM****= Standart error of mean,* ***Cu****= Copper*

Table3. *Effects of plant extract mixture and copper on egg quality parameters*
Tablo 3. *Bitki ekstrakt karışımı ve bakırın yumurta kalite parametreleri üzerine etkisi*

PEM mg/kg	**0**		**500**		**750**		**1000**		**SEM**	**PEM / Polinomial**	**Cu**	**PEM*Cu**
Cu mg/kg	**0**	**200**	**0**	**200**	**0**	**200**	**0**	**200**				
SI (%)	74.22	74.44	73.89	73.83	73.42	73.61	74.17	74.94	0.65	0.38	0.54	0.93
SS (kg/cm^2)	0.84	1.03	1.14	1.20	0.95	0.91	0.90	0.84	0.07	0.01/**(Q)**	0.44	0.26
ST (mm×10^{-2})	0.39	0.40	0.39	0.40	0.39	0.40	0.43	0.42	0.01	0.00 /**(L)**	0.49	0.58
SW (g)	8.55	8.37	8.32	8.80	8.22	8.22	8.37	8.17	0.17	0.18	0.84	0.20
YC	8.83	8.39	8.44	8.11	8.33	7.50	8.17	7.78	0.23	2.02	0.00	0.70
YI (%)	42.71	42.44	43.09	43.74	43.15	43.58	43.20	42.50	0.54	0.35	0.95	0.58
AI (%)	8.32	8.44	8.44	8.90	8.85	7.93	8.20	8.86	0.39	0.87	0.76	0.20
HU	82.01	82.65	83.03	84.52	84.20	80.17	80.83	85.04	1.66	0.77	0.62	0.11

SI*= Shape index,* ***SS****= Shell strenght,* ***ST****= Shell thickness,* ***SW****= Shell weight,* ***YC****= Yolk color,* ***YI****= Yolk index,* ***AI****= Albumen index,* ***HU****= Haugh unit,* ***PEM****= Plant extract mixture,* ***SEM****= Standart error mean,* ***Cu****= Copper,* ***L****= Linear effect of PEM supplementation;* ***Q****= Quadratic effect of PEM supplementation*

Table 4. *Effects of plant extract mixture on cholesterol and trigliserid level of egg and serum (%)*
Tablo 4. *Bitki ekstrakt karışımı ve bakırın yumurta sarısı ve serum kolesterol ile trigliserid oranları üzerine etkisi (%)*

PEM mg/kg		**0**		**500**		**750**		**1000**		**SEM**	**PEM**	**Cu**	**PEM*Cu**
Cu mg/kg		**0**	**200**	**0**	**200**	**0**	**200**	**0**	**200**				
Egg yolk	Chol	18.84	18.19	18.25	20.99	17.77	18.95	17.45	16.22	1.26	0.19	0.57	0.39
	Trig	60.54	56.00	56.33	60.35	58.52	61.33	61.29	59.03	1.72	0.57	1.0	0.06
Serum	Chol	24.35	23.52	23.33	22.83	23.33	23.12	23.34	23.00	0.65	0.54	0.32	0.97
	Trig	43.87	45.17	45.36	45.26	42.63	44.12	44.15	43.09	0.92	0.15	0.53	0.47

Chol*= Cholesterol,* ***Trig****= Trigliserid,* ***PEM****= Plant extract mixture,* ***SEM****= Standart error mean,* ***Cu****= Copper*

copper and combination of PEM and copper on fatty acid composotion of yolk. While supplemented PEM and copper did not affect fatty acid level in yolk, PEM with copper combination increased oleic acid and total monounsaturated fatty acid (MUFA) level (P<.05).

DISCUSSION

Present study, significant differences were observed among the groups with respect to performance parameters at the end of the 14-week experimental period. A similar observation was reported by Bozkurt et al.[9] who determined that body weight was not affected by addition of an essential oil combination to the layer diets. Arpasova et al.[24] concluded that body weight was not influenced diet including 0.25 ml/kg thyme essential oil in the laying hens. But Çabuk et al.[25] found that essential oils had the positive effects on body weight of laying hens. In accordance with the present findings, Orhan and Eren [26] reported that the addition of essential oil mixture including *Origanum vulgare* (leaf), *Thymus vulgaris* (leaf), thyme oil, origanum oil, garlic oil, anise oil and fennel oil into laying hen diets had no significant effect on egg weight, egg production, feed conversion ratio, feed consumption. In agreement with the present study, Aksu and Bozkurt [27] found that body weight, feed intake, and feed efficiency of broilers were not affected by the adding of essential oils at the level of 1.000 mg/kg. Similarly, Köksal and Küçükersan [28] reported that supplementation of essential oil into basal diet at level of 0.75 g/kg did not have significant effect on performance including body weight, body weight gain, feed intake and feed conversion ratio in broilers. Specific effects of the essential oils on laying performance have not received much attention because poultry may not acutely respond to flavor [29]. In contrast to our results, a study has observed that the addition of essential oil mixture into a layer diet has positive effect on egg weight and egg production rate [30]. Chandra et al.[31] reported that the supplementation of dietary copper at different levels (50, 75, 100, 125, 150 and 175 mg Cu per kg diet) into layer diets had no significant influence on performance parameters. Kaya and Macit [21] reported that egg weight, egg production and feed consumption decreased with 200 mg/kg Cu sulfate supplementation but feed conversion ratio and cracked egg were not affected. Pekel and Alp [32] postulated that supplementation with 250 mg/kg of Cu sulfate improved egg production but decreased egg weight and feed intake.

Except for shell stiffness and shell thickness, none of the egg quality traits were affected by PEM. In contrast to our study, other researchers have observed that the addition of essential oil mixture to layer diet did not have significant effect on shell strenght [9,26,30]. Similarly Nasiroleslami and Torki [33] reported that adding 300 mg/kg fennel essential oil to laying hen diet improved shell thickness. In contrast

Table 5. *Effects of plant extract mixture and Cu on fatty acid composition of egg yolk (%)*
Tablo 5. *Yumurta tavuk rasyonlarına bitki ekstrakt karışımı ve bakır ilavesinin yumurta sarısı yağ asidi komposizyonu üzerine etkisi (%)*

PEM mg/kg	**0**		**500**		**750**		**1000**		**SEM**	**PEM**	**Cu**	**PEM*Cu**
Cu mg/kg	**0**	**200**	**0**	**200**	**0**	**200**	**0**	**200**				
C14:0	0.28	0.28	0.27	0.32	0.27	0.27	0.23	0.26	0.02	0.08	0.17	0.49
C14:1	0.01	0.01	0.02	0.03	0.01	0.02	0.02	0.02	0.01	0.28	0.22	0.89
C15:0	0.03	0.04	0.03	0.04	0.02	0.02	0.02	0.02	0.01	0.12	0.90	0.38
C16:0	25.93	25.78	25.45	26.58	26.10	26.04	25.19	25.93	0.45	0.66	0.20	0.41
C16:1	2.16	2.34	2.33	2.72	2.44	2.63	2.28	2.35	0.20	0.37	0.15	0.88
C17:0	0.18	0.17	0.18	0.17	0.16	0.15	0.18	0.16	0.01	0.367	0.10	0.98
C17:1	0.08	0.08	0.08	0.08	0.08	0.08	0.07	0.08	0.01	0.68	0.58	0.56
C18:0	9.63	9.57	9.33	9.11	8.95	8.80	9.16	9.58	0.30	0.13	0.99	0.71
C18:1t (n-9)	0.06	0.06	0.06	0.07	0.06	0.02	0.06	0.05	0.02	0.38	0.36	0.43
C18:1c (n-9)	37.95^{b}	38.27^{ab}	37.98^{b}	36.02^{c}	37.68^{b}	39.05^{a}	37.95^{b}	38.46^{ab}	0.55	0.07	0.88	**0.03**
C18:2t (n-6)	1.50	1.59	1.60	1.60	1.62	1.76	1.60	1.59	0.06	0.16	0.18	0.57
C18:2c (n-6)	16.97	16.57	17.21	17.79	17.25	16.02	17.48	16.54	0.60	0.50	0.25	0.46
C18.3 (n-6)	0.12	0.11	0.12	0.11	0.10	0.07	0.09	0.10	0.02	0.13	0.34	0.51
C18.3 (n-3)	0.40	0.42	0.44	0.48	0.45	0.39	0.45	0.40	0.03	0.45	0.60	0.33
C20:1	0.19	0.22	0.20	0.19	0.22	0.21	0.20	0.21	0.01	0.50	0.51	0.47
C20:2	0.19	0.20	0.21	0.21	0.21	0.19	0.19	0.20	0.01	0.61	0.87	0.65
C20:3 (n-6)	0.18	0.19	0.19	0.19	0.18	0.17	0.14	0.17	0.02	0.14	0.40	0.56
C20:4	1.89	1.90	1.90	1.84	1.80	1.79	1.94	1.85	0.05	0.13	0.28	0.74
C24:1	0.71	0.73	0.69	0.65	0.71	0.69	0.76	0.68	0.02	0.18	0.13	0.17
∑ SFA	36.05	35.83	35.26	36.21	35.51	35.26	34.78	35.96	0.47	0.56	0.22	0.29
∑ UFA	62.43	62.69	63.03	62.01	62.79	63.08	63.19	62.69	0.45	0.67	0.45	0.41
∑ MUFA	41.17^{b}	41.71^{ab}	41.36^{b}	39.77^{c}	41.19^{b}	42.70^{a}	41.30^{b}	41.83^{ab}	0.50	0.06	0.49	**0.03**
∑ PUFA	21.27	20.99	21.66	22.24	21.60	20.38	21.89	20.86	0.66	0.48	0.30	0.51
∑ (ω-6)	20.67	20.36	21.01	21.54	20.94	19.79	21.25	20.26	0.62	0.49	0.28	0.52
∑ (ω-3)	0.41	0.42	0.44	0.49	0.45	0.39	0.45	0.40	0.03	0.37	0.62	0.29

C14:0*= Miristic Acid,* ***C14:1****= Miristioleic Acid,* ***C16:0****= Palmitic Acid,* ***C16:1****= Palmitoleic Acid,* ***C17:0****= Heptadecanoic Acid,* ***C17:1****= Heptadecenoic Acid,* ***C18:0****= Stearic Acid,* ***C18:1t (n-9)****= Elaidik Acid,* ***C18:1c (n-9)****= Oleic Acid,* ***C18:2t (n-6)****= Linoleic Acid,* ***C18:2c(n-6)****= Linoleic Acid,* ***C18.3(n-6)****= γ-Linolenic Acid,* ***C18.3(n-3)****= α-Linolenic Acid,* ***C20:1****= Gadoleic Acid,* ***C20:2****= Eikosadienoic Acid,,* ***C20:3 (n-6)****= Eikosatrienoic Acid,* ***C20:4****= Arashidonic Acid,* ***C24:1****= Nervonic Acid,* ***∑ SFA****= Total saturated fatty acid,* ***∑ UFA****= Total unsaturated fatty acid,* ***∑ MUFA****= Total Monounsaturated Fatty Acid,* ***∑ PUFA****= Total unsaturated fatty acid,* ***∑ (ω-6)****= Total omega-6,* ***∑ (ω-3)****= Total Omega-3,* ***PEM****= Plant extract mixture,* ***SEM****= Standart error mean,* ***Cu****= Copper*

to present study, there have been some researches who reported the unbeneficial effects of essential oil mixture on shell thickness in laying hens [9,30,34]. Information acquired from literature showed that herbal extracts used in poultry nutrition caused feed intake and feed efficiency improvement [35,36] because of increased production of digestive enzymes [37]. Increase in shell thickness may lead to higher nutrient retention and nutrient availability through the intestines during shell formation in this study. Yolk color was decreased by suplementation of 200 mg/kg copper to laying hen diets. In contrast to our results, Kaya and Macit [21] observed that yolk color was not affected by addition of 200 mg/kg copper to layer diets.

The effects of supplemental treatments on cholesterol and triglyceride ratios of yolk and serum were not significant in the present study. In accordance with the present findings, some researchers who reported that cholesterol and triglyceride concentrations of serum were not affected by addition of 24 mg/kg essential oil mixture [9] or 300 mg essential oils of fennel per kg diet [33]. A different observation was reported by Bölükbaşı et al.[38] who determined that the addition of 200 ppm thyme essential oil to the layer diet resulted in remarkable reduction in serum cholesterol and trigliserid ratios. Bölükbaşı et al.[39] showed that adding essential oils of thyme (200 mg/kg) into layer diet decreased triglyceride rations of yolk but not effected cholesterol ratios of yolk. The results of cholesterol and triglyceride ratios of egg and serum were supported by Kaya and Macit[21] who founded that the supplementation of 200 ppm copper did not have a significant effect on cholesterol and triglyceride ratios of egg and serum. On the contrary to the result of this experiment, in previous studies, egg yolk cholesterol in laying hens was decreased

by dietary Cu supplementation [5,13,40,41]. Also Abaza et al.[42] stated that Cu supplementation at 0 to 100 and 200 mg/kg levels in the laying quail diets decreased cholesterol and triglyceride concentrations of serum.

Inclusion of PEM with copper combination into the diets of laying hens increased oleic acid and total mono-unsaturated fatty acid (MUFA) level. The increase in MUFA observed in the eggs from layer fed the combination of PEM and copper diets was mainly contributed by oleic acid (C18:1c [n-9]). Recently, MUFA have been a subject of interest due to their possible hypolipidemic and anti-thrombotic effects [43]. Ansari et al.[44] reported that fatty acid level of yolk in layer was not effected by 250 ppm copper. Maurice and Lightsey [45] postulated that adding of 250 ppm copper into layer diets did not effect fatty acid level of yolk except for arashidonic acid level.

The differences between present study and other experiments may be due to differences in the laying period, age and genotype of hen and the combination, and kind of used extract and levels of Cu and extract in the diets of laying hens. In previous years, effects of supplementation of PEM together with Cu as combination in laying hens diets have not been studied. Therefore there have not been yet any literature to compare the results related to performance and some egg quality traits containing cholesterol and fatty acid levels obtained from present study with findings reported by other researchers.

Results from present study showed that addition of plant extract mixture containing leaves of *Origanum vulgare* (leaf), *Thymus vulgaris* (leaf), thyme oil, origanum oil, garlic oil, anise oil and fennel oil into the laying hen diets can be beneficial to improve the shell stiffness and thickness from egg quality traits. In addition, feeding of laying hens with diet containing combination of plant extract mixture and copper increased MUFA level of yolk, but did not decrease the cholesterol level of egg yolk in laying hens.

In conclusion, combination of 750 mg/kg PEM and Cu may be used in the diets of 38 wks of age laying hens for 3-month experimantal period, but more experiment on the supplementation of extracts and Cu combinations into diets of laying hens should be conducted by researchers related to this subject.

Acknowledgements

We would like to thank Prof.Dr. Mevlüt KARAOĞLU for technical assistance in providing copper data.

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Kafkas Univ Vet Fak Derg
19 (4): 681-686, 2013
DOI: 10.9775/kvfd.2013.8650

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

The Statistical Analysis of Some Volumetric Measurements in the Japanese Quails' Head with Different Feather Color: A Computed Tomography Study

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Makale Kodu (Article Code): KVFD-2013-8650

Summary

The aim of this study was to contribute to quail head morphology and the formation of basic data sources for comparative measurement in the quail head. In this study, 60 Japanese quail (Coturnix japonica) heads, which were white, brown and wild type were used. Four different anatomical structures of the quail heads, varying in feather color, were measured manually by two independent radiologists. These anatomical structures were head volume (HV), brain volume (BV), parietooccipital air space volume (POAV) and calvarial bone volume (CBV). According to computed tomography (CT) measurements, the quail head with white feather color had the smallest volumetrical values. This condition is thought to be caused by genetic differences.

Keywords: *Computed Tomography, Japanese quail, Morphometry*

Farklı Tüy Renklerine Sahip Japon Bıldırcınlarının Başlarında Bazı Hacimsel Ölçülerin İstatistiksel Analizi: Bilgisayarlı Tomografi Çalışması

Özet

Bu çalışmanın amacı bıldırcın başlarında karşılaştırmalı ölçümler için temel veri kaynağı oluşturmak ve bıldırcın başı morfolojisine katkı sağlamaktı. Çalışmada beyaz, kahverengi ve yabani tip olmak üzere toplam 60 Japon bıldırcını başı kullanıldı. Farklı renklere göre bıldırcın başlarının dört farklı anatomik yapısı iki radyolog tarafından ölçüldü. Bu yapılar kafa hacmi, cavum cranii hacmi - beyin hacmi, parietooccipital hava alanı hacmi ve kafa kemikleri hacmi şeklindeydi. Bilgisayarlı Tomografi ölçülerine göre, beyaz tüy rengine sahip bıldırcın başının en küçük hacimsel değerlere sahip olduğu belirlendi. Bu duruma genetik farklılıkların neden olduğu düşünülmektedir.

Anahtar sözcükler: *Bilgisayarlı Tomografi, Japon Bıldırcını, Morfometri*

INTRODUCTION

The quail (Coturnix japonica) is a species categorized under Phasianidae family [1,2]. The quail is widely used as an *in vivo* [3-6] in physiological, pathological, toxicological and anatomical studies owing to its lower feed consumption, early sexual maturity and fast growth. In recent years, the increase in consumption of meat and eggs of the quail has also strengthened its economic aspect [1,2,7].

Quail feather color is considered as a race or genetical feature. Differences in the Quail feather color are attributed to mutations in the color [8]. The feather colors are white [9], brown, yellow [9,10], wild type (gray) [11] and roux [10].

In terms of anatomical model preparation, computed tomography (CT) is a quite useful method for reliable topographical patterns [12]. CT is used for antropomorphometrical studies for creating macroscopic and microscopic models and revealing the phenotipic differences quickly in

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small animals [13-17]. The detailed imaging of structures that make up the skull and the minimum degree of overlapping images provides a great advantage to the CT compared to other radiological methods [18,19]. One of the advantages of the CT is the short-term applicability [20]. Using CT, high solution images are obtained from cross-sections of head, and subsequently anatomical or patological data could be evaluated [21-24]. Three-dimensional images of anatomical structures must be accurately obtained for the zoo-morphological requirements, and functional, developmental, comparative studies [25]. Additionally, the development of high resolution imaging techniques such as magnetic resonance imaging (MRI) and CT facilitated to create a small animal model and anatomical evaluations [26-30]. Volumetric measurements of small organisms or non-living structures can be evaluated via the three-dimensional images obtained from CT [25]. Three-dimensional imaging method of animal materials can be gathered into two broad categories: the first one is based on reconstruction from serial section images and the second one is based on whole - volume imaging [25]. A volume image obtained from CT consists of a stack of reconstructed cross sections around the axis of rotation [31]. Therefore, the organism could easily be evaluated in terms of the development, growth and structural differences owing to obtained images [25]. In the next step, some morphometric and volumetric measurements are practised by using these images [20].

Roentgen and CT are diagnosis methods which are frequently referred to in veterinary practice [32,33]. CT has just begun to be used in the field of avian medicine [20]. Therefore, the evaluation and interpretation of images are possible only by becoming familiar with a good level of macro - anatomy of the region and the cross - sectional anatomy [33].

Turbinates, sinuses, nasal cavity, and other anatomical structures can be monitored by using CT in detail in the bird head [20]. In previous studies, anatomical CT images were excessively employed in imaging the head, nasal - para-nasal sinuses, cranial cavity, nasolacrimal duct system, and turbinates have been analyzed in terms of and its three dimensional reconstructed models [24,34-39] in the differences animal specieses (reptile, sea turtle, donkey, rodentia, dog and cat [32-36,39]. However, there was no a study which reveals the anatomical data of the quail head by using CT in the literature.

The aim of this study was to contribute to quail head morphology and the formation of basic data sources for comparative measurement in the quail head using CT. Additionally, in this study, using the above-mentioned characteristics of CT was purposed to determined both status of imaging quality and parameters of method in quail head and to identify whether or not the difference term some volumetrical measurements (see materials and methods section) among quail heads, varying in feather color.

MATERIAL and METHODS

In this study 60 quail heads were used. A total of 20 of them were white, 20 of them were brown and 20 of them were wild type. Quails were supplied from Quail Unit in the Education, Research and Application Farm of Kafkas University. Quails were six weeks, female and average 120 - 160 g (white: 130±5.3 g, brown: 148±7.6 g, wild type: 140±8.2 g) live weight. Animals were grown under the same live conditions (light intensity, feeding, the cage state, water etc.). Quails were killed by slaughtering for protect to wastable quilty of carcase and their heads were referred to the Research and Application Hospital Radiology Unit of Kafkas University to obtain CT images. To scan the heads 64 sliced CT (0.05 mm section) device (Aquilion 64®, Toshiba Medical Systems, 2011, Zoetermeer. The Netherlands) was used.

Four different anatomical structures of each group were measured manually by two independent radiologists with more than five-years experience. Radiologists were defined as observer 1 and observer 2. These anatomical structures were head volume (HV), brain volume (BV) *(Fig. 1-D),* parietooccipital air space volume (diverticula from recessus tympanicus dorsalis invade the occipital, parietal, prootic and squamosal bones [40]) (POAV) *(Fig. 1-A, Fig. 1-B, Fig. 2-F)* and calvarial bone volume (CBV). Aquarius iNtuition Edition ver. 4. 4. 6. software was used for measurements.

Different tissues have different densities in CT, so four different density ranges were used to calculate four different tissue volumes. To calculate the POAV from -1.000 to -850 Hounsfield unit (HU), HV 0-1000 HU, BV 0-150 HU and CBV 200-1000 HU ranges were [41] setted. Both 2D axial and coronal plane images were used to calculate all the volumes. In addition to 2D images, measurements were verified also on 3D volume rendered images. For the volume unit, cm^3 were used and all the data were recorded carefully.

To calculate the BV and POAV region growing tool *(Fig. 1-A, Fig. 1-D),* CBV and HV single click tool *(Fig. 1-C)* was used. Because BV and POAV are spread to area, but CBV and HV are not.

Four different anatomical structure of three kind of quail heads were measured by two radiologists and many different parameters obtained. To proccess the findings easily, we named the variables as: head volume of observer 1 (HV1), head volume of observer 2 (HV2), brain volume of observer 1 (BV1), brain volume of observer 2 (BV2), parietooccipital air space volume of observer 1 (POAV1), parietooccipital air space volume of observer 2 (POAV2), calvarial bone volume of observer 1 (CBV1), calvarial bone volume of observer 2 (CBV2).

Statistical Analysis

Statistical analysis of the study was performed using

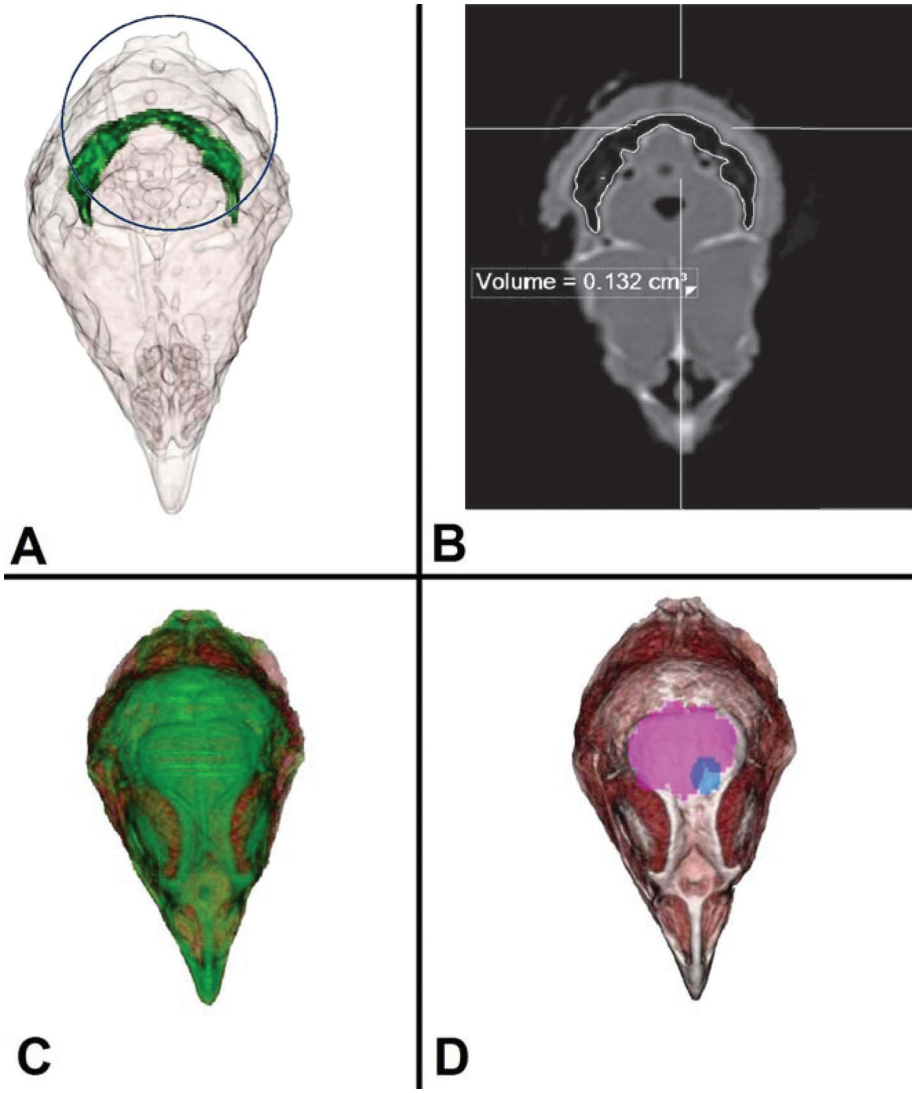

Fig 1. A- 3D volume rendered dorso-ventral aspect image of parietooccipital air space volume of quail head measured by region groving tool, **B-** 2D axial slice of parieto-occipital air space volume of quail head measured by region groving tool, **C-** 3D volume rendered dorso-ventral aspect image of head volume of quail measured by single click tool, **D-** 3D volume rendered dorso-ventral aspect image of brain volume of quail head measured by region groving tool

Şekil 1. A- Bıldırcın başındaki parietooccipital hava alanı hacminin bölge büyütme aracı kullanılarakölçümünündorso-ventralyönden 3B görüntüsü, **B-** Bıldırcın başındaki parieto-occipital hava alanı hacminin bölge büyütme aracı kullanılarak ölçümünün 2B eksen kesit görüntüsü, **C-** Bıldırcın kafa hacminin tek tık aracı kullanılarak ölçümünün dorso-ventral yönden 3B görüntüsü, **D-** Bıldırcın başındaki beyin hacminin bölge büyütme aracı kullanılarak ölçümünün dorso-ventral yönden 3B görüntüsü

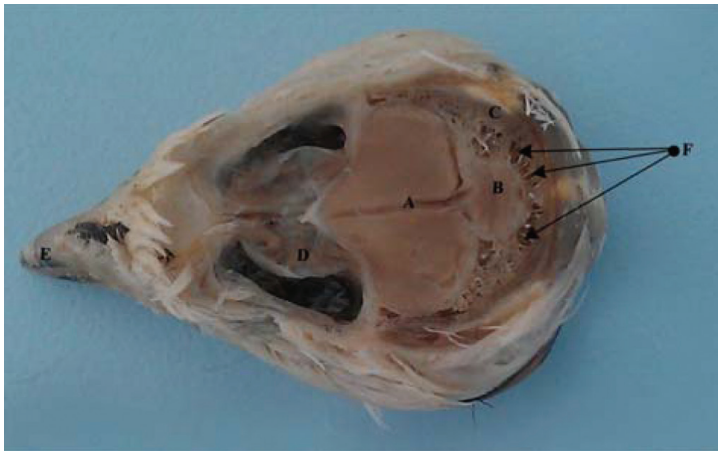

Fig 2. Dorso - ventral view of transversal section through of the quail head. **A-** brain, **B-** cerebellum, **C-** occipital bone, **D-** oculus, **E-** rostrum, **F-** parietooccipital air space

Şekil 2. Bıldırcın kafasının transversal kesitinin dorso - ventral görünüşü. **A-** beyin, **B-** beyincik, **C-** occipital kemik, **D-** göz, **E-** gaga, **F-** parietooccipital hava alanı

Statistical Package for Social Sciences (SPSS) version 17.0 software package. Continuous variables were expressed as arithmetical mean±standard deviation.

Paired sample t test was used to determine the differences of the measurements of BV, HV, POAV and CBV between observer 1 and observer 2 regardless of the quail types and the groups were dependent according to observers.

Kruskal Wallis test used to calculate the differences of BV, POAV, CBV and HV of white, brown and wild type sub-groups for observer 1 and 2 independently. Firstly three groups were analyzed, secondly white-brown, white-wild

and brown-wild groups were analyzed in pairs to find out the differences between the groups.

RESULTS

According to the findings of the study, average values of the BV, POAV, CBV and HV of the quails (n:60) were 0.43, 0.23, 1.93 and 6.64 cm^3, respectively.

According to the observers, the average results obtained from the study showed in *Table 1*. In this table, it seems that values of observer 1 are a little more than the values of observer 2.

Table 2 indicated that each volume of three groups of quail heads showed significant difference between each other, except BV2. Moreover, the same table showed significant difference between white and brown quail head measurements except BV2 and CBV2. Additionally, *Table 2* showed significant difference between wild type and brown quail head measurements except POAV1, BV2 and POAV2, and significant difference between white and wild type quail head measurements except BV2 and POAV2 volumes ($P<0.05$). This results indicated that approximately all of the four measurements of the three kind of quail heads were different from each other.

Paired samples t test demonstrates that there were no significant difference in CBV and HV measurements and there were significant difference in BV and POAV between the observers ($P<0.05$).

DISCUSSION

One should consider the limitations of this study before using these data. We have firstly reported the volumetric measurements of head in the quail by using CT. Thus, we were not able to discuss the variation of morphometrical properties of the bird head. While this study provides new morphometrical data for four volumetric measurement in the quail head, volumetric morphometrical values for other species, such as the chicken, goose, and pigeon, are also needed. When we perfomed this study, there was not a method established in the birds. Therefore, we could not compare in terms of method.

According to the findings of the study, average values of the BV, POAV, CBV and HV of the quail (n:60) were 0.43, 0.23, 1.93 and 6.64 cm^3, respectively. According to this situation, BV was forming %6.47 of HV, POAV was forming %3.46 of HV, and CBV was forming %29.07 of HV.

BV and POAV differences were due to the subjective results of the region growing tool and method. CBV and HV anology were due to the single click method based on our results. Single click is a useful tool that allows measuring the volume by clicking only once to calculate the volume. However, region growing is time consuming and relatively difficult. In this regard, the results of single click are more objective than region growing method.

In this study, feather color having the race property for quails was primary exit point. Therefore, the heads of quails

Table 1. *Shows all the data of each groups based on observers*
Table 1. *Araştırıcılara göre grupların verilerinin tamamı görünmektedir*

Measurement	Observer 1			Observer 2		
	White	Brown	Wild	White	Brown	Wild
BV (region growing)	0.45±0.07	0.56±0.07	0.50±0.05	0.36±0.67	0.37±0.05	0.34±0.10
POAV (region growing)	0.21±0.07	0.33±0.07	0.29±0.04	0.15±0.05	0.19±0.06	0.20±0,19
CBV (single click)	1.56±0.27	1.79±0.32	2.49±0.29	1.63±0.26	1.79±0.32	2.35±0.36
HV (single click)	5.92±0.41	6.60±0.65	7.50±0.65	5.88±0.34	6.50±0.81	7.45±0.68

The unit of the mean values and the standard deviations are cm^3

Table 2. *Kruskal Wallis Test of the white, brown and wild type subgroups (W-White, B-Brown, WT-Wild Type)*
Table 2. *Beyaz, kahverengi ve yabani tipin Kruskal Wallis Testi (W-Beyaz, B-Kahverengi, WT-Yabani Tip)*

Test	HV1	BV1	POAV1	CBV1	HV2	BV2	POAV2	CBV2
Chi-Square (W, B&WT)	*33.75	*22.1	*19.71	*36.83	*32.76	2.84	*7.07	*26.63
Chi-Square (W&B)	*11.71	*18.62	*15.81	*6.4	*10.11	0.27	*6.94	3.05
Chi-Square (WT&B)	*13.14	*9.51	3.59	*22.55	*11.35	2.64	3.64	*16.15
Chi-Square (W&WT)	*26.56	*5.10	*10.36	*27.27	*27.83	1.32	0.05	*20.91

** Means within a row are significantly different ($P<0.05$)*

in three different feather colors were used. There are many quails with different feather colors in the world. Some of them are white, brown, roux, yellow and gray or wild type [8-11]. In this study, different races of quails were used to compare morphological and morphometric differences. Thus, both a method for subsequent studies and a data base were formed.

CT measurements shows that the wild type quail has the biggest head and then brown and white quails were ranking lower, respectively. The brown quail has the biggest cranial cavity volume and then it is followed by wild type and white had smallest. Moreover, the wild type quail has the biggest head bone volume and then brown and white quails have respectively. According to these results, quail race with white feather color had the smallest volumetrical values. Although more compherensive studies are needed to determine the cause of the above mentioned situation, we could speculate that the applied selection pressure in the quail unit and genetic differences can be effective.

When data of head morphometry in the quails are evaluated study material is provided from single sex and equal number of members. Female quails constituted the animal material of this study. Hence, the data obtained from female quails was analyzed.

The lack of previous CT based studies in the avian literature hampered the comparisions. For this reason, the results that we have reported here have potential to contribute not only to the assessments of species but also the comparative and topographic anatomy of birds.

In conclusion, this study showed us that CT can be used for morphological and morphometric studies in quail head, additionally birds and differences term some volumetrical measurements among quail heads, varying in feather color. Furthermore, we concluded that more compherensive studies are necessary to determine the exact relationship between feather color (or race) and some morphometric measurements in the Japanese quails.

Acknowledgments

We thank Radiology Technician Ufuk Kandemir, in Radiology Unit of Research and Application Hospital of Kafkas University for his help in performing CT images.

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Kafkas Univ Vet Fak Derg
19 (4): 687-692, 2013
DOI: 10.9775/kvfd.2013.8658

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

The Anatomy of the Cardiac Veins in Storks (*Ciconia ciconia*)

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Makale Kodu (Article Code): KVFD-2013-8658

Summary

This study was carried out to describe the origin, course and ramifications of the cardiac veins in storks. For this purpose, coloured latex injection method was applied on heart of nine adult storks. The results indicate that venous drainage of the heart was provided by the *v. cardiaca sinistra*, the *v. cardiaca media*, the *vv. cardiacae dextrae* and the *vv. cardiacae minimae*. The coronary sinus was not found in all samples. The *v. cardiaca sinistra* and *v. cardiaca media* were opened into the *v. cava cranialis sinistra*. The *v. cardiaca sinistra* was the largest vein providing to venous drainage of heart which consisted of two main parts *pars interventricularis* and *v. cardiaca circumflexa sinistra*. The *v. cardiaca circumflexa sinistra* was collected to venous blood of the ventriculus sinister and atrium sinistrum by means of several vessels. One branch of the *v. cardiaca circumflexa sinistra* coming from ventriculus sinister was much larger than rest and drained big part of ventriculus sinister. The *v. cardiac media* was a single vein. The *vv. cardiacae dextrae* were opened directly into the right atrium. The *vv. cardiacae minimae* were collected venous blood from the wall of atrium dextrum and interventricular septum and emptied into the right chambers of heart. The venous blood of the interventricular septum was collected by the *vv. septales* of *pars interventricularis* and *v. cardiaca media*. It was concluded that circulation of cardiac veins in stork closely resembles that of rodent and is unlike both fowl and ostrich patterns.

Keywords: *Anatomy, Cardiac veins, Heart, Stork*

Leyleklerde (*Ciconia ciconia*) Kalp Venlerinin Anatomisi

Özet

Bu çalışmada, leylekte koroner venlerin başlangıç, seyir ve dallanmaları incelendi. Bu amaçla dokuz adet yetişkin leyleğe renklendirilmiş latex verildi. Kalbin venöz drenajının *v. cardiaca sinistra, v. cardiaca media, vv. cardiacae dextrae* ve *vv. cardiacae minimae* tarafından sağlandığı tepit edildi. Sinus coronarius'un bulunmadığı belirlendi. *V. cardiaca sinistra* ve *v. cardiaca media*'nın *v. cava cranialis sinistra'* ya açıldığı görüldü. *V. cardiaca sinistra*'nın kalbin venöz kanını toplayan en güçlü ven olduğu tespit edildi. Bu damarın, *pars interventricularis* ve *v. cardiaca circumflexa sinistra* olarak iki bölümden oluştuğu görüldü. *V. cardiaca circumflexa sinistra*'nın bir çok damar aracılığı ile ventriculus sinister ve atrium sinistrum'un venöz kanını topladığı görüldü. Ventriculus sinister'den çıkan *v. cardiaca circumflexa sinistra*'nın bir dalının diğer dallardan güçlü olduğu ve ventriculus sinister'in büyük bir bölümünü diranajını sağladığı tespit edildi. *V. cardiaca media*'nın tek olarak şekillendiği belirlendi. *Vv. cardiacae dextrae*'nın doğrudan atrium dextrum'a açıldığı belirlendi. *V. cardiacae minimae*'nın atrium dextrum duvarı ve septum interventriculare'nin venöz kanını topladıkları ve kalbin sağ boşluklarına açıldığı görüldü. Septum interventriculare'nin drenajını *v. cardiaca media* ile *pars interventricularis*'in *vv. septales*'inin yaptığı tespit edildi. Sonuç olarak leyleğin koroner ven dolaşımı tavuk ve devekuşundan ziyade kemirgenlere daha çok benzediği görüldü.

Anahtar sözcükler: *Anatomi, Kalp venleri, Kalp, Leylek*

INTRODUCTION

The venous drainage of the heart is provided by the *v. cardiaca sinistra,* the *v. cardiaca media,* the *vv. cardiacae dextrae and* the *v. cardicae minimae* in birds [1,2]. The *v. cardiaca sinistra* of birds are formed by the *pars interventricularis* and the *pars basilaris* [1-3]

The *pars interventricularis* is started near the apex of the heart and run in the paraconal interventricular groove. The *pars interventricularis* at the coronary groove border continues as the *pars basilaris*, which lies on the base of the heart, between the conus arteriosus and bulbus aortae

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and opened into right atrium [1-4]. However, the *pars basilaris* is not found in some samples of fowl [1].

Vena cardiaca circumflexa sinistra is begun by the fine superficial tributaries from the proximal part of the left aspect of the ventriculus sinister. Then, it runs in the coronary groove and opened into atrium dextrum [1,3]. *V. cardiaca media* was begun just above the apex of the heart. After it was received many ventral and dorsal tributaries from the adjacent wall of the ventriculus sinister and ventriculus dexter, led to right atrium [1-4]. *Vv. cardiacae dextrae are* consisted by veins draining the ventriculus dexter and led to the atrium dextrum. *Venae cardiacae minimae* are found constantly in the wall of atrium dextrum and the interventricular septum [1].

Generally, the coronary sinus collecting of principal cardiac veins is a large blood vessel or dilation of right atrium [5]. Although the coronary sinus is emphasized to exist in ostrich [3,6], some authors state that domestic birds [1,4] have not a coronary sinus.

Birds are the most diverse group of animals rather than mammals which are alive today [7]. Although venous drainage of heart has been well documented in mammalian, there is no study about the venous drainage of bird's heart, except fowl *(Gallus domesticus)* [1,8-10] and ostrich [3]. Therefore, this study aimed to reveal the conformation and branching of the cardiac veins in stork *(Ciconia ciconia)* which is wild and a migrant bird.

MATERIAL and METHODS

Nine adult storks of both sexes and weighing between 2.5 and 3 kg were utilized for the study. The animals used in the study were obtained from Mustafa Kemal University and Adana Veterinary Control and Research Institute. Animals which were could not survive due to badly injured or suspected various diseases were used. All experimental procedures were approved by Adana Veterinary Control and Research Institute's Animal Experiments Local Ethics Committee (Approval no. 02.02.2012/15).

Coloured latex injection was applied in order to describe the coronary veins as suggested by literatures [11,12].

Coelom of the storks were opened right just after death. The heart was removed from the coelom. Plastic catheters were inserted into the *v. cava caudalis*. All cardiac vessels and chambers were washed with 0.9% saline (NaCl). After, two *v. cava cranialis* were clamped. After then, blue (Setacolor TM, Cobalt blue, num. 20; PEBEO Cedex, France) colored latex (Rubber latex TM; MERCAN, Istanbul, Turkey) was injected into the *v. cava caudalis*. The hearts were then immersed in 10% formalin for 3 days. After solidification of latex solution, the cardiac veins were investigated macroscopically using a dissection microscope (Nikon SMZ-2T; Nikon Corp., Tokyo, Japan). Photographs were taken digitally and stored on a personal computer. The vessels were measured with a digital caliper (DV892 calipers, Tecnotest corp., Germany) sensitivity of 0.1% mm.

Nomina Anatomica Avium [2] was used for anatomical nomenclature.

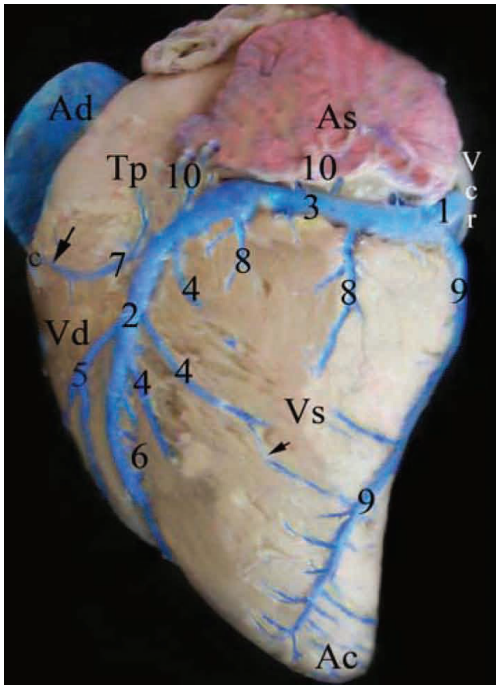
RESULTS

The veins draining the heart of the stork were the *v. cardiaca sinistra*, the *v. cardiaca media*, the *vv. cordiacae dextrae* and the *vv. cardicae minimae*. The coronary sinus was absent in all of the studied samples.

The *vena cardiaca sinistra (Figs. 1,2/1):* In all the samples, the *v. cardiaca sinistra* was the largest vein carrying the venous blood of the heart and emptied into the *v. cava cranialis sinistra*. During its course, the *v. cardiaca sinistra* was named as the *pars interventricularis (Figs. 1,3/2)* in the paraconal interventricular groove and the *v. cardiaca circumflexa sinistra (Figs.1,2/3)* in the coronary groove.

The *pars interventricularis (Figs. 1,3/2):* The *pars interventricularis* began at the level of the apex of the heart and anastomosed with the *v. cardiaca media (Fig. 3/→)* at the same level. The *pars interventricularis* was formed by the 5-6 *vv. ventriculares (Fig. 1/4)* coming from the ventriculus sinister and 4-5 *vv. ventriculares (Fig. 1/5)* from ventriculus dexter. The terminal branches of the *pars interventricularis* of *v. cardiaca sinistra* anastomosed with the branches of *vv. cardiacae dextrae (Fig. 3/→)*. Along its course, the *pars interventricularis* also received 6-7 small *vv. septales (Fig. 1/6)* from interventricular septum. A strong *v. septalis* draining the proximal half to a third of the interventricular septum was also joined the *pars interventricularis* in three samples. Just before continuing as *v. cardiaca circumflexa sinistra,* the *pars interventricularis* also received the *v. conalis (Fig. 1/7)* draining the left part of conus arteriosus and proximal portion of the ventriculus dexter. The terminal branches of *v. conalis* were found to anastomose with the *v. conalis (Fig. 1/→)* of *vv. cardiacae dextrae* in the four of the investigated hearts.

The *vena cardiaca circumflexa sinistra (Figs. 1,2/3):* The *pars interventricularis* coursed in the coronary groove as the *v. cardiaca circumflexa sinistra*. It was entirely covered by the auricula sinistra. Along its course, the *v. cardiaca circumflexa sinistra* received 3-4 thin *vv. ventriculares (Figs. 1,2/8)* starting from the proximal part of the ventriculus sinister. In all hearts, it was noted that one of the *vv. ventriculares* coming from the ventriculus sinister was the most distinct one. In all samples, this vein *(Figs. 1,2/9)* started from the apex of the heart and flowed towards the base of the heart on the ventriculus sinister, parallel to the *v. cardiaca media*. Along its course, it collected several subbranches from the wall of ventriculus sinister. Its



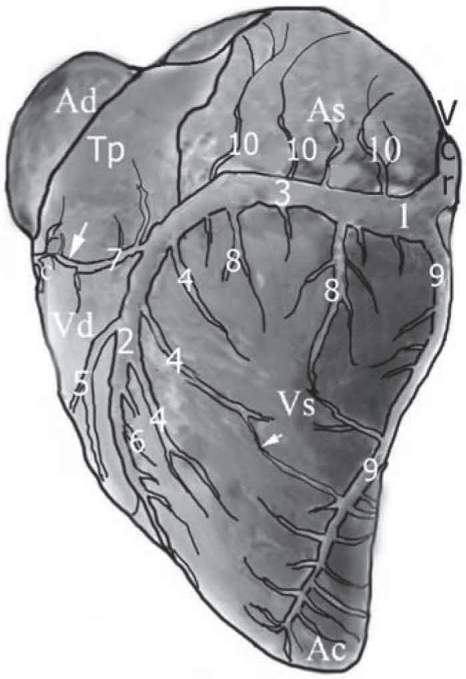
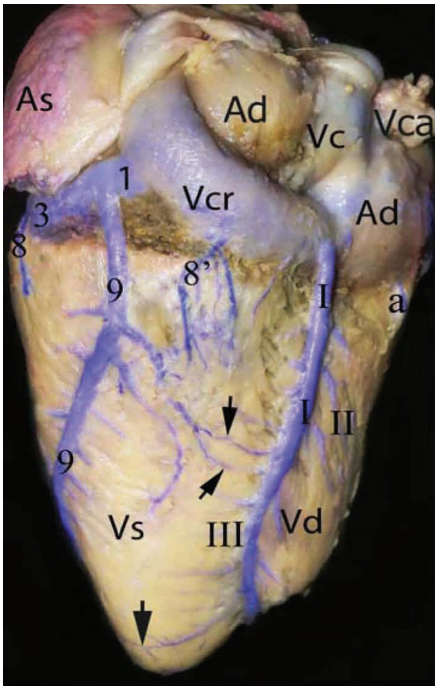


Fig 1. The *pars interventricularis,* the *v. cardiaca circumflexa sinistra* and their branches (cranial surface)

Şekil 1. *Pars interventricularis* ile *v. cardiaca circumflexa sinistra*'nın dalları (cranial yüz)

1: v. cardiaca sinistra, 2: v. pars interventricularis, 3: v. cardiaca circumflexa sinistra, 4-5: The vv. ventriculares of pars interventricularis, 6: The vv. septales of pars interventricularis, 7: The v. conalis of pars interventricularis, 8: The vv. ventriculares of v. cardiaca circumflexa sinistra, 9: The strong vein of v. cardiaca circumflexa sinistra, 10: The vv. atriales of v. cardiaca circumflexa sinistra, c: The v. conalis of v. cardiaca circumflexa sinistra, Tp: truncus pulmonalis, As: atrium sinistrum, Ad: atrium dextrum, Vd: ventriculus dexter, Vs: ventriculus sinister, AC: apex cordis, Vrc: v. cava cranialis sinistra and arrows: anastomose



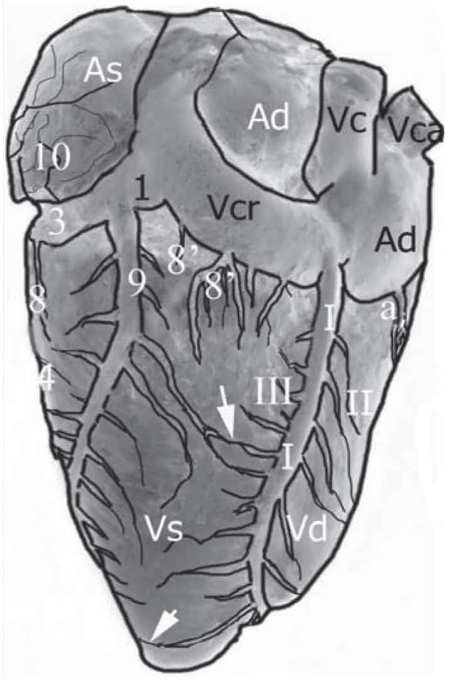


Fig 2. The *v. cardiaca sinistra* and the *v. cardiaca media* and their branches (caudal surface)

Şekil 2. *V. cardiaca sinistra* ve *v. cardiaca media*'nın dalları (caudal yüz)

1: v. cardiaca sinstra, 3 v. cardiaca circumflexa sinistra, 8: The vv. ventriculares of v. cardiaca circumflexa sinistra 8': The vv. ventriculares of v. cava cranialis sinistra, 9: The strong vein of v. cardiaca circumflexa sinistra, 10: The vv. atriales of v. cardiaca circumflexa sinistra, I: v. cardiaca media, II,III: The vv. ventriculares of v. cardiaca media, a: The vv. ventriculares of vv. cardiacae dextrae, As: atrium sinistrum, Ad: Atrium dextrum, Vd: ventriculus dexter, Vs: ventriculus sinister, Vcr: v. cava cranialis sinistra, Vca: v. cava cranialis dextra, Vc: v. cava caudalis and arrows: anastomose

terminal branches were also seen to reach the papillary muscle in all samples. In one heart, it was also larger than the *v. cardiaca media*, according to its subbranches and diameter. Its branches also anastomosed with the *pars interventircularis (Fig. 1/→)* and *vv. ventriculares* of *v. cardiaca media (Fig. 2/→)*. In five samples, 2 or 3 fine *vv. ventriculares (Fig. 2/8')* which were located on the proximal of ventriculus sinister were also seen to open directly into *v. cava cranialis sinistra*. The *v. cardiaca circumflexa sinistra* also received dorsally directed 5-6 thin *vv. atriales (Figs. 1,2/10)* from the wall of atrium sinistrum. The *v. cardiaca circumflexa sinistra* led into *v. cava cranialis sinistra*.

The *venae cardiaca media (Fig. 1/I):* The *v. cardiaca media* was formed by the branches stemming from the apex of the heart and ascending towards the base of the heart in the subsinuosal interventricular groove. It was opened into the *v. cava cranialis sinistra*. The *v. cardiaca media* was thinner than the *v. cardiaca sinistra* and a single vein. Six or seven *vv. ventriculares (Fig. 3/II)* from the ventriculus dexter and 5-7 *vv. ventriculares (Fig. 2/III)* from the ventriculus sinister joined to the *v. cardiaca media*. The *v. cardiaca media* was also carried to the *vv. septales (Fig. 4/IV)* from the interventricular septum. These *vv. septales* were smaller and fewer in number than those opening to the *pars interventricularis*.

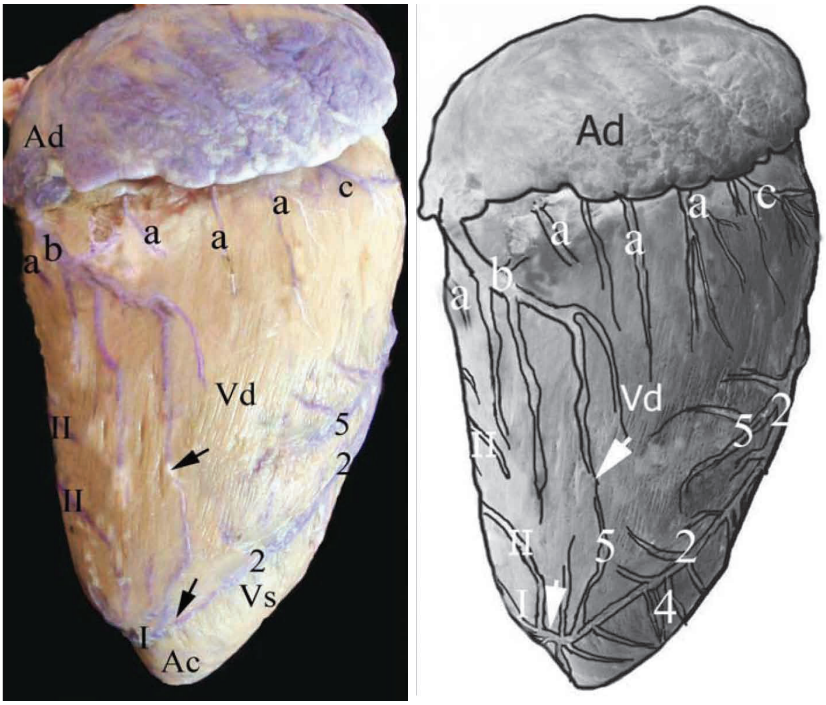


Fig 3. *Vv. cardiacae dextrae* (atrial surface)

Şekil 3. *Vv. cardiacae dextrae* (atrial yüz)

I: v. cardiaca media, II: The vv. ventriculares of v. cardiaca media, a: The vv. ventriculares of vv. cardiacae dextrae, b: The strong vein of vv. cardiacae dextrae, c: v. conalis, 2: pars interventricularis, 5: The vv. ventriculares of pars interventricularis, Ad: Atrium dextrum, Vd: ventriculus dexter, AC: apex cordis and arrows: anastomose

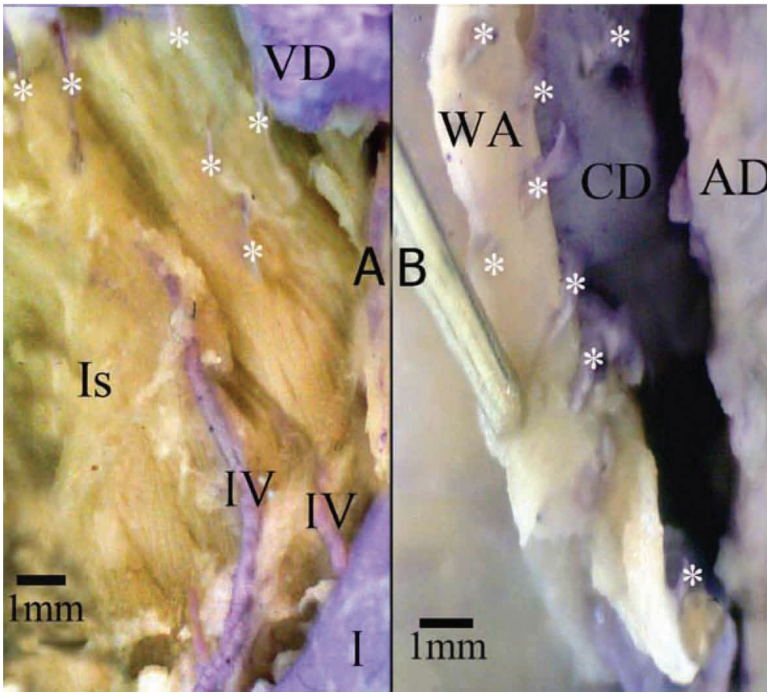


Fig 4. The *vv. septales* and the *vv. cardiacae minimae* in the interventricular septum (A), The *vv. cardiacae minimale* on the wall of atrium dextrum (B)

Şekil 4. Septum interventriculares'te *vv. septales* ve *vv. cardiacae minimae* (A), Atrium dextrum duvarında *vv. cardiacae minimae* (B)

I: v. cardiaca media, IV: The vv. septales of v. cardiaca media, Is: interventricular septum, VD: The ventriculus dexter, CD: The cavity of atrium dextrum, AD: atrium dextrum, Aw: The wall of atrium dextrum and *: vv. cardiacae minimae

The *venae cardiacae dextrae (Fig. 3/a,b,c):* The *vv. cardiacae dextrae* consisted of 4-5 *vv. ventriculares, v. conalis* and 3-4 fine veins draining the right atrioventricular valve. The *vv. ventriculares* were joined to the *vv. cardiacae dextrae* at the distal border of the proximal third of the ventriculus sinister. After their origin, the *vv. ventriculares* run toward base of heart and emptied directly into right atrium *(Fig. 3/a)*. In six specimens, one of *vv. ventriculares* was longer than the others. This vein *(Fig. 3/b)* originated from the 1/3 distal part of the ventriculus dexter and collected venous blood from the distal and middle part of wall of ventriculus dexter. In addition to *vv. ventriculares,* another vessel involved in the formation of the *vv. cardiacae dextrae* was *v. conalis.* The *v. conalis (Fig. 3/c)* was formed by the thin branches stemming from the right part of conus arteriosus and the proximal portion of ventriculus dexter.

The *venae cardiacae minimae (Fig. 4/*):* The *vv. cardiacae minimae* were identified only on the right half of the heart. They seemed to play a role in the venous drainage of the interventricular septum and the wall of atrium dextrum. These veins were opened into the cavity of the

ventriculus dexter *(Fig. 4-A/*)* and the atrium dextrum *(Fig. 4-B/*)*.

During their course, the *v. cardiaca circumflexa sinistra*, the *v. cardiaca media*, a thick subbranch of *v. cardiaca circumflexa sinistra* and the *pars interventricularis* were seen to course subepicardially. However, the thin branches of *v. cardiaca sinistra* and *v. cardiaca media* were situated intramyocardially. Although the *vv. ventriculares* of *vv. cardiacae dextrae* were located intramyocardially, the some thin branches belonging to *vv. cardiacae dextrae* were found subepicardially.

DISCUSSION

The *vena cava cranialis sinistra* is usually absent in carnivore, equine, ruminant [5]. However, this vein could be constant observed in birds [1-4], rat [13], beaver [14], mice [15], rabbit [12,16,17], marsupial [18]. In fowl, the *v. cava cranialis sinistra* is also termed the left precaval vein [1]. In the present study, the *v. cava cranialis sinistra* collected a great amount of venous blood of the heart, as reported in rabbits [12,16] beaver [14], mice [15] but does not correspond to the finding of Lindsay [1] who stated that the cardiac veins generally empty directly into the atrium dextrum in the fowl.

The coronary sinus is a tubular dilatation of the right atrium or it is the rudiment of the right sinus horn of the embryonic heart in some domestic animals [5,19] and humans [19]. Some researchers are reported that a great amount of venous blood is collected by coronary sinus, domestic animals [5,20], roe deer [21], porcupines [11] and human [19]. However, the coronary sinus was absent in stork, as observed in the domestic birds [1,4] and rat [13], beaver [14], mice [15], rabbit [12,16,17].

As indicated in the birds [1-3,22], the *v. cardiaca sinistra*, the *v. cardiaca media*, the *vv. cardiacae dextrae* and the *vv. cardiacae minimae* were identified in the stork heart.

In birds [1-3] *v. cardiaca sinistra* is constituted by two main veins, the p*ars interventricularis* and the *pars basilaris*. In most fowl [1,22] and ostrich [3], after the *pars interventricularis* reaches to the junction of the paraconal interventricular groove and coronary groove, it advances caudally as the *pars basilaris* in the fat-filled space between the atrium sinistrum and truncus pulmonalis and aorta, and empties into the left recess of the atrium sinistrum. These arrangement and distribution are also reported in marsupial [21]. However, the *pars basilaris* was not found in our samples. The *pars interventricularis* was continued as the *v. cardiaca circumflexa sinistra* in coronary groove in all samples. Same finding is reported by Nickel et al.[5], Yoldas and Nur [12], Yadm and Gad [17], Aksoy et al.[20], Kabak and Onuk [21], Beşoluk and Tıpırdamaz [23] in mammalian and by Lindsay [1] and Kaupp [22] in some fowl.

The *pars interventricularis* of *v. cardiaca sinistra* is single vein that beginning at the level of the notch of cardiac apex in fowl [1]. This vein is a double vein in 13 percent of ostrich [3]. The one of them lies paraconal interventriculer groove. The other one runs caudally to it on the wall of the ventriculus sinister. However, in the present study, the *pars interventricularis* was observed to be a single vein all samples. On the other hand, Lindsay [1] is reported that in 6 percent of fowl the *pars interventricularis* is not found.

In mammalian [5], two branches of paraconal interventricular vein is confirmed to be distributed to the wall of the ventriculus sinister that are called the left proximal collateral vein and the left distal collateral vein. In the investigated samples, there was not found the similar these veins. But, numerous fine branches of the *pars interventricularis* were observed instead of the two branches, as in birds [1,3], rabbit [12] , beaver [14] and mice [15].

Along its course, *v. cardiaca circumflexa sinistra* collects multiple the *vv. atriales* and *vv. ventriculares* from the atrium sinistrum and ventriculus sinister as indicated by the literature [1-4,22], respectively. One of the most interesting findings of the present study was that a vein of *vv. ventriculares* which was stronger than the others was found in samples. We thought that this vein was the same as left marginal ventricular vein in mammalian [5,12,14,17,20,21,23], according to its course.

In fowl, the *v. cardiaca media* is a prominent venous channel, which independently enters into the atrium dextrum [1]. In our samples, it opened into the *v. cava cranialis sinistra* as reported in rabbit [12,16], beaver [14], marsupial [15]. In birds [1,3,4] the *v. cardiaca media* is the strongest vein of the cardiac veins. Moreover, Bezuidenhout [3] reported that the *v. cardiaca media* is as a double vein in ostrich. In this study, the *v. cardiaca media* was weaker than the *v. cardiaca sinistra* and a single vein. This situation is similar to finding for rabbit [12], beaver [14] and marsupial [15]. Furthermore, in birds [1,3,4], rabbit [12,16], beaver [14] and marsupial [15], along its course, it was collected fine branches from the both ventricles, as in stork

In birds, the *vv. cardiacae dextrae (ventrales)* are constituted with the *vv. atriales, vv. ventriculares* and *vv. conalis* [1-3]. These veins are formed to the *v. cardiaca circumflexa dextra* that drains directly into the atrium dextrum in birds [1,3]. In the present study, the *vv. cardiacae dextrae* were constituted of a few *vv. ventriculares* opened into atrium dextrum separately. Thus, the *v. cardica circumflexa dextra* was not formed in stork, as in some fowl [1], beavers [14], mice [15] rabbits [17], marsupial [18]. In six specimens, one of veins collecting venous blood of the ventriculus dexter was stronger than the others. This vein was similar to vessel named as the right marginal ventricular vein in mammalian [5,12,20,21,23]. This strong vein has been also reported in mice [15] and marsupial [18]

In many mammals [5,12,14-17,19-21,23], main cardiac veins which are the great cardiac vein, middle cardiac vein, right cardiac veins and their secondary branches are well-defined in terms of the course they follow and the place they drain and that are present in a regular and specialised way, though they may display some variations. Unlike the mammals, as reported in the resources on birds [1,3,4,22] as well as in our samples, the size, number, distribution and origins of these secondary branches were observed to differ from sample to sample. For this reason, the identification of the secondary branches, except for these two strong veins, were not undertaken in our samples.

The venae cardiacae minimae carrying venous blood from myocardium to the heart chambers opened into the atrium dextrum [12,16] or to all of the heart chambers in mammalian [5,18,20]. In our samples, these veins were found constantly in the atrium dextrum and the which is similar to the findings reported by literature [1,3,12,16].

The venous drainage of the interventricular septum was provided mostly by the *vv. septales* of both the *pars interventricularis* of *v. cardiaca sinistra* and the *v. cardiaca media* in birds [1,3]. Although the *vv. cardiacae minimae* were made a small contribution to the venous drainage of the interventricular septum by means of the few thin, the *vv. septales* were primarily responsible for septal drainage in stork, which was similar to the cases in the fowl[1] and mammalian [12,20].

In conclusion, the venous drainage of the heart was consisted of the *v. cardiaca sinistra,* the *v. cardiaca media,* the *vv. cardiacae dextrae* and the *vv. cardiacae minimae.* The *v. cardiaca sinistra* was the largest vessel that drained of the heart. The origin and course of *v. cardiaca sinistra* and the *v. cardiaca media* of stork basically resembled the great cardiac vein and middle cardiac vein of the mammalian, respectively. One of the most interesting finding of the present study was that the two strong veins found in all samples, called the left marginal ventricular vein and right marginal ventricular vein, especially present in the mammalian. Because v. *cardiaca circumflexa dextra* was absent, veins formed the *vv. cardiacae dextrae* opened into atrium dextrum separately. The *vv. cardiacae minimae* emptied into both the atrium dextrum and the ventriculus dexter. There was no the coronary sinus.

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Kafkas Univ Vet Fak Derg
19 (4): 693-698, 2013
DOI: 10.9775/kvfd.2013.8678

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

Cirit Atlarında Vücut Ölçüleri [1] [2]

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Makale Kodu (Article Code): KVFD-2013-8678

Özet

Bu araştırmada, Erzurum ilinde cirit kulüplerinde bulunan erkek Arap atlarının, vücut ölçüleri ve cirit oyunlarındaki koşu süreleri belirlenmiştir. Bu parametreler üzerine, atların performanslarına göre oluşturulan grupların, yaşın ve vücut kondisyon skorunun (VKS) etkisi araştırılmıştır. Ayrıca koşu süreleri ile vücut ölçüleri arasındaki fenotipik korelasyonlar da hesaplanmıştır. Cidago yüksekliği (154.19±0.33), sağrı yüksekliği (154.27±0.35) ve göğüs derinliği (67.01±0.25) ortalama değerleri, diğer bazı at ırkları ve özellikle Arap atları için bildirilen değerlerden yüksek; sağrı uzunluğu (47.51±0.25), ön göğüs genişliği (38.41±0.21), göğüs çevresi (157.53±0.58), baş uzunluğu (54.75±0.20) düşük; ön incik çevresi (19.40±0.14) ölçüsü ise benzer veya yüksek bulunmuştur. Atların yarış performansı ve cirit atı sahiplerinin tercihine göre yapılan sınıflandırmada, en iyi cirit atlarının göğüs derinliği ve sağrı uzunluğunun daha yüksek olduğu belirlenmiştir. Atların yaşı arttıkça, ön göğüs genişliği ve ön incik çevresi ölçüsünde artış gözlenmiştir. VKS 3 ve 4 olan atlar arasında vücut ölçülerinde farklılık gözlenmemiştir. Vücut ölçüleri arasında önemli düzeyde fenotipik korelasyonlar bulunurken, koşu süresi ile incelenen vücut ölçüleri arasında ilişki tespit edilmemiştir. Koşu süreleri bakımından, VKS 3 olan atların (4.71±0.080) VKS 4 olan atlardan (4.96±0.048) daha hızlı koştuğu belirlenmiştir.

Anahtar sözcükler: *At, Cirit, Vücut ölçüleri, Koşu süreleri, Vücut kondisyon skoru*

Body Sizes of the Javelin Horses

Summary

In this research, the running times and body sizes of the male Arabian horses in Javelin Clubs of the Erzurum province were determined. The effects of the performance based groupings of horses, age and body condition score (BCS) were investigated on these parameters. The phenotypic correlations between the running times and body sizes were calculated. The average values of the withers height (154.19±0.33) , the height at the rump (154.27±0.35) and the depth of the chest (67.01±0.25) were found higher than those reported for some horse races, especially for the Arabian horses; the rump length (47.51±0.25), width of the front chest (38.41±0.21), chest circumference (157.53±0.58) and the length of the head (54.75±0.20) were found lower, and the front cannon bone circumference size (19.40±0.14) was found similar or higher. Horses having higher chest depth and rump length were determined to be preferred for javelin sport in the classification made according to the running performance of the horses and the preferences of the breeders. Increases in the width of the front chest and the front cannon bone circumference were observed as the age of horses advanced. No differences in body sizes were observed between BCS 3 and 4 horses. While there were many significant correlations between the body sizes, no relationship was detected between the examined body sizes and the running times. When investigated according to the running times, it was determined that BCS 3 horses (4.71±0.080) were running faster that BCS 4 horses (4.96±0.048).

Keywords: *Horse, Javelin, Body sizes, Pun time, Body condition score*

GİRİŞ

Türklerin en eski oyunlarından olan cirit [1] yakın zamana kadar Anadolu'nun birçok bölgesinde oynanmaktaydı. Ancak günümüzde sadece Erzurum, Uşak, Manisa, Sivas, Bayburt, Erzincan, Kars ve Malatya illerinde oynanmaktadır [2].

Eski yıllarda cirit atları Erzurum'da yetiştirilirken, günümüzde atlı cirit sporunda kullanılacak atlar, genellikle at yarışlarında başarılı olamayan veya çeşitli nedenlerle yarıştan çıkarılan erkek Arap atları arasından seçilmektedir [3].

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Atlarda tanımlayıcı bir ırk özelliği olan vücut ölçüleri, vücut yapısının uyumluluğunun değerlendirilmesinde [4] veya atların normal ve anormal gelişimin karşılaştırılmasında kullanılmaktadır [5]. Yarış atlarında vücut ölçüleri ile performansları arasında genetik korelasyon vardır. Atların seçiminde veya fiyatlarının belirlenmesinde de vücut ölçülerinden faydalanılabilir [6].

Atlarda vücut konformasyonu; kullanım yönü, genetik yapı ve beslenme durumuna göre şekillenmektedir [7,8]. Cirit atlarında, aniden hızlanma, durma ve dönme hareketleri düz koşu yapan yarış atlarına göre farklıdır [9]. Yarış atı, binek atı veya çekim atlarında vücut bölgelerinin gelişimi farklı olabilmektedir [10].

Farklı araştırıcılar tarafından Arap atları [4,6,11-18], farklı at ırkları [19-25] ve Türkiye'de yetiştirilen yerli atlardan alınan vücut ölçü değerleri [26-29] ile ilgili araştırmalar yürütülmüştür. Atlarda koşu hızı üzerine etkili faktörler üzerine çalışmalar da yapılmıştır [30-32]. Ancak cirit atlarında, vücut ölçüleri ile ilgili herhangi bir çalışmaya rastlanılmamıştır.

Erzurum bölgesinde yapılan bu çalışmada, cirit atlarının vücut ölçüleri ve cirit oyunlarındaki koşu süreleri belirlenerek, bu parametreler üzerine; anket bilgilerine ve cirit oyunlarında alınan derecelere göre oluşturulan skor gruplarının, yaşın ve vücut kondisyon skorunun etkisi araştırılmıştır.

MATERYAL ve METOT

Araştırma Erzurum merkezde 13 spor kulübüne kayıtlı, 94 erkek Arap atına ait veriler kullanılarak gerçekleştirilmiştir. Cirit atlarının büyük çoğunluğunun (%91.26) Arap atı olması ve cirit oyunlarında erkek atların (%100) tercih edilmesi nedeniyle, araştırma erkek Arap atları üzerinde yürütülmüştür. Cirit müsabakaları sırasında 40 adet ata ait düz koşu süresi ölçülmüştür.

Vücut ölçüleri, atlar beton ya da düz bir zemin üzerine getirilerek normal bir pozisyonda durmaları temin edildikten sonra alınmıştır. Vücut ölçülerinden cidago yüksekliği, sağrı yüksekliği, göğüs derinliği, ölçü bastonu ile (Hauptner®), sağrı uzunluğu ve ön göğüs genişliği ölçü pergeli (Elmark®) ile, sırt uzunluğu, beden uzunluğu, göğüs çevresi, boyun kalınlığı, baş uzunluğu, ön incik çevresi, carpal eklemin çevresi ve tarsal eklemin çevresi ölçü şeridi ile ölçülmüştür. Cirit yarışlarında atların koşu sürelerini belirlemek için tripot ile sabitlenmiş video kamera (Sony DCR-SR58®) kullanılmıştır. Atların koşu süreleri kronometre ile belirlenmiştir.

Aşağıda belirtildiği şekilde skor, yaş ve VKS grupları oluşturulmuştur.

Skor grupları: Cirit atı sahiplerinden elde edilen bilgiler ve Geleneksel Spor Dalları Federasyonunun düzenlemiş olduğu müsabakalarda alınan takım derecelerine göre atları 3 gruba ayrılmıştır. Skor grupları belirlenirken üç farklı değerlendirmenin puan toplamları dikkate alınmıştır: 1. değerlendirmede "*kulübünüzdeki atlardan 6 tanesini iyiden kötüye sıralayınız*" sorusuna verilen cevaba göre 1. sıradaki ata 6 puan, 6. sıradaki ata 1 puan verilmiştir. 2. değerlendirmede, "*kulüp dışı atlardan 6 tanesinin isimlerini iyiden kötüye sıralayınız*" sorusuna verilen cevaba göre 1. sıradaki ata 6 puan, 6. sıradaki ata 1 puan verilmiştir. Her atın aldığı toplam puan hesaplanarak 6'lı puanlamaya çevrilmiştir. 3 değerlendirmede ise yapılan resmi müsabakalar sunucunda birinci takımın bütün atları 3 puan, ikinci takım 2.5 puan, üçüncü takım 2 puan, dördüncü takım 1.5 puan, beşinci takım 1 puan alırken, sonuncu takımın bütün atları da 0.5'er puan almıştır. Bu üç değerlendirmeye ait puanların toplamı ile skor grupları oluşturulmuştur. Cirit atları, iyi performanstan, zayıf performansa sıralanarak, skor gruplarında toplam puanı 7-15 arası olan cirit atları 1. grupta (iyi), puanı 4-6.5 arası olanlar 2. grupta (orta) ve puanı 0-3.5 arası olanlar ise 3. grupta (zayıf) yer almıştır.

Yaş grupları: 4-5, 6-7, 8-9 ve 10 yaş ve üzeri yaştaki atlar yaş gruplarını oluşturmuştur.

Vücut kondisyon skoru grupları: Çalışmada cirit atlarına ait VKS, Carroll ve Huntington [33] tarafından bildirilen Avustralya sistemine göre 0 puan ile 5 puan arasında skorlama yapılarak belirlenmiştir. Cirit oyunlarında kullanılan atların VKS değerleri 3 ve 4 olarak tespit edilmiştir. VKS 3 olan atlar grup 3, VKS değeri 4 olan atlar ise grup 4 olarak tanımlanmıştır.

Cirit oyunları sırasında, atların rakip oyuncudan kaçtığı veya rakip oyucuya yetişmek için kısa mesafede hızlanarak koştukları alan olan, cirit sahasındaki atış alanı çizgisi ile orta çizgi arasındaki mesafede (51 metre) 40 adet ata ait koşu süreleri ölçülmüştür. Koşu süreleri her cirit atına ait en az 10 kayıtın ortalamaları alınarak değerlendirilmiştir.

Veriler skor grubu, yaş grubu, VKS grupları şeklinde tanımlanmıştır. Vücut ölçüleri ve koşu sürelerine etkili faktörler aşağıdaki matematik modelle değerlendirilmiştir.

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

Bu modelde yer alan terimlerden;

Y_{ijkl} = i. skor grubu, j. yaş grubu, k. vücut kondisyon skoru gurubundaki 1. hayvana ait fenotipik değeri, μ = Populasyon ortalamasını, a_i = Skor etkisini (1, 2, 3), b_j = Yaşın etkisini (4-5, 6-7, 8-9, 10<), c_k = Vücut kondisyon skorunun etkisini (3, 4), e_{ijkl} = Şansa bağlı hatayı temsil etmektedir.

Verilerin istatistiksel analizi için SPSS 10.0 paket program [34] kullanılmıştır. Alt grup ortalamalarının karşılaştırılmasında Duncan çoklu karşılaştırma testi [35] uygulanmıştır. İncelenen özellikler arası ilişkilerin belirlenmesinde parametrik doğrusal korelasyon tekniğinden (Pearson Korelasyonu) yararlanılmıştır [35].

BULGULAR

Erzurum ilindeki cirit atalarının farklı skor, yaş ve VKS gruplarına göre incelenen vücut ölçüleri ve koşu sürelerine ait ortalamaları ile çoklu karşılaştırma testi sonuçları *Tablo 1* ve *Tablo 2*'de, koşu süresi ve vücut ölçüleri arasındaki fenotipik korelasyonlar ise *Tablo 3*'te verilmiştir.

TARTIŞMA ve SONUÇ

Vücut Ölçüleri: Çalışmada atlarda cidago yüksekliği için bulunan ortalama değer (154.19±0.33 cm); Andolusian atları [25] için belirtilen değerle benzerlik gösterirken, İspanyol [15], İran [6,16], Mısır Arap atları [4], Türkiye'de yetiştirilen Arap atları [13,17,18] ve yerli atlarda [26-29] tespit edilen değerlerden daha yüksek bulunmuştur. Ayrıca İngiliz aygırları [13], Noriker [20], İspanyol Andolusian [22], Finn [23] ve Murgese [19]. Mangalarga Marchador [21], Bardigrano atlarının [24] cidago yüksekliği bu çalışmadaki cirit atlarında tespit edilen değerden daha düşüktür. Bu durum cirit atlarının seçiminde daha yüksek cidagolu Arap atlarının tercih edilmesinin bir sonucudur. Adımların genişliği ve sırtın sağlamlığına yardım ettiğinden, hızlı yürüyüşlü binek atlarda uzun ve yüksek cidago istenir. Cidago alçak olduğunda, eyerin kayma ihtimali de çoktur [36]. Yaşın cidago yüksekliği üzerine etkisi; Van, Kars yerli [26,28], Ankara ve İzmir hipodromlarındaki Arap atlarında [13] bu araştırma bulgularına paralel olarak önemsiz (P>0.05) bulunurken, Murgese atlarında [19] çok önemli, İngiliz atlarında [13], Çifteler, Anadolu, Karacabey, Mahmudiye ve Sultansuyu Tarım İşletmesi Arap atlarında [11,17,18], İran Arap atlarında [6] ise istatistiksel olarak önemli olduğu saptanmıştır. Araştırmada, yaşın cidago yüksekliği üzerine etkisinin önemsiz çıkmasının sebebi bu çalışmada incelenen cirit atlarının gelişimini tamamlamış (4 yaş ve üzeri) olmasından kaynaklanmıştır.

Tablo 1. *Farklı skor, yaş ve VKS gruplarındaki cirit atlarına ait bazı vücut ölçüleri (cm)*
Table 1. *Body sizes of javelin horses at different score, age, and VKS groups (cm)*

Grup [1]	Skor			Yaş				Vücut Kondisyon Skoru		Genel Ortalama
	1	2	3	4-5	6-7	8-9	10<	3	4	
N	32	27	35	29	29	22	14	33	61	
CY	154.58±0.57	154.35±0.58	153.65±0.50	155.17±0.55	154.04±0.53	154.35±0.63	153.21±0.80	154.04±0.56	154.34±0.38	154.19±0.33
SY	154.61±0.60	154.61±0.62	153.59±0.53	**155.19±0.58** [a]	**153.97±0.56** [ab]	**155.09±0.66** [a]	**152.84±0.84** [b]	154.51±0.59	154.04±0.40	154.27±0.35
SU	**48.05±0.42** [a]	**47.82±0.43** [a]	**46.67±0.37** [b]	47.39±0.40	47.43±0.39	47.79±0.46	47.45±0.59	47.43±0.41	47.60±0.28	47.51±0.25
SIU	80.71±1.12	82.98±1.15	82.66±0.98	82.18±1.07	82.24±1.05	82.73±1.24	81.31±1.57	81.44±1.10	82.79±0.75	82.12±0.66
GD	**67.96±0.43** [a]	**66.56±0.44** [b]	**66.50±0.38** [b]	67.44±0.41	67.57±0.40	66.41±0.48	66.61±0.61	66.99±0.43	67.03±0.29	67.01±0.25
GC	157.88±0.99	157.59±1.01	157.13±0.87	157.35±0.95	158.05±0.92	156.98±1.09	157.75±1.39	156.78±0.97	158.28±0.66	157.53±0.58
GG	38.62±0.36	38.68±0.37	37.91±0.31	**37.90±0.34** [b]	**38.00±0.33** [b]	**38.53±0.40** [ab]	**39.20 ±0.50** [a]	38.42±0.35	38.39±0.24	38.41±0.21
BEU	149.83±0.10	149.07±1.03	147.43±0.88	149.19±0.96	147.79±0.93	149.74±1.14	148.39±1.41	149.25±0.98	148.30±0.67	148.78±0.59
BK	84.35±1.05	83.64±1.08	81.51±0.93	**84.26±1.01** [ab]	**82.43±0.98** [ab]	**81.11±1.17** [b]	**84.86±1.48** [a]	83.68±1.04	82.66±0.70	82.17±0.62
BAU	**55.16±0.35** [a]	**54.01±0.36** [b]	**55.07±0.31** [a]	55.04±0.33	54.84±0.32	54.22±0.38	54.89±0.49	54.69±0.34	54.80±0.23	54.75±0.20
IC	19.35±0.24	19.34±0.24	19.52±0.21	**19.14±0.23** [b]	**19.30±0.22** [b]	**19.20±0.26** [b]	**19.97±0.34** [a]	19.30±0.24	19.51±0.16	19.40±0.14
CC	**30.64±0.31** [ab]	**31.26±0.32** [a]	**30.36±0.27** [b]	30.62±0.30	30.56±0.29	31.23±0.34	30.62±0.43	30.68±0.30	30.83±0.21	30.76±0.18
TC	40.96±0.40	40.77±0.41	40.34±0.35	41.08±0.38	40.62±0.37	40.63±0.44	40.42±0.56	40.75±0.39	40.63±0.26	40.69±0.23
BU/CY	0.97	0.97	0.96	0.96	0.96	0.97	0.97	0.97	0.96	0.96

[a,b,c] Aynı satırda farklı harfleri taşıyan ortalamalar arası farklılıklar önemlidir (P<0.05), ***[1]:*** *Skor:* ***1****= İyi,* ***2****= Orta,* ***3****= Zayıf, Vücut Kondisyon Skoru:* ***3****= VKS 3,* ***4****= VKS 4 olan ataları belirtmektedir,* ***CY:*** *Cidago yüksekliği,* ***SY:*** *Sağrı yüksekliği,* ***SU:*** *Sağrı uzunluğu,* ***SIU:*** *Sırt uzunluğu,* ***GD:*** *Göğüs derinliği,* ***GC:*** *Göğüs çevresi,* ***GG:*** *Ön göğüs genişliği,* ***BEU:*** *Beden uzunluğu,* ***BK:*** *Boyun kalınlığı,* ***BAU:*** *Baş uzunluğu,* ***IC:*** *Ön incik çevresi,* ***CC:*** *Carpal eklem çevre uzunluğu,* ***TC:*** *Tarsal eklem çevre uzunluğu,* ***BU/CY:*** *Beden uzunluğunun cidago yüksekliğine oranı*

Tablo 2. *Farklı skor, yaş ve VKS gruplarındaki cirit atlarına ait koşu süreleri (sn)*
Table 2. *Running time of javelin horses at different score, age, and VKS groups (sec)*

Özellikler	N	Gruplar	*X*	SxX	(P<)[1]
Skor	14	1	**4.70** [b]	0.067	*
	11	2	**4.97** [a]	0.060	
	15	3	**4.84** [b]	0.101	
Yaş	12	4-5	4.74	0.087	ÖS
	11	6-7	4.90	0.062	
	9	8-9	4.77	0.071	
	8	10<	4.93	0.098	
VKS	13	3	4.71	0.080	**
	27	4	4.96	0.048	

[1] ***ÖS:*** *P>0.05,* * *P<0.05,* ** *P<0.01,* ***[a,b]:*** *Aynı sütunda farklı harfleri taşıyan ortalamalar arası farklılıklar önemlidir (P<0.05)*

Sağrı yüksekliği için bulunan ortalama (154.27±0.35 cm) değer; Finn atlarında [23] bildirilen değerlere benzer olarak bulunurken, Mısır [4], İzmir Şirinyer ve Ankara 75. Yıl Hipodromundaki Arap aygırlarında [13], Anadolu, Karacabey, Sultansuyu, Mahmudiye Tarım İşletmelerinde yetiştirilen Arap atları [18], Mangalarga Marchador atları [21] ve bazı yerli atlarda [26-28] bildirilen değerlerden yüksek olarak saptanmış, TJK bünyesindeki İzmir Şirinyer ve Ankara 75. Yıl Hipodromundaki İngiliz aygırı [13] ve Noriker atlarından [20] ise düşük bulunmuştur. Bu sonuçlara göre Cirit oyunlarında sağrı yüksekliği daha fazla olan Arap atların tercih

Tablo 3. *Koşu süresi ve vücut ölçüleri arasındaki fenotipik korelasyonlar*
Table 3. *Phenotypic correlations between measurements and running time*

Özellikler	KS	CY	SY	SU	SIU	GD	GC	GG	BEU	BK	BAU	IC	CC
CY	-0.23												
SY	-0.30	**0.70****											
SU	-0.11	0.11	0.17										
SIU	-0.08	0.25	**0.39***	0.02									
GD	-0.30	0.30	0.23	-0.04	**0.39***								
GC	-0.05	0.17	0.19	**0.24***	**0.45****	**0.38***							
GG	-0.14	0.08	0.10	**0.35***	0.09	-0.10	0.13						
BEU	-0.21	**0.48****	**0.62****	**0.47****	**0.5****	**0.43****	**0.56****	0.24					
BK	-0.07	0.23	**0.36***	0.18	0.02	0.24	0.30	0.18	0.22				
BAU	0.06	0.28	-0.01	-0.05	-0.18	0.25	0.14	0.22	0.20	0.18			
IC	-0.25	0.21	0.15	0.17	0.17	**0.46****	**0.32***	0.02	0.19	**0.35***	0.26		
CC	-0.30	0.28	0.19	0.27	0.22	**0.46****	**0.41****	0.11	**0.41****	0.17	0.15	**0.73****	
TC	-0.17	0.23	0.14	0.10	**0.42****	**0.54****	**0.34***	0.17	**0.50****	0.28	0.18	**0.34***	**0.50****

*** P<0.01, * P<0.05, **CY:** Cidago yüksekliği, **SY:** Sağrı yüksekliği, **SU:** Sağrı uzunluğu, **SIU:** Sırt uzunluğu, **GD:** Göğüs derinliği, **GC:** Göğüs çevresi, **GG:** Ön göğüs genişliği, **BEU:** Beden uzunluğu, **BK:** Boyun kalınlığı, **BAU:** Baş uzunluğu, **IC:** Ön incik çevresi, **CC:** Carpal eklem çevre uzunluğu, **TC:** Tarsal eklem çevre uzunluğu. **KS:** Koşu süresi*

edildiği anlaşılmaktadır. Sağrı yüksekliğine yaşın etkisi Van yerli atlarında [28], Anadolu, Karacabey, Mahmudiye Tarım İşletmelerinde yetiştirilen Arap atlarında [18] elde edilen, araştırma bulgularına benzer olarak önemli (P<0.05) bulunurken, Ankara ve İzmir hipodromlarında yapılan çalışmada Arap (3-4 yaş) ve İngiliz (3-5 yaş) atlarında ise önemsiz (P>0.05) bulunmuştur [13]. Alarslan'ın [28] belirttiği gibi 10 yaş ve üzeri atlarda yaşın ilerlemesine bağlı olarak sağrı yüksekliğinde düşüş gözlenmiştir.

Sağrı uzunluğu için tesbit edilen 47.51±0.25 cm değer İspanyol Arap atları [15] için bildirilen değere benzer, Mangalarga Marchador atlarında [21] bildirilenden ise düşüktür. Cirit atlarında sağrı uzunluğu farklı skor gruplarında istatistik olarak önemli (P<0.05) farklılık göstermiştir. En uzun sağrılı atların birinci ve ikinci skor grubunda bulunduğu belirlenmiştir. Sağrının uzun olması üzerindeki kasların uzun olmasını ve hayvanın daha hızlı koşmasını sağlar [10]. Cirit oyunlarında uzun sağrılı atlar tercih edil-mektedir.

Göğüs derinliği için bulunan ortalama değer (67.01±0.25 cm) bazı yerli [26-29] ve İspanyol Andolisian [22] atlarında bildirilen değerlerden yüksek olarak bulunurken; Sultansuyu ve Mahmudiye Tarım İşletmelerinde yetiştirilen Arap atlarınada [18] bildirilen değerlere benzer, Anadolu, Karacabey Tarım İşletmesi Arap atları [18], Mısır'daki Arap aygırları [4] ve Noriker atlarında [20] bildirilen değerlerden ise düşük olduğu tespit edilmiştir. Skor grupları incelendiğinde göğüs derinliğinin, en iyi atların bulunduğu birinci skor grubunda daha yüksek olduğu tespit edilmiştir. Akciğer ve kalbin gelişme derecesi, göğüs boşluğunun büyüklüğü ile ilişkili olduğu için göğsün gelişme derecesi önemlidir [7,11]. Cirit atlarının seçiminde göğüs derinliği gelişmiş atlar tercih edilebilir. Göğüs derinliğine yaşın etkisi Van yerli atlarında [28] elde edilen sonuca benzerlik göstererek önemsiz (P>0.05) bulunurken, Kars yerli [26] (P<0.05) ve farklı tarım işletmelerinde yetiştirilen Arap atlarında [18], istatistiksel olarak önemli (P<0.01) olduğu belirtilmiştir.

Çalışmada 157.53±0.58 cm olarak tesbit edilen göğüs çevresi ölçüsü, bazı yerli atlarda [26,28] bildirilen değerlerden daha yüksek, Anadolu, Karacabey, Mahmudiye [18] ve Sultansuyu [17,18] Tarım İşletmelerinde yetiştirilen Arap atlarında, Mısır'da yetiştirilen [4] ve yaşı büyük olan Arap aygırlarında [11], Noriker [20], Van yerli [27], Murgese [19] ve Bardigrano atlarında [24] bildirilen değerden ise daha düşük bulunmuştur. Göğüs çevresi bakımından bu çalışmadaki skor, yaş ve VKS gruplarında istatistik farklılık önemsizdir (P>0.05). Arap atlarında (3-4 yaşlı) [14], Kars yöresi yerli atlarda [26] ve Sultansuyu Tarım İşletmesi Arap atlarda [17] yaşın etkisinin önemli (P<0.05) olduğu bildirilmiştir. Göğüs çevresinin genişliği, içindeki organların gelişme durumu ve büyüklüğü hayvanların sağlığı ve verimleri üzerine önemli derecede etkilidir [7].

Ortalama ön göğüs genişliği değeri (38.41±0.21); Kars yerli atlarına [26] benzer tespit edilirken; İspanyol Arap [15] ve Türk Rahvan atlarından [15] yüksek olarak bulunmuş, Mısır Arap aygırlarından [4], Noriker [20], Mangalarga Marchador [21] ve Van yerli atlarından [27] düşük bulunmuştur. Cirit atlarında ön göğüs genişliği incelendiğinde yaş gruplarındaki fark istatistiksel olarak önemli (P<0.05) bulunmuştur. On ve üzeri yaştaki atlarda ön göğüs genişliğinin daha yüksek olduğu tespit edilmiştir.

Beden uzunluğu için ortalama değer (148.78±0.59 cm), Mısır'da yetiştirilen Arap aygırlarına [4], İspanyol Arap atlarına [15] benzerlik gösterirken, yerli atlar [26-29] için bildirilen değerlerden daha yüksek bulunmuştur. Diğer yandan bu çalışmada beden uzunluğu için bildirilen değerin İran Arap atları [16], İzmir Şirinyer ve Ankara 75. Yıl hipodromundaki Arap aygırları [13], farklı tarım işletmelerinde yetiştirilen Arap atları [18], İngiliz aygırları [3], Noriker [20], Mangalarga Marchador [21], İspanyol Andolisian [22] ve Finn atları [23] için bildirilen değerden ise düşük olduğu saptanmıştır. Beden uzunluğu ortalamaları arasındaki farklılıklar yaş, ırk, bölge ve işletme farklılıklarından kaynaklanabilir. Cirit

atlarında skor grupları arasında beden uzunluğu bakımından fark gözlenememesine (P>0.05) rağmen cirit atlarının beden uzunlunun yerli atlar hariç birçok Arap atı ve farklı at ırklarından düşük olması, cirit atı seçiminde beden uzunluğu fazla olan atların tercih edilmediğini göstermektedir. Cirit atlarında beden uzunluğunun cidago yüksekliğine oranı ortalama 0.96 bulunmuştur bu değer farklı tarım işletmelerinde yetiştirilen Arap atları [18] için bildirilen değerlerden (1.01-1.04) düşüktür. Bu durum cirit atlarında cidago yüksekliği fazla, beden uzunluğu daha az olan Arap atlarının tercih edildiğini göstermektedir. İncelenen beden uzunluğu değerleri için skor, yaş ve VKS gruplarında istatistiksel olarak farklılık belirlenmemiştir (P>0.05). Yaşın beden uzunluğu üzerine etkisi, bu araştırma bulgularına paralel olarak; Van yerli atları [28], Arap ve İngiliz atlarında [13] istatistiksel olarak önemsiz (P>0.05) bulunurken, Altındere [27] ve Sultansuyu [12,17] tarım işletmelerinde yetiştrilen atlarda önemli (P<0.05) farklı tarım İşletmelerinde yetiştirilen Arap atlarında [18] çok önemli (P<0.01) bulunmuştur. Safkan Arap atlarının 4 yaşında büyümelerini tamamladıkları belirtilmiştir [18]. Yaşın etkisinin önemsiz çıkması araştırmada yer alan atların 4 yaş üzeri olmasından kaynaklanabilir.

Atlarda boyun kalınlığı ortalama 82.17±0.62 cm'dir. Boyun kalınlığı yaş gruplarında istatistiksel olarak farklı bulunmuştur (P<0.05). Boyun kalınlığı 10 ve üzeri yaştaki atlarda en yüksek değeri almıştır. Boyun kalınlığı incelendiğinde skor ve VKS gruplarında istatistiksel farlılık saptanmamıştır (P>0.05). Boyunun kendisi kuru, kasları iyi gelişmiş ve yağlanmamış olmalıdır, bu boyun şekli bütün verim yönleri için elverişli sayılmaktadır [36].

Baş uzunluğu (54.75±0.20 cm) için ortalama değerin; İspanyol Arap atları [15], Kars yöresindeki yerli atlarında bildirilen değerlere [26] benzer; İran Arap atları [6], Noriker atları [20], Mangalarga Marchador atları [21], Uzunyayla atları ve Türk Rahvan atında [29] bildirilen değerden ise düşük olduğu tespit edilmiştir. Başın yapısı hayvanların ırkı, verim ve hizmet yönüne göre değişir. Cirit atlarında baş uzunluğunun, skor gruplarında istatistiksel olarak farklı olduğu tespit edilmiştir (P<0.05). Baş uzunluğunun, 1. skor grubunda en yüksek değere sahip olduğu belirlenmiştir. Kars yöresindeki değişik yaş ve cinsiyetteki Türk yerli atlarda yapılan çalışmada baş uzunluğu üzerine yaşın etkisinin istatistiksel olarak önemli (P<0.05) olduğu belirlenmiştir [32]. Ancak bu çalışmada, farklı yaştaki atların baş uzunlukları arasında istatistiksel olarak fark bulunmamıştır (P>0.05). Başın yapısı yarış atlarında genelde asil yani küçük, zarif yapılı ve kuru; ağır atlarda ise iri, kaba ve etlidir [7].

Bu çalışmada ön incik çevresi için tespit edilen değer (19.40±0.14 cm); Safkan Arap atı için bildirilen bazı değerlere benzer [11,15,17,18], bazı değerlerden [4,17] ise yüksektir. Bu durum cirit atlarında ön incik çevresi bakımından değişikliğin olmadığını göstermektedir. Diğer yandan, bu çalışmada ön incik çevresi içi bulunan değer, yerli atlar için bildirilen değerlerden yüksek iken [26-29], bazı yabancı at ırkları [19,20,22,24] için bildirilen değerlerden ise düşüktür. Ön inciğin kısa ve geniş olması dayanıklılığı artıracağından aranılan özelliktir [36]. Yaş gruplarında ön incik çevresi incelendiğinde istatistiksel farklılık önemli bulunmuş olup (P<0.05), 10 ve üzeri yaştaki atların en kalın inciğe sahip olduğu saptanmıştır. Bu araştırma bulgularına paralel olarak; Çifteler [11] ve Sultansuyu [17] Tarım İşletmesinde yetiştirilen Türk Arap atlarında yaşın etkisinin önemli (P<0.05) olduğunu bildirilmiştir. Diğer yandan bu araştırma bulgularından farklı olarak Kars yöresi yerli atlarda yapılan çalışmada ön incik çevresi üzerine yaşın etkisinin önemsiz (P>0.05) olduğu belirlenmiştir [26].

Koşu Süreleri: Atların 51 metrelik mesafeyi ortalama 4.83±0.048 sn'de koştuğu tespit edilmiştir. En çok tercih edilen atların bulunduğu 1.skor grubundaki atların koşu süreleri 3. skor grubundakilere benzer, 2. skor grubundakilerden ise düşüktür. Atların koşu hızı, atın yaşı, eğitmeni, binicisi, hava şartları, hipodromun durumu, taşınan ağırlık ve atın sağlık durumu gibi birçok çevresel faktöre bağlı olabilir [31]. Koşu süreleri arasında varyasyonun düşük olması nedeniyle hayvan sayısının veya koşu mesafesinin artırılması isabetli olacaktır. At yarışlarında yarış hızının müsabakada ya da sezonda elde ettiği başarıyı ifade ettiği belirtilmektedir [32]. Cirit oyunlarında atların kısa sürede hızlanarak rakip cirit oyuncusundan kaçması veya yakalaması için koşu hızı önemlidir. Pilarski ve ark.[30] yaşın, atların hızı üzerine etkisinin olduğunu belirtmiş ancak bu çalışmada farklı yaş gruplarında koşu süreleri bakımından farklılık istatistiksel olarak önemsiz (P>0.05) çıkmıştır.

Vücut kondisyon skoru 3 olan atların hızı, VKS 4 olan atlardan yüksek çıkmıştır (P>0.01). Avustralya sistemine göre de VKS 3 olan atların performansları yüksektir [37]. Cirit atlarının dengeli bir şekilde beslenerek cirit sezonu öncesi ve sonrası VKS'nun 4 yerine 3'te tutulması daha yüksek performans sağlayabilir.

Fenotipik Korelasyonlar: Cirit atlarından alınan vücut ölçüleri arasındaki fenotipik korelasyon değerlerinden cidago yüksekliği ile sağrı yüksekliği, beden uzunluğu ve boyun uzunluğu arasında istatistiksel olarak önemli veya çok önemli pozitif ilişki bulunmuştur. Benzer şekilde Antalyalı [14] yapmış olduğu çalışmada cidago yüksekliği ile sağrı yüksekliği (0.59) arasında çok önemli ilişkinin olduğunu bildirmiştir.

Cidago yüksekliği ile göğüs derinliği (0.302), göğüs genişliği (0.077) ve göğüs çevresi (0.171) arasında istatistik olarak bir ilişki bulunmamasına rağmen Bayram ve ark.[27] ile Kırmızıbayrak ve ark.[26] söz konusu vücut ölçüleri ile cidago yüksekliği arasında önemli ve çok önemli derecelerde ilişkilerin bulunduğunu saptamışlardır. Cidago yüksekliği ile incik çevresi (0.212) arasında belirlenen korelasyon istatistik olarak önemli bulunmazken, Antalyalı [14], Bayram ve ark.[27], Suantama ve ark.[23] ve Sadek ve ark.[4] önemli ve çok önemli derecede korelasyonlar tespit etmişlerdir.

Yapılan çalışmada sağrı yüksekliği ile beden uzunluğu (0.620) arasında istatistiksel olarak çok önemli (P<0.01) düzeyde pozitif korelasyon bulunmuştur. Kırmızıbayrak ve ark.[26] ile Suontama ve ark.[23] bu çalışmaya benzer olarak bu ilişkiyi istatistiksel olarak çok önemli bulmuşlardır. Sağrı yüksekliği ile göğüs genişliği (0.101) arasındaki ilişki Bayram ve ark.'nın [27] bulgularına benzer olarak istatistiksel olarak önemsiz bulunmuştur.

Antalyalı [14] ile Sadek ve ark.[4] bu çalışmaya benzer olarak beden uzunluğu ile göğüs çevresi arasındaki korelasyonu istatistiksel olarak önemli bulmuşlardır.

İncik çevresi ile göğüs derinliği (0.458) arasında çok önemli (P<0.01), boyun kalınlığı (0.346) ve göğüs çevresi (0.324) arasında önemli (P<0,01) pozitif bir korelasyon olduğu belirlenmiştir. Antalyalı [14] incik çevresi ile göğüs çevresi (0.075) arasındaki ilişkiyi istatistiksel olarak önemsiz bulmuştur. Bayram ve ark.[27] incik çevresinin göğüs derinliği (0.11) ve göğüs çevresi (0.28) arasındaki ilişkiyi önemli ve çok önemli olduğunu belirtmişlerdir. Suontama ve ark.[23] incik çevresi ve göğüs çevresi (0.58) arasındaki korelasyonu istatistiksel olarak çok önemli düzeyde bulmuştur. İncik çevresi ile carpal eklem çevre uzunlukları arasındaki yüksek dereceli pozitif ilişki (0.74) cirit atı seçiminde incik çevresi ölçüsü yanında carpal eklem cevre ölçüsünün de dikkate alınabileceğini göstermektedir.

Sonuç olarak cirit atları seçiminde yüksek cidago ve sağrılı, beden uzunluğu daha kısa olan Arap atları tercih edilmektedir. Cirit oyunlarında performansı iyi olan atlarda, göğüs derinliği ve sağrı uzunluğu daha fazladır. Cirit atı seçiminde bu ölçüler dikkate alınmalıdır. Cirit oyunlarında atların VKS'sinin 3 olması, performanslarını olumlu etkileyebilir. Cirit atlarında vücut ölçüleri arasındaki fenotipik korelasyonlar, bu karakterlerin biri bakımından üstün değerli bireylerin seçilmesi ile seçilen bireylerde ikinci karakterin ne durumda olacağı hakkında fikir vermesi açısından önemlidir.

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Kafkas Univ Vet Fak Derg
19 (4): 699-703, 2013
DOI: 10.9775/kvfd.2013.8692

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

Effect of Varieties on Potential Nutritive Value of Pistachio Hulls

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Makale Kodu (Article Code): KVFD-2013-8692

Summary

The aim of this study was to determine the potential nutritive value of pistachio hulls obtained from six different varieties using chemical composition and *in vitro* gas production technique. *In vitro* gas productions of pistachio hulls were determined at 0, 3, 6, 12, 24, 48, 72 and 96 h incubation times and their gas production kinetics were described. There is considerable variation among pistachio hulls obtained from six different varieties in terms of chemical composition and *in vitro* gas production, metabolisable energy (ME) and organic matter digestibility (OMD). Dry matter (DM) contents of pistachio hulls ranged 26.45 to 29.25%. Ash content of pistachio hulls ranged from 8.50 to 19.86%. The crude protein (CP) contents of pistachio hulls ranged 7.27 to 14.99%. The hulls from Kırmızı, Ohadi and Keten Gömlegi (KG) had significantly higher CP contents than the others. The NDF and ADF contents of pistachio hulls ranged from 18.25 to 22.49% and 14.32 to 18.29% respectively. The pistachio hulls from Ohadi and Uzun had significantly higher NDF and ADF contents than the others. The CT contents of pistachio hulls ranged from 2.03 to 2.63%. The hulls from Beyaz Ben (BB) had significantly higher CT content than that of KG. The gas production rate ranged from 0.071 to 0.107%. The gas production rate of pistachio hulls from Sultani and KG were significantly higher than the others. The potential gas production of pistachio hulls ranged from 65.92 to 73.46 mL. The potential gas production of pistachio hulls from BB and Ohadi were significantly higher than the others. The ME and OMD contents of pistachio hulls ranged from 9.76 to 11.05 MJ/kg DM and 69.00 to 74.50% respectively. The ME contents of pistachio hulls from BB and Ohadi were significantly higher than those of Kırmızı, Uzun and KG whereas the OMD contents of pistachio hulls from Ohadi were significantly higher than those of pistachio hulls for Kırmızı, Sultani, Uzun and KG. In conclusion, chemical characterization with CP, OMD and ME suggests that the pistachio hulls had moderate level of CP concentration and was quite digestible therefore it can be said that pistachio hulls will provide feedstuffs of an acceptable quality for ruminant animals.

Keywords: *Chemical composition, Condensed tannin, Digestibility, Nutritive value, In vitro gas production, Pistachio hulls*

Varyetenin Antep Fıstığı Dış Kabuğunun Potansiyel Besleme Değerine Etkisi

Özet

Bu çalışmanın amacı, farklı varyetelerden elde edilen Antep fıstığının dış kabuğunun potansiyel besleme değerini kimyasal kompozisyonu ve *in vitro* gaz üretim tekniğini kullanarak belirlemektir. *İn vitro* gaz ölçümleri fermentasyonun başlamasından sonra 3, 6, 12, 24, 48, 72 ve 96 saatlerinde yapılmıştır. Farklı varyetelerden elde edilen Antep fıstığı kabuğunun kompozisyonları, *in vitro* gaz üretimi, metabolik enerji içeriklerinde ve organik madde sindirim dereceleri arasında önemli varyasyonlar vardır. Antep fıstığı kabukların kuru madde içeriği %26.45 - %29.25 arasında değişmiştir. Kül içeriği ise %8.50 - %19.86 arasında değişmiştir. Antep fıstığı kabukların ham protein içeriği %7.27 - %14.99 arasında değişmiştir. Kırmızı, Ohadi ve keten gömleği varyeteleri en yüksek protein içeriğine sahip olmuştur. Antep fıstığı kabukların NDF ve ADF içerikleri sırasıyla %18.25 - %22.49 ve %14.32 - %18.29 arasında değişmiştir. Ohadi ve Uzun varyeteleri en yüksek NDF ve ADF içeriğine sahip olmuştur. Antep fıstığı kabukların kondense tanen içerikleri %2.03 - %2.63 arasında değişmiş olup Beyaz Ben varyetesi en yüksek tanen içeriğine sahip olmuştur. Antep fıstığı kabukların gaz üretim hızı %0.071 ile %0.107 arasında değişmiş olup en yüksek gaz üretim hızına Sultani ve Keten Gömleği varyeteleri sahip olmuşlardır. Antep fıstığı kabukların potansiyel gaz üretim miktarları 65.92 ile 73.46 ml arasında değişmiş olup en yüksek gaz üretim miktarına Beyaz Ben ve Ohadi varyeteleri sahip olmuştur. Antep fıstığı kabukların metabolik enerji ve organik madde sindirim dereceleri sırasıyla 9.76 ile 11.05 MJ/kg KM, %69.00 - %74.50 arasında değişmiştir. Beyaz Ben ve Ohadi varyetelerinden elde edilen kabukların metabolik enerji içerikleri Kırmızı, Sultani, Uzun ve Keten Gömleği varyetelerinden elde edilen kabukların metabolik enerjisi içeriğinden yüksek olmasına rağmen Ohadi varyetesinden elde edilen Kırmızı, Sultani, Uzun ve Keten Gömleği varyetelerinden elde edilen kabuklarının OMD değerinden yüksek bulunmuştur. Sonuç olarak, kimyasal olarak ham protein, OMD ve ME bakımından yapılan karekterizasyon Antep fıstığı kabuklarının orta seviyede ham protein içerdiği ve oldukça sindirilebilir olduğundan dolayı ruminantlar için kabul edilebilir kalitede yem sağlayacağı söylenebilir.

Anahtar sözcükler: *Kimyasal kompozisyon, Kondense tanen, Sindirim derecesi, Besleme değeri, İn vitro gaz üretimi, Fıstık dış kabuğu*

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INTRODUCTION

After processing of fruits, vegetables, crops and nuts, considerable amount agricultural byproduct become available and used to meet the energy and protein requirements of livestock animal in most parts of the world especially during the dry season when food shortage occurs [1]. There is about 2.212.229 hectares of pistachio garden in Turkey and annual dry pistachio production is 128000 tones [2]. Pistachio fruit is well known for its oleaginous and edible seed. The hull of pistachio is constituted by epicarp and mesocarp which adheres tightly the hard inner shell until the pistachio nut is ripe [3]. De-hulling of pistachio after harvest produces considerable amount hulls which are dried or ensiled for storage. Several researches showed that dried or ensiled pistachio by product can be used ruminant diets to some extent [1-6]. Although there is considerable amount pistachio hull production in Turkey there is no information about chemical composition, digestibility, and metabolic energy contents of pistachio hulls obtained from different varieties. Chemical composition, in combination with *in vitro* gas production, OMD and ME content were widely used to determine the potential nutritive value of feedstuffs which are previously limited or un-investigated [7-10].The aim of this study was to determine the potential nutritive value of pistachio hulls obtained from six different varieties using chemical composition and *in vitro* gas production technique.

MATERIAL and METHODS

This experiment was conducted in the laboratory of Department of Animal Science, Faculty of Agriculture, University of Kahramanmaras Sutcu Imam, Kahramanmaras, Turkey. Studies performed using *in vitro* experimental model was approved by the Animal Experimentation Ethics Committee of University of Kahramanmaras Sutcu Imam, Faculty of Agriculture (Protocol No: 2013/01). Pistachio hulls obtained from six different varieties (Beyaz Ben, Kırmızı, Ohadi, Sultani, Uzun and Keten Gömleği) were shade dried and taken to laboratory and milled in a hammer mill through a 1 mm sieve for subsequent analysis.

Dry matter (DM) contents of pistachio hulls was determined by drying the samples at 105ºC overnight and ash by igniting the samples in muffle furnace at 525ºC for 8 h. Nitrogen (N) contents of pistachio hulls was measured by the Kjeldahl method [11]. Crude protein contents of pistachio hulls were calculated as N X 6.25. Neutral detergent fiber (NDF) contents of pistachio hulls was determined by the method van Soest and Wine [12] and ADF contents of pistachio hulls were determined by the method of van Soest [13]. Condensed tannin contents of pistachio hulls were determined by butanol-HCl method as described by Makkar *et al.*[14]. All chemical analysis is carried out in duplicate.

Pistachio hulls milled through a 1 mm sieve were incubated *in vitro* rumen fluid in calibrated glass syringes following the procedures of Menke *et al.*[15].

Rumen fluid was obtained from one year old and approximately 50 kg of three fistulated Awassi ram after one week adjustment period of a diet. Fistulated Awassi ram fed twice daily with a diet containing alfalfa hay (60%) and concentrate (40%). Rumen fluid was collected before morning feeding and squeezed through four layers of cheesecloth. The rumen fluid was flushed with CO_2. The rumen fluid was added to buffered mineral solution in the ratio of 1:2 respectively. Approximately 0.200 gram dry weight of Pistachio hulls samples was weighed in triplicate into calibrated glass syringes of 100 ml. The syringes were prewarmed at 39ºC before the injection of 30 ml rumen fluid-buffer mixture into each syringe followed by incubation in a water bath at 39ºC. Gas production was recorded at 3, 6, 12, 24, 48, 72 and 96 h after incubation and corrected for blank incubation. Cumulative gas production data of Pistachio hulls were fitted to non-linear exponential model as: $Y = A\ (1 - \exp^{-ct})$ [16].

Where *Y* is gas production at time't', A is the potential gas production (ml/200 mg DM), c is the gas production rate constant (h^{-1}) and t is the incubation time (h).

ME (MJ/kg DM) values of pistachio hulls were estimated using equation of Menke *et al.*[15] as follows:

ME (MJ/kg DM) = 2.20 + 0.136 GP + 0.057 CP,

where, GP = 24 h net gas production (ml/0.200 g); CP = Crude protein (%).

Organic matter digestibility (%) values of pistachio hulls were calculated using equation of Menke *et al.* [14] as follows:

OMD (%) = 14.88 + 0.889GP + 0.45CP +0.0651 XA

where XA: ash content (%).

One-way analysis of variance (ANOVA) was carried out to determine the effect of variety on the chemical composition, gas production kinetics, ME and OMD of pistachio hulls. Significance between individual means was identified using the Tukey's multiple range tests [17]. Mean differences were considered significant at $P<0.05$.

RESULTS

The effect of variety on the chemical composition of pistachio hulls is presented in *Table 1*. The variety has significant effect on the chemical composition of pistachio hulls. The DM contents of pistachio hulls ranged from 26.45 to 29.25%. The CP contents of pistachio hulls ranged 7.27 to 14.99%. The hulls from Kırmızı, Ohadi and KG had significantly higher CP contents than the others. The NDF and ADF contents ranged from 18.25 to 22.49% and 14.32

to 18.29% respectively. The hulls from Ohadi and Uzun had significantly higher NDF and ADF contents than the others. The ash contents of pistachio hulls were ranged 8.50 to 19.86%. The hulls from Uzun had significantly higher ash content than that of Sultani. The CT contents of hulls ranged from 2.03 to 2.63%. The hulls from BB had significantly higher CT content than that of KG.

The effect of variety on gas production of pistachio hulls at different time intervals is presented in *Fig. 1*. At 3, 6 and 12 hours incubation the gas production for Kırmızı and Uzun were significantly lower than the others. At 24, 48 and 72 h incubation times, the gas production for Kırmızı, Uzun and KG were significantly higher than the others. At 72 h incubation times the gas productions for BB and Ohadi were significantly higher than the others.

The effect of variety on gas production kinetics, ME, OMD of pistachio hulls is presented in *Table 2*. The variety had a significant effect on gas production kinetics, ME, OMD of pistachio hulls. The gas production rate ranged from 0.071 to 0.107%. The gas production rate of pistachio hulls from Sultani and KG were significantly higher than the others. The potential gas production of pistachio hulls ranged from 65.92 to 73.46 ml. The potential gas production of pistachio hulls from BB and Ohadi were significantly higher than the others.

The ME and OMD contents of pistachio hulls ranged from 9.76 to 11.05 MJ/kg DM and 69.00 to 74.50% respectively. The ME contents of pistachio hulls from BB and Ohadi were significantly higher than those of Kırmızı, Uzun and KG whereas the OMD contents of pistachio

Table 1. *The effect of variety on the chemical composition of pistachio hulls (on a dry matter basis)*
Tablo 1. *Çeşidin Antep fıstığı dış kabuğunun kimyasal kompozisyonuna etkisi (kuru madde bazında)*

Nutrient Composition	Variety						SEM	Sig.
	BB	Kırmızı	Ohadi	Sultani	Uzun	KG		
DM	26.45^{c}	26.73^{c}	29.25^{a}	28.54^{ab}	28.98^{ab}	26.85^{b}	0.549	**
Ash	16.66^{ab}	18.62^{ab}	11.38^{ab}	8.50^{b}	19.86^{a}	15.44^{ab}	3.070	*
CP	11.06^{c}	14.99^{a}	13.30^{b}	7.27^{d}	10.77^{c}	14.49^{b}	0.357	***
NDF	18.25^{b}	19.03^{b}	22.13^{a}	18.51^{b}	22.49^{a}	18.84^{b}	0.225	***
ADF	15.15^{b}	14.32^{b}	18.29^{a}	15.38^{b}	18.02^{a}	14.47^{b}	0.479	***
CT	2.63^{a}	2.34^{ab}	2.37^{ab}	2.07^{ab}	2.55^{ab}	2.03^{b}	0.183	**

^{a,b,c} Row means with common superscripts do not differ (P<0.05), ***SEM*** *- standard error mean,* ***Sig.*** *- significance level,* ***DM*** *- Dry matter %,* ***CP*** *- Crude protein,* ***NDF*** *- Neutral detergent fiber,* ***ADF*** *- Acid detergent fiber,* ***CT*** *- Condensed tannin,* ***BB*** *- Beyaz Ben,* ***KG*** *- Keten Gömlegi, *P<0.05, **P<0.01, *** P<0.001*

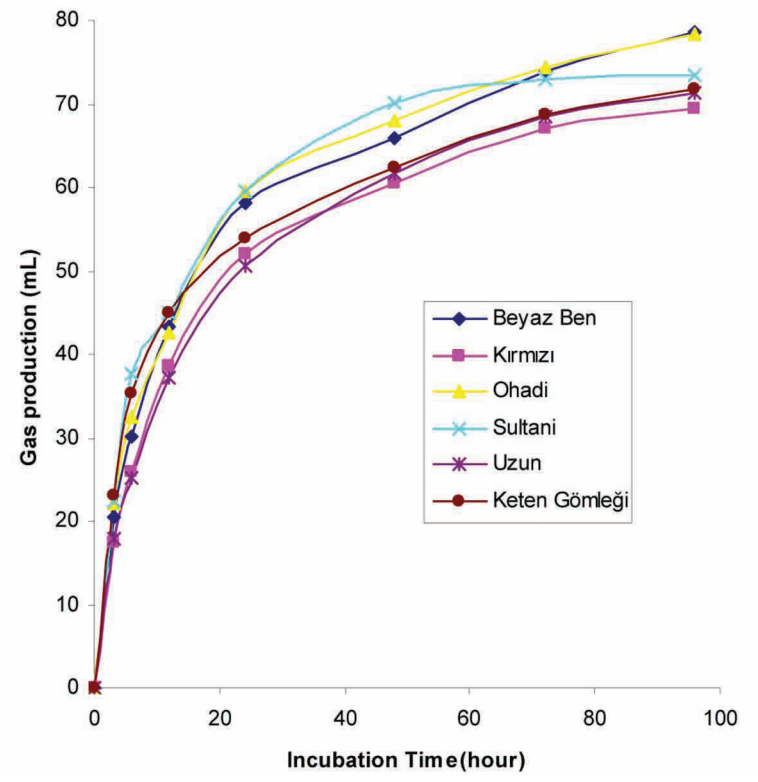

Fig 1. The effect of variety on gas production of pistachio hulls

Şekil 1. Çeşidin Antep fıstığı dış kabuğunun gaz üretimine etkisi

Table 2. *The effect of variety on the gas production kinetics, metabolisable energy and organic matter digestibility of pistachio hulls*
Tablo 2. *Çeşidin Antep fıstığı dış kabuğunun gaz üretim par/ametrelerine, metobolik enerji ve sindirim derecesine etkisi*

Parameters	Variety						SEM	Sig.
	BB	Kırmızı	Ohadi	Sultani	Uzun	KG		
c	0.077^{b}	0.074^{b}	0.081^{b}	0.098^{a}	0.071^{b}	0.107^{a}	0.005	***
A	73.11^{a}	66.01^{c}	73.46^{a}	70.96^{b}	67.39^{c}	65.92^{c}	0.538	***
ME	10.74^{a}	10.11^{cd}	11.05^{a}	10.70^{ab}	9.76^{d}	10.34^{bc}	0.108	***
OMD	72.65^{ab}	69.00^{c}	74.50^{a}	71.60^{b}	71.60^{b}	70.26^{bc}	0.711	***

^{a,b,c} Row means with common superscripts do not differ (P<0.05), ***SEM*** *- standard error mean,* ***Sig.*** *- significance level,* ***c*** *- gas production rate (%),* ***A*** *- potential gas production (mL),* ***ME*** *- Metabolisable energy (MJ /kg DM),* ***OMD*** *- Organic matter digestibility %, *** P<0.001*

hulls from Ohadi were significantly higher than those of pistachio hulls for Kırmızı, Sultani, Uzun and KG.

DISCUSSION

There are significant variations among varieties in terms of chemical compositions of pistachio hulls. The NDF, ADF and CP contents obtained in the current study were considerably lower than those reported by Gholizadeh *et al.*[6] who reported that NDF and ADF, CP contents were 25, 20 and 16.6% respectively. On the other hand, except for Uzun and Sultani, ash contents of pistachio hulls obtained in the current study were considerably higher than those reported by Gholizadeh *et al.* [6] who reported that ash content was 12.7%. The differences between two studies are possibly associated differences in variety, growing conditions, kernel maturity and de-hulling process applied.

Except for Sultani, the crude protein contents of pistachio hulls are comparable with those proposed as the minimum requirements for lactation (12% of DM) and growth (11.3% of DM) in ruminants [18]. Therefore pistachio hulls investigated in the current experiment have the potential for ruminant animals to meet the protein requirements during the critical periods when there is a shortage of high quality forages. However, Kumar and Singh [19] suggested that CP in diet may be restricted due to high levels of condensed tannin (50 g/kg DM) owing to excessive formation of tannin-protein complexes, *e.g*. with endogenous protein, which pass through the animal largely undigested. On the other hand Barry [20] suggested that CT which is lower than 2-3% may have beneficial effects to ruminants due to reduction in protein degradation as a result of the formation of protein-tannin complexes in the rumen. However, in the current experiment, the condensed tannin levels of pistachio hulls were lower than those considered detrimental to ruminant animals.

There are significant variation among pistachio varieties in terms of in vitro gas production and estimated parameters such as OMD and ME contents of hulls. The variation in gas production and estimated parameters among pistachio varieties can be attributed to compositional differences of pistachio hulls, especially cell wall, CP contents and some other anti-nutritive factors such as CT. Babayemi *et al*. [21] indicated that there are many factors that may determine the amount of gas to be produced during the fermentation, depending on the nature and level of cell content, the presence of secondary metabolites in feedstuffs. Blummel and Orskov [22] suggested that in vitro gas production is associated with volatile fatty acid (VFA) production following fermentation of substrate so the more fermentation of a substrate the greater the gas production, although the fermentation end products do correlate more closely with gas production.

Organic matter digestibility of pistachio hulls obtained in the current study were considerably higher than those reported by Noghabi and Rauzbehan [23] who reported that OMD of pistachio hulls ranged from 52.6 to 65.6% and polyethylene glycol (PEG) supplementation significantly increased in vitro OMD of pistachio hulls.

Although there is considerable amount pistachio hull production in Turkey, there is no information about the biomass production of pistachio hulls as a feed source for ruminant animals. It would be more useful if further studies should be focused on the biomass production potential of pistachio hulls in Turkey.

In conclusion, chemical characterization with CP, OMD and ME suggests that the pistachio hulls moderately high CP concentration and was quite digestible therefore pistachio hulls provide feedstuffs of an acceptable quality for ruminant animals.

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Kafkas Univ Vet Fak Derg
19 (4): 705-708, 2013
DOI: 10.9775/kvfd.2013.8610

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SHORT COMMUNICATION

Geleneksel Yöntemle Üretilen Sucuklarda *Listeria monocytogenes, Staphylococcus aureus* ve Koliform Varlığının Araştırılması

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Makale Kodu (Article Code): KVFD-2013-8610

Özet

Uygun olmayan koşullarda üretilen sucuklarda gıda kaynaklı patojenlerden *Listeria monocytogenes*, koagülaz (+) *Staphylococcus aureus* ve koliformlar gelişebilmekte ve bu ürünler insan sağlığı açısından risk arz edebilmektedir. Bu çalışmada, Mersin ve Adana illerine bağlı yaylalarda geleneksel yöntemlerle üretilen (kırsalda satışı ve servisi yapılan) 60 sucuk örneği *L. monocytogenes*, koagülaz (+) *S. aureus* ve koliformlar açısından incelenmiştir. Türk Gıda Kodeksine göre sucukta hiç bulunmaması gereken *L. monocytogenes* 7 örnekte tespit edilmiştir. Ayrıca 10 örnekte koagülaz (+) *S. aureus* ve 18 örnekte *koliform* grubu mikroorganizmaya rastlanmıştır. Kırsal alanda sucuk satış ve servis noktalarındaki denetimlerin arttırılması ve üretim yapan kişilerin hijyen konusunda eğitimi sorunun çözümüne katkı sağlayacaktır.

Anahtar sözcükler: *Et ürünleri, Sucuk, Mikrobiyel kalite, Gıda kaynaklı patojenler*

An Investigation on the Microbiological Quality of Sucuk Produced by Traditional Methods

Summary

Foodborne pathogens like *Listeria monocytogenes*, coagulase(+) *Staphylococcus aureus* and coliforms may grow in sucuk produced under unsuitable conditions and possess risks for human health. In this study, 60 sucuk samples produced by traditional methods in plateaus of Mersin and Adana provinces were investigated in terms of *L. monocytogenes*, coagulase (+) *S. aureus* and coliforms. *L. monocytogenes*, which must not to be present in sausages according to Turkish Food Codex, was detected in 7 samples. In addition, 10 of samples were found to be contaminated with coagulase (+) *S. aureus* and 18 of samples with coliforms. Increasing controls of sausage sale and service points in rural areas, and training of producers on hygiene will contribute to the solution of the problem.

Keywords: *Meat products, Turkish dry fermented sausage (sucuk), Microbial quality, Foodborne pathogens*

GİRİŞ

Sucuğun bileşimi (yağ, baharat ve diğer katkı maddelerinin miktar ve çeşitleri) pazar isteklerine göre değişmektedir. Geleneksel sucuk üretimi iklim koşullarına bağlı olarak özellikle sıcaklığın, hava akımının ve rutubetin uygun olduğu sonbaharda yapılır. Sucuk hamuru kılıflara doldurulduktan sonra askılara alınarak olgunlaşmaya bırakılır. Bu yöntemde sucuklar 20-30 günde olgunlaşır [1]. Fermente sucuklarda kalite niteliklerinin geliştirilip arttırılmasında bazı mikroorganizmaların enzimatik ve teknolojik etkileri büyük önem taşır. Bu mikroorganizmaların başlıcaları laktobasil, mikrokok ve stafilokok grubunda yer alan bakteri türleridir. Bunlardan laktobasiller doğrudan oluşturdukları laktik asit ile fermente sucuklarda pH'nın düşmesi, aroma oluşumu ve dolaylı olarak renk oluşumunda; mikrokok/stafilokoklar ise nitratı indirgeyerek renk ve aroma oluşumunda rol oynarlar [2]. Geleneksel yöntemlerle yapılan fermente Türk sucuğu, olgunlaşmada önemli rol oynayan faydalı bakterilerin yanı sıra istenmeyen bazı mikroorganizmaları da barındırabilir. Bu çalışmada incelenen bakterilerden biri olan *S. aureus*'un, dünyada bakteriyel gıda zehirlenmelerinden sorumlu tutulan bakteriyel ajanlar arasında ilk sırada yer aldığı belirtilmektedir [3].

Koliform grubu bakteriler 37°C'de 48 saat içinde laktozdan asit ve gaz oluşturabilen bakterilerdir. Koliform

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grubu bakteriler gıdalarda hijyen indikatörü olarak değerlendirilir. *Listeria* cinsinin 8 türü vardır. Bu türler *Listeria monocytogenes, L. innocua, L. seeligeri, L. welshimeri, L. ivanovii*, *L. marthii*, *L. rocourtiae* ve *L. grayi*'dir. *Listeria* türleri içerisinde insan listeriozisi ile genel olarak *Listeria monocytogenes* seyrek olarak da *L. seeligeri* ve *L. ivanovii* ilişkilendirilmektedir [4]. *Listeria* ubiküter bir bakteri olduğundan insanlara geçişinde gıdalar başta olmak üzere birçok muhtemel yol vardır. İnsan listeriozunun asıl kaynağını *L. monocytogenes* ile kontamine hayvansal gıdalar oluşturmaktadır [3]. Sucuk hamurunun başlangıç pH'sının yüksek olması *L. monocytogenes* gelişmesi için uygun ortam sağlamaktadır. Olgunlaşmanın ileri günlerinde değişik faktörlerin (örn. bakteriyosin) kombine etkisi ile sayı azalmaktadır.

Gıdalarda *L. monocyogenes* belirlenmesinde sıfır tolerans politikası geçerlidir. Bu yüzden *L. monocyogenes* analizlerinde 25 gr'da var/yok testi uygulanmaktadır. Türk Gıda Kodeksi Mikrobiyolojik Kriterler Yönetmeliğine göre [5] fermente sucukta *L. monocytogenes* bulunmamalıdır. Aynı yönetmenlikte *S. aureus* ve koliform için herhangi bir kriter mevcut değildir. Türk Standartları Enstitüsü'nün 2002 yılında yayımladığı TS 1070 Türk sucuğu standardına göre; incelenen her beş örnekte *S. aureus* için bir örnekte $5x10^3$ kob/g dört örnekte 10^2 kob/g'ı ve koliform grubu bakterilerin 10 kob/g'ı geçmemesi gerektiği öngörülmektedir. Kök ve ark.[6] Aydın ilinde satışa sunulan fermente sucukların %14'ünde *Listeria* spp. ve %4'ünde *L. monocytogenes* tespit etmişlerdir. Ayrıca değişik oranlarda koagülaz (+) *S. aureus* ve koliform grubu bakteri belirlediklerini bildirmişlerdir. Öksüztepe ve ark. Elazığ'da yaptıkları çalışmada sucuk örneklerinin %9'unda *Listeria* spp. ve %10'unda koagülaz (+) *S. aureus* tespit etmişlerdir [7]. Koluman ve ark.[8] inceledikleri 50 kıyma örneğinde %70 oranında *S. aureus,* Güven ve ark.[9] ise 80 kıyma örneğinin %46.25'inde bu bakteriyi tespit ettiklerini bildirmişlerdir.

Son yıllarda fast food kültürünün ve işlenmiş et ürünlerindeki rekabetin artması ile geleneksel Türk sucuğu daha çok şehirden uzak noktalarda ve kırsalda yemek hizmeti veren işletmelerde bulunur hale gelmiştir. Ülkemizde halkın damak tadına hitabeden ve sevilerek tüketilen bir ürün olan fermente sucukta halk sağlığı açısından muhtemel risklerinin belirlenmesi, bilimsel ve yasal bir gerekliliktir. Ham maddesinin et olması dolayısıyla sucuğun, hammadde ve üretim aşamasından tüketiciye ulaşıncaya kadar geçen her dönemde oluşabilecek mikrobiyolojik riskler halk sağlığı açısından önem taşımaktadır. Bu çalışma, kırsal piyasadan temin edilen fermente sucukların *L. monocytogenes,* koagülaz (+) *S. aureus* ve koliformlar açısından mikrobiyolojik kalitesini ortaya koymak amacıyla yapılmıştır.

MATERYAL ve METOT

Çalışmamızda 60 adet fermente sucuk kullanıldı. Örnekler Adana, Tarsus, Mersin yaylalarından Eylül-Ekim-Kasım 2011 zaman diliminde toplandı. Aseptik şartlarda steril numune poşetlerine alınarak soğuk zincirle laboratuara getirilen örnekler hemen analize alındı.

L. monocytogenes aranması [10] ve *S. aureus* sayımı için [11] FDA/BAM yöntemleri kullanıldı. Koliform grubu bakterilerin sayımı için Halkman'ın belirttiği metot kullanıldı [12].

BULGULAR

Çalışmamızda kullanılan 60 adet fermente sucuk örneğinin 7'sinde *L. monocytogenes,* 10'unda koagülaz (+) *S. aureus* ve 18'inde koliform grubu mikroorganizma tespit edilmiştir. Katı vasatta tipik *L. monocytogenes* kolonisi tespit edilen örneklerdeki sonuçlar, aynı örneklerin vidas sonuçları ile uyumlu bulundu. Fermente sucukların mikrobiyolojik analizleri sonucu elde edilen pozitif örnekler *Tablo 1*'de, fermente sucuk örneklerinde mikroorganizma sayılarının yüzde dağılımı ise *Tablo 2*'de verilmiştir.

TARTIŞMA ve SONUÇ

Özellikle et ve et ürünleri ile insanlara bulaşan *Listeria*' lar insanlarda ciddi infeksiyonlara yol açabilmektedir. Bu çalışmada analize alınan örneklerin %11.6'sında *L. monocytogenes* tespit edilmiştir. Sancak ve ark. [13], fermente Türk sucuklarında yaptıkları çalışmada, örneklerin %15'inde *L. monocytogenes* tespit ettiklerini bildirmişleridir. Aynı çalışmada salam örneklerinin %10'unun, sosis örneklerinin ise %25'inin *L. monocytogenes* ile konta-

Tablo 1. *Fermente sucuk örneklerinin mikrobiyolojik sonuçları (n=60)*
Table 1. *The results of microbiological of samples fermented sausage (n=60)*

Mikroorganizma	(+) Örnek Sayısı	%	Toplam Örnek Sayısı
L. monocytogenes	7	11.6	60
Koagülaz(+) *Staphylococcus aureus*	10	16.6	60
Koliform	18	30.0	60

Tablo 2. *Fermente sucuk örneklerindeki mikroorganizma sayılarının yüzdesi (kob/g, n=60)*
Table 2. *The fermented sausages samples of microorganism count percent (cfu/g, n=60)*

Mikroorganizma	<0.1x10		$0.1x10^1$ - $9.9x10^1$		$1.0x10^2$ - $9.9x10^2$		$1.0x10^3$ - $9.9x10^3$		>$1.0x10^4$	
	n	%	n	%	n	%	n	%	n	%
Koagülaz(+) *Staphylococcus aureus*	50	83.4	1	1.66	5	8	4	6.4	-	-
Koliform	42	70	1	1.66	9	15	8	13.33	-	-

mine olduğu bildirilmiştir. Çalışmamızda tespit ettiğimiz %11.6 *L. monocytogenes* oranı Sancak ve ark.[13] tarafından tespit edilen %15'lik ve Bozkurt [14] tarafından 25 sucuk örneğinde bildirilen %31.58'lik oranlardan düşüktür. Kök ve ark.[6] Aydın'da, Öksüztepe ve ark.[7] Elazığ'da yaptıkları çalışmalarda fermente sucukların %4'ünde *L. monocytogenes* bulunduğunu bildirmişlerdir. Araştırmacıların tespit ettikleri oran, bu çalışmada belirlenen orandan oldukça düşüktür. Bu farklılıklar örneklerde kullanılan sorbat, nitrat ve starter gibi katkı maddelerinin varlığından ve farklı oranlarda bulunmasının yanı sıra ambalaj farklılıklarından da kaynaklanabilir. Sucuk üretiminde kullanılan tuz, sodyum nitrit gibi maddelerin antilisterial etkilerinin yanı sıra, olgunlaşma sırasında floraya hâkim olan laktobasillerin pH'yı düşürmesi ve yine bu bakterilerin üretmiş olduğu bakteriosinlerin antagonistik etkisinden dolayı sucukta *L. monocytogenes* sayısı azalabilir. Çolak ve ark.[15] İstanbul piyasasından elde ettikleri 300 sucuk örneğinin 35'inde (%11.6) *L. monocytogenes* tespit etmişlerdir. Bu veriler çalışmamızda elde ettiğimiz verilerle oransal bakımdan tam uyum içerisindedir.

pH 4.2'nin üzerindeki fermente et ürünlerinde bulunma ihtimali yüksek olan *S. aureus* incelediğimiz örneklerden 10'unda (%16.6) tespit edilmiştir. Kök ve ark.[6] inceledikleri örneklerin %12'sinde, Öksüztepe ve ark.[7] %10'unda Sancak ve ark.[16] %16'sında (koagülaz (+) *S. aureus* tespit ettiklerinin bildirmişlerdir. Bulgularımız Sancak ve ark.[16] bildirdiği bulgular ile uyum içerisindedir. Erdoğrul ve ark. [17] fermente sucuklarda bildirdiği %6.6 *S. aureus* oranından ise oldukça fazladır. Tespit oranlarının farklı olmasında incelenen örnek sayısı değişkenliğinin etkili olduğu düşünülmektedir. Ozmotolerant bir bakteri olan *S. aureus* ile sucuk yapımı sırasında az sayıda kontaminasyon olsa dahi fermentasyon sürecindeki düşük a_w düzeylerinde gelişip toksik etki gösterebilir. Uygun koşullarda olgunlaştırılmayan ve starter kültür içermeyen sucuklarda stafilokokların gelişerek enterotoksin oluşturabilecekleri belirtilmiştir [3]. Stafilokokal gıda zehirlenmelerinin önüne geçmek için üretim, personel ve alet ekipman hijyeni önem arzetmektedir. Kırsalda üretilen sucuklarda koruyucu ve starter kültür kullanılmadığı düşünülmektedir.

Bu çalışmada kullanılan örneklerde hijyen indikatörü olarak bilinen koliform bakterilerine %30 oranında rastlanmıştır *(Tablo 1)*. Bu durum ham maddenin kontamine olmasından, üretim hijyenine dikkat edilmemesinden, alet ekipmanın kontamine olmasından ve yetersiz olgunlaştırmadan kaynaklanabilir. Koliform grubu bakteriler ürünün raf ömrünü azaltan önemli bir faktördür. Kök ve ark.[6] sucuk örneklerinde ortalama koliform bakteri sayısı 1.62 log kob/g olarak bumuşlardır. Erdoğrul ve ark.[17] inceledikleri sucuk örneklerinde ortalama koliform bakteri sayısını 244/g, olarak bildirmiştir. Bu çalışmada koliform sayısı üreme olan örneklerde $9.0x10^1$ - $6.0x10^3$ kob/g arasında bulunmuştur. Çalışmalar arasındaki farklılıklar üretim yerlerindeki hijyen, ham maddenin bakteriyel yükü, personel ve alet-ekipman hijyeninden kaynaklanabilir. Özellikle koagülaz (+) *S. aureus* ve koliform sayısında tespit edilen miktarlar bu parametrelerin sucukla ilgili mikrobiyolojik kriterler yönetmeliğine girmesi gerektiğini ortaya koymaktadır. Toplam 42 örnekte koliform grubu bakteriye rastlanmadı. Bunun sebebi olgunlaşma sırasında su aktivitesinin azalması, pH'nın düşmesi, rekabetçi flora ve bakteriyosinlerin varlığı olabilir.

Sonuç olarak elde edilen veriler sucuk örneklerinde *L. monocytogenes,* koagülaz (+) *S. aureus* ve koliform grubu bakterilerin varlığını ortaya koymuştur. Bu durum ürünün raf ömrünün kısalmasına neden olmasının yanında halk sağlığı açısından da büyük önem arz etmektedir. Sucuğun uygun olmayan koşullarda üretilmesi ve değişik özelliklerdeki ham maddeler kullanılmasından kaynaklanan mikrobiyel riskleri bertaraf etmek için kırsal alandaki satış ve servis noktalarında denetimlerin artırılması gerekir. Geleneksel sucuk üretimi yapan kişilerin ham madde ve üretim hijyeni yanı sıra muhafaza ve satış koşulları konularında eğitilmesi ürünlerin halk sağlığı riski oluşturma ihtimalini düşürecektir.

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Kafkas Univ Vet Fak Derg
19 (4): 709-712, 2013
DOI: 10.9775/kvfd.2013.8753

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

SHORT COMMUNICATION

Comparison of Three Methods for Routine Detection of *Staphylococcus aureus* Isolated from Bovine Mastitis

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Makale Kodu (Article Code): KVFD-2013-8753

Summary

The present study aimed to compare three identification methods that are routinely used for the detection of *Staphylococcus aureus* as bovine mastitis agent. The evaluated methods were as followed: conventional biochemical method, commercial identification system BioLog (Gen III MicroPlate) and amplification of species-specific gene (*nuc*) by polymerase chain reaction (PCR). A total of 73 staphylococcal isolates were collected from 453 individual milk samples from dairy cows with subclinical and clinical mastitis from different farms in Bulgaria. This isolates were determined as 60 coagulase-positive, 3 catalase-negative and 10 coagulase-negative by conventional methods. BioLog system identified 72 isolates as *S. aureus* subsp. *aureus* and one coagulase-positive isolate as *S. schleiferi* subsp. *coagulans*. PCR amplification of *nuc* gene further confirmed *S. aureus* subsp. *aureus* isolates identified by the BioLog system. The primary identification of *S. aureus* on the basis of coagulase level led to erroneous determination of 14 (19.2%) of the isolates. Based on the findings, BioLog system and PCR appear to be more reliable detection systems for *S. aureus* from milk. In conclusion, the present study showed that a routine approach using a combination of phenotypic and molecular detection systems could improve *S. aureus* detection in milk.

Keywords: *Staphylococcus aureus, Bovine mastitis, Identification, BioLog, nuc gene, PCR*

Bovin Mastitislerde *Staphylococcus aureus*'un Rutin Tespitinde Üç Metodun Karşılaştırılması

Özet

Bu çalışma Bovin mastitis etkeni olarak *Staphylococcus aureus*'un belirlenmesinde rutin olarak kullanılan üç tespit yöntemini karşılaştırmayı amaçlamaktadır. Denenen yöntemler şunlardır; konvensiyonel biyokimyasal metot, ticari tespit sistem BioLog (Gen III MicroPlate) ve polimeraz zincir reaksiyonu (PZR) ile amplifiye edilmiş tür spesifik gen (*nuc*). Bulgaristan'daki farklı çiftliklerde subklinik ve klinik mastitisli sütçü ineklerden alınan 453 adet örnekten toplam 73 staphilococal izolat elde edildi. Konvensiyonel metotlarla bu izolatların 60'ı koagulaz pozitif, 3'ü katalaz negatif ve 10'u koagulaz negatif olarak belirlendi. BioLog sistemi ile 72 izolat *S. aureus* subsp. *aureus* olarak tespit edilirken bir adet koagulaz pozitif izolat *S. schleiferi* subsp. *coagulans* olarak belirlendi. PZR ile *nuc* geninin amplifikasyonu kullanılarak BioLog sistemi ile *S. aureus* subsp. *aureus* olarak tespit edilen izolatlar teyit edildi. Koagulaz seviyesine dayanarak *S. aureus*'un primer identifikasyonu 14 (%19,2) hatalı tespite neden oldu. Bu bulgulara dayanarak BioLog sistem ve PZR, *S. aureus*'un sütten tespitinde daha güvenilir yöntemler olarak belirlendi. Sonuç olarak, bu çalışma ile rutin olarak fenotipik ve moleküler tespit yöntemlerinin beraber kullanılmasının sütten *S. aureus*'un tespitinde başarıyı artıracağı ortaya konuldu.

Anahtar sözcükler: *Staphylococcus aureus, Bovin mastitis, Tespit,, BioLog, nuc geni, PZR*

INTRODUCTION

Bovine mastitis is among the leading issues that cause serious economic losses in dairy industry throughout the world, resulting in reduced milk production, reduction of the quality of milk through contamination and due to

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treatment-associated hazards [1]. Of all mastitis causative agents, *S. aureus* is most commonly isolated [2], while the coagulase-negative staphylococci (CoNS) are also common but not considered as significant mastitis pathogens [3]. Therefore, the identification of the staphylococci by their ability to produce coagulase is deemed the main criterion for differentiation of those with pathogenic potential from others. On the other hand, some coagulase-positive staphylococci (CoPS) other than *S. aureus* such as *S. intermedius* and *S. hyicus* could cause intramammary infections in cows [4]. In that respect, the differentiation of the staphylococcal mastitis causative agents is important not only for the therapeutic approach and the remedial schemes, but also for the ability of some of them to produce a wide range of virulent factors [5].

Several conventional phenotypic and molecular methods have been used as routine approaches for the identification of staphylococci. The conventional culture methods require isolation of the bacteria on suitable media and subsequent identification with numerous biochemical tests. Commercial identification systems recognizing *S. aureus* are available such as BD Phoenix [6], BBL Crystal and Api Staph system [7] and Vitek2 [8]. Over the last few years, molecular techniques have become more popular and are currently used as detecting systems of mastitis pathogens in many diagnostic laboratories [9].

The present study aimed to compare three methods for routine identification of *S. aureus*, isolated from dairy cows, the first based upon the conventional identification and coagulase activity; the second using an identification system (BioLog) and the third - a molecular approach using PCR amplifying the species-specific gene, *nuc*, as a reference method.

MATERIAL and METHODS

Isolation and Primary Identification of S. aureus

A total of 453 individual milk samples were collected from cows with subclinical and clinical mastitis from different farms in Bulgaria. The indication for sampling from the subclinical mastitis animals was the positive result for increased somatic cell count after screening with Mastitis test NK (Biovet, Czech Republic). The milk sampling was done according to the guidelines of bacteriological examination. The samples (10 µL) were inoculated on trypticase soy agar (TSA, Fluka, India) supplemented with 5% defibrinated sheep blood (TSBA) and incubated at 37ºC for 24-48 h under aerobic conditions. The primary identification of staphylococcal isolates was made by Gram staining, catalase and oxidase activity, colony morphology and pigmentation, hemolysis, growth and mannitol fermentation on Chapman agar (NCIPD, Bulgaria). Further, the pathogenic potential of the isolates was tested by tube coagulase test with rabbit plasma (NCIPD, Bulgaria). A weak coagulase activity was recorded as positive reaction.

Identification of the Isolates with BioLog System

The suspicious for *S. aureus* isolates were checked with identification system BioLog Gen III microplates following precisely the manufacturer's instructions (Biolog, Hayward, USA). In brief, protocol A was used for identifying of *S. aureus*. The isolates were cultured on TSBA and the inocula were prepared in a special type of liquid medium, provided by the company until achieving the desired cell density determined by turbidimeter. The plates were filled with 100 mL of the readily prepared inocula and incubated at 33ºC for 24 h under aerobic conditions. After incubation, the plates were read by the computer system software OmniLog.

Extraction of DNA and PCR Analysis

Bacterial colonies were suspended in 100 µL bidistilled water and boiled for 10 min to extract DNA. After centrifugation at 12.000 g for 5 min at room temperature, the supernatant that contains the nucleic acids was used as DNA template for subsequent PCR analysis. DNA was also isolated by universal commercially available kit - prepGem (ZyGem, USA), according to the company's protocol. The concentration and purity of DNA extract was determined by DNA/RNA spectrophotometer Gene Quant 1300 at A260 and A280. The DNA extracts were then stored at - 20ºC until the beginning of the experiments.

The PCR methodology used in the study was described previously by Brakstad et al.[10] with an expected amplicon size of 270 bp. Positive and negative control strains were included for each PCR experiment.

RESULTS

From collected 453 milk samples, 73 staphylococcal strains were isolated. Gram-stained smears of the pure cultures exhibited clusters of Gram-positive cocci. Catalase test revealed three isolates with catalase-negative reaction. The hemolytic activity of isolates was different, variations were observed in the pigmentation of the colonies as well. Ten of the beta-hemolytic isolates gave negative results in coagulase tests in two independent trials. With exception of one coagulase-positive isolate, all others fermented mannitol of Chapman agar under aerobic conditions *(Table 1)*.

BioLog system determined catalase-negative and coagulase-negative isolates as *S. aureus* subsp. *aureus* with probability of 0.919 - 0.999. The typical coagulase-positive isolates were confirmed as *S. aureus* subsp. *aureus*. The coagulase-positive non-hemolytic isolate with white colonies and non-fermenting mannitol was identified as *S. schleiferi* subsp. *coagulans* with probability of 0.970. Three

Table 1. *Comparison of the methods for detection of S. aureus isolated from bovine mastitis*
Tablo 1. *Bovin mastitislerden S. aureus'un tespiti amacıyla kullanılan metotların karşılaştırılması*

Isolates n/%	Criteria for Primary Identification							BioLog ID	*nuc* gene
	Catalase /oxidase	Haemolysis			Pigment	Coagulase	Mannitol		
		Double	Beta	No					
3/4.1	-/-	+			creamy-white	+	+	*S. aureus*	+
10/13.7	+/-		+		greyish	-	+	*S. aureus*	+
32/43.8	+/-	+			yellow	+	+	*S. aureus*	+
6/8.2	+/-	+			greyish	+	+	*S. aureus*	+
14/19.2	+/-		+		greyish	+	+	*S. aureus*	+
7/9.6	+/-		+		yellow	+	+	*S. aureus*	+
1/1.4	+/-			-	white	+	-	*S. schleiferi coagulans*	-

Key: *+ = positive reaction; – = negative reaction*

independent trials were performed to confirm the species affiliation of the isolate.

The PCR protocol based on the *nuc* gene further confirmed *S. aureus* subsp. *aureus* isolates identified by BioLog system (*Table 1*). The two DNA isolation techniques were of similar quality as template for PCR.

DISCUSSION

The primary conventional identification of CoPS is very important and suggests about *S. aureus*, however, prior to testing with rabbit plasma some tests as Gram staining, catalase and oxidase activity that are particularly valuable for the discrimination of the agent from streptococci and micrococci have to be done as routine tests [11]. An interesting and unique finding in this study was the three isolates with catalase-negative reaction which was preserved throughout subsequent subcultivations. The isolates showed double hemolysis, which gave us the clue and motivated us further to continue with the identification process. The review of the literature revealed reports for catalase-negative *S. aureus,* but they referred to isolates of human origin [7,12-14]. Piau et al.[8] determined a catalase-negative *S. aureus* strain with point mutations in the *katA* gene as a possible mechanism for the loss of catalase activity. To the best of our knowledge, this is the first report of catalase-negative *S. aureus* from bovine origin in particular from cases of subclinical mastitis, determined by the primary routine identification.

According to Bannerman [15] the detection of free coagulase in the plasma tube coagulase test is the gold standard for *S. aureus* identification. In this regard, another interesting finding in this study was the isolation of 10 coagulase-negative strains from subclinical mastitis with a wide range of beta hemolysis. Worldwide reports of coagulase-negative variants of *S. aureus* in bovine mastitis are still rare [16]. Akineden et al.[4] determined two *S. aureus* coagulase-negative strains, that would be interpreted as CoNS naturally. The very weak beta hemolysin activity of the isolates motivated the authors to further identify the strains on a molecular level, that confirmed the species affiliation of the isolates as *S. aureus*.

An important criterion for the presumptive primary identification of *S.aureus* is also the hemolytic activity of isolates in the initial cultures. In our study 41 (56.2%) of isolates showed double hemolysis (alpha + beta hemolysins), 31 (42.5%) beta and 1 (1.4%) was non-hemolytic. The isolates with double hemolysis coagulated rabbit plasma in the test tube by the 4th h, a quick (less than 4 h) coagulation was also observed in some beta hemolytic isolates (8/31) as well as in the non-hemolytic and the others required overnight incubation. In our study we did not find *S. aureus* isolates without hemolytic activity.

The staphylococcal isolates were subjected to further analysis and identification by the BioLog system. The atypical catalase-negative (n=3) and coagulase-negative (n=10) isolates were determined as *S. aureus* subsp. *aureus* with a very high probability (91.9-99.9%). Vitek 2 (bioMérieux, France) Gram-positive identification card identified the catalase-negative isolate as *S. aureus* with probability of 93 % [8]. To et al.[17] compared two identification systems for the identification of a catalase-negative *S. aureus* strain from a patient with endocarditis and pericarditis of the mitral valve. While Becton Dickinson Phoenix PID panel identified the strain as *S. aureus*, Vitek system Gram-positive identification test could not identify the bacterium. Our coagulase-positive isolate without hemolytic activity and mannitol fermenting ability was identified as *S. schleiferi* subsp. *coagulans*. *S. schleiferi* subsp. *coagulans* is considered pathogenic mainly in dogs as causative agent of otitis externa [11]. In our case the bacterium was isolated from clinical mastitis in pure culture. Working with BioLog Gen III does not require any preliminary tests, even Gram staining, which is important in selection of

other identification systems. BioLog system recognizes the biochemical profile of approximately 2500 bacterial species, including many important veterinary pathogens and those responsible for mastitis. In that respect, we do not agree with Ahmadi et al.[9] who affirmed that most commercial identification systems were not designed to determine the important veterinary pathogens.

S. aureus isolates identified with BioLog were confirmed by PCR amplification of the fragment of agent's species-specific *nuc* gene. The results of our research showed an excellent correlation between the BioLog system and PCR detection of *S. aureus* in bacterial colonies from bovine mastitis. A protocol for the identification of *S. aureus* in milk by PCR was proposed by Ahmadi et al.[9]. Real-time PCR-based commercial reagent kit is now available for investigation of bovine mastitis pathogens without conventional culturing [18]. Although the identification system BioLog and the PCR protocol for detection of *S. aureus* require a culture step, they proved to be very reliable methods, especially in the developing countries, where advanced molecular techniques are not yet applicable.

The comparison of the methods for routine detection of *S. aureus* showed that the conventional identification on coagulase activity level alone would result in false determination of 14/73 (19.2%) *S. aureus* isolates - 3 catalase-negative and 10 coagulase-negative, and also of one coagulase-positive identified as *S. schleiferi* subsp. *coagulans*. Special attention is required when working with atypical *S. aureus* strains in udder health laboratories, where the identification systems and PCR based methods are not currently used as diagnostic approaches. In conclusion, this study has shown that a routine approach using a combination of phenotypic and molecular detection systems could improve *S. aureus* detection in milk.

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Kafkas Univ Vet Fak Derg
19 (4): 713-716, 2013
DOI: 10.9775/kvfd.2012.8461

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

CASE REPORT

Bir Ceylanda *(Gazella gazella)* Generalize Reaktif Amiloidoz Olgusu

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Makale Kodu (Article Code): KVFD-2012-8461

Özet

Bu raporda 1.5 yaşında, erkek bir dağ ceylanında saptanan generalize reaktif amiloidozis tanımlandı. Makroskobik incelemede karın boşluğundaki serozalarda hiperemi ve rumende ise kenarları kanamalı 7 cm çapında ülser belirlendi. Her iki böbreğin yüzeyinde 1-2 mm büyüklüğünde boz-beyaz renkte odaklar gözlendi. Mikroskobik incelemelerde böbreklerde glomerulus, tubulus ve akıtıcı kanal bazal membranları ve damar duvarlarında, dalakta foliküllerde, karaciğerde Disse aralığında ve ince barsaklarda lamina propriyada pembe renkte, homojen ve amorf yapıda birikimler gözlendi. Bu birikimlerin Kongo kırmızısı ile tuğla kırmızısı renkte boyandıkları, potasyum permanganat ile muamele edildikten sonra Kongo kırmızısı ile boyanmadıkları görülerek AA-amiloidoz (sekonder amiloidozis) olduğu kanısına varıldı. %10'luk formaldehitte bulunan stok doku parçalarının Lugol ve sülfirik asitle muamele edilmeleri sonucunda amiloid birikimlerinin koyu mavi renkte oldukları gözlendi. Rumende ülserasyonla birlikte generalize amiloidoz saptanan bu olguda, amiloid birikimlerinin makroskobik olarak sadece taze dokularda değil formaldehit solüsyonunda tespit edildikten sonra da gösterilebileceğine dikkat çekilmiştir.

Anahtar sözcükler: *Generalize amiloidozis, Ceylan, Patoloji*

Generalized Reactive Amyloidosis in a Gazelle *(Gazella gazella)*

Summary

This report was described generalized amyloidosis in a male gazelle, 1.5 years old. In necropsy, hyperaemia on all the serosal surfaces in abdomen, a hemorrhagic ulcer, 7 cm in diameter, on mucosa of rumen, and whitish colored foci, 1-2 mm diameter, on the surfaces of both kidneys were present. Microscopically, there were homogenous pink depositions in the glomeruli, intertubular interstitium, tubular basement membranes and walls of blood vessel in kidney, lymphoid follicles in spleen, space of Disse in liver and lamina propria of the small intestine. These deposits were seen brick-red color stained with Congo red on tissue sections, but these were not stained with Congo red after treated with potassium permanganate. For this reason, AA (secondary) amyloidosis was evaluated. When stock tissue samples stored in 10% formalin were treated with Lugol and acid sulphuric, amyloid depositions were seen as dark blue. Generalized reactive amyloidosis with ulceration in the rumen was diagnosed. Amyloid deposits in the tissues are not only fresh tissue but also tissue stored formalin can be determined as a macroscopic.

Keywords: *Generalized amyloidosis, Gazelle, Pathology*

GİRİŞ

Amiloid hücre dışında toplanan, çözünmeyen ve proteolitik enzimlerle eritilemeyen anormal fibriler bir proteindir. Amiloid protein yapısına göre AL (Amyloid Light Chain) protein ve AA (Amyloid Associated) protein olarak ikiye ayrılır [1]. Dağılımına göre sistemik ve lokal, patogenezine göre primer ve sekonder olarak sınıflandırılmaktadır [2].

Amiloidoz yabani hayvanlardan Sibirya kaplanlarında, vizonlarda (mink), siyah bacaklı kediler ve siyah bacaklı gelinciklerde [2], çitalarda [3], Dorcas ceylanında [4], Arap ceylanında [5], dağ ceylanında [6], Rocky dağı büyük boynuzlu koyunlarında [7], tavşanlarda [8], aslanlarda [9], kuğularda ve kınalı kekliklerde [10,11], deniz aslanı [12], yunus [13] ve balinalarda [14] bildirilmiştir.

Bu makalede, hayvanat bahçesinde ölen bir dağ ceylanında, organlardaki amiloid birikiminin patolojik yöntemlerle tanımlanması ve amiloidozun tiplendirilmesi amaçlandı.

OLGUNUN TANIMI

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Veteriner Fakültesi Patoloji Anabilim Dalı'na getirilen 1.5 yaşlı erkek bir ceylan oluşturdu.

Ceylanın nekropsisinde; karın boşluğundaki tüm serozalarda hiperemi, rumende 7 cm çapında ve kenarları kanamalı ülser ile böbreklerde kapsula altında toplu iğne başı büyüklüğünde boz-beyaz renkte odaklar gözlendi.

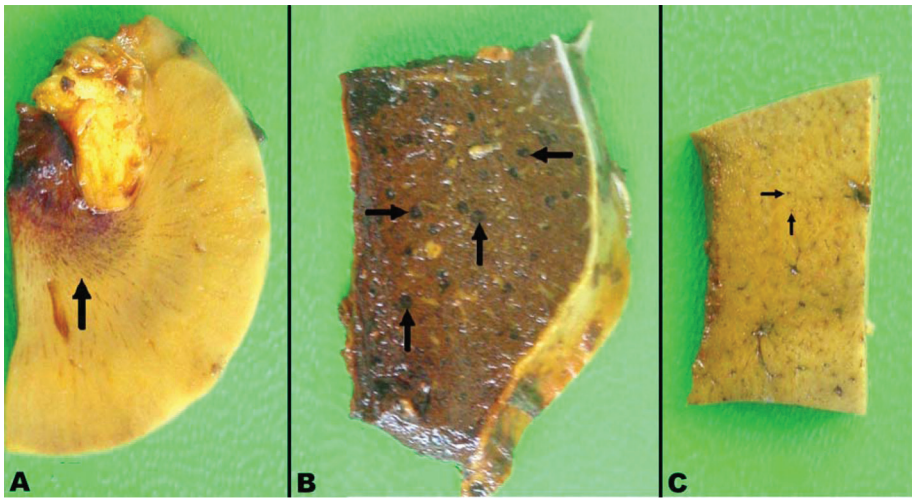
Nekropside alınan parçalar %10'luk formolde tespit edildikten sonra bilinen yöntemlerle takibi yapılarak parafin bloklar elde edildi. Bu bloklardan 5 mikron kalınlığında kesitler alınarak Hematoksilen-Eozin (HE) ve Kongo Kırmızı (KK) ile boyandı [15]. Amiloidin tipini belirlemek amacıyla kesitler önce potasyum permanganat ile muamele edildikten sonra KK ile boyanarak [16] ışık mikroskobunda incelendi.

Mikroskobik incelemelerde rumende, çevresinde geniş nekrozlar içeren, ülserasyonlu yangısal granulasyon dokusu tespit edildi *(Şekil 4 C-D)*. Böbreklerde glomerular kapillar damar duvarında belirgin, medullada ise tubulus ve akıtıcı kanal bazal membranları ve interstisyumda damar duvarlarında pembe renkte, homojen ve amorf yapıda birikimler gözlendi *(Şekil 2 A-C)*. Benzer birikimlere dalakta sentral arter duvarı ve foliküllerin tamamında *(Şekil 3 A-B)*, karaciğerde Disse aralığında fokal olarak *(Şekil 3 D-E)*, akciğerlerde damar duvarlarında *(Şekil 4 A)*, ince barsaklarda ise lamina propriyada rastlandı *(Şekil 4 B)*. Bu organların KK ile boyanan kesitlerinde amiloid birikimlerinin tuğla kırmızısı renginde boyandıkları dikkati çekti *(Şekil 2 D-F, Şekil 3 B,E)*. Diğer organ kesitlerinde ise KK ile boyamalarda herhangi bir boyanma tespit edilmedi. Alınan kesitlerin potasyum permanganat ile muamele edildikten sonra KK ile boyanmadıkları *(Şekil 3 C,F)* ve bu birikimlerinin reaktif (sekonder) amiloidoziste tanımlanan AA-protein yapısında olduğu kanısına varıldı.

Histopatolojik incelemeler sonucunda amiloid belirlenen dokuların %10'luk formolde saklanan parçalarına Lugol ve sülfirik asit damlatıldığında amiloidli kısımların koyu mavi renk aldıkları dikkati çekti *(Şekil 1 A-C)*.

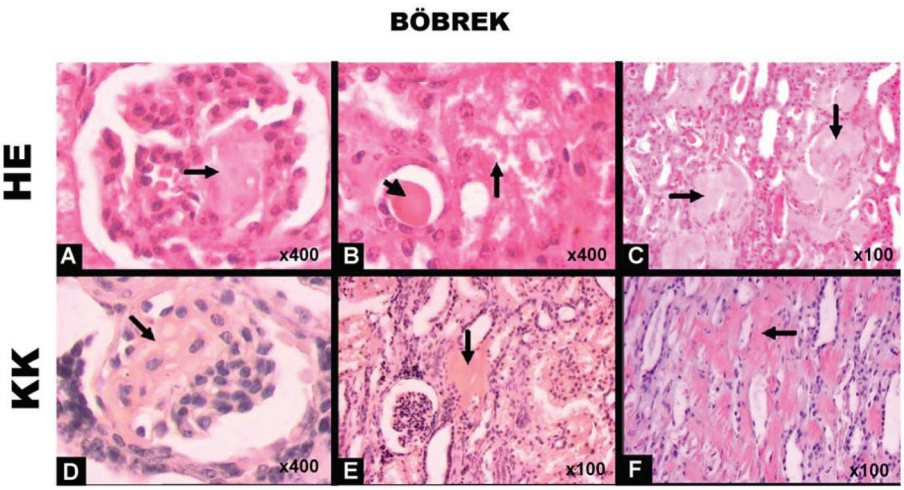
TARTIŞMA ve SONUÇ

Yaban hayvanlarında genellikle sekonder amiloidoza ilişkin vaka raporları bildirilmektedir [2]. Dorcas ceylanlarında amiloidozun genellikle kronik enfeksiyonlar sonucunda geliştiği [4], ancak genetik predispozisyonun da önemli olabileceği bildirilmiştir [2]. Bir dağ ceylanı [6] ve bir Arap ceylanındaki [5] amiloidozun sebebinin ise idiyopatik olduğu öne sürülmüştür. Çeribaşı ve ark.[17] bir İran ceylanında fibrino-



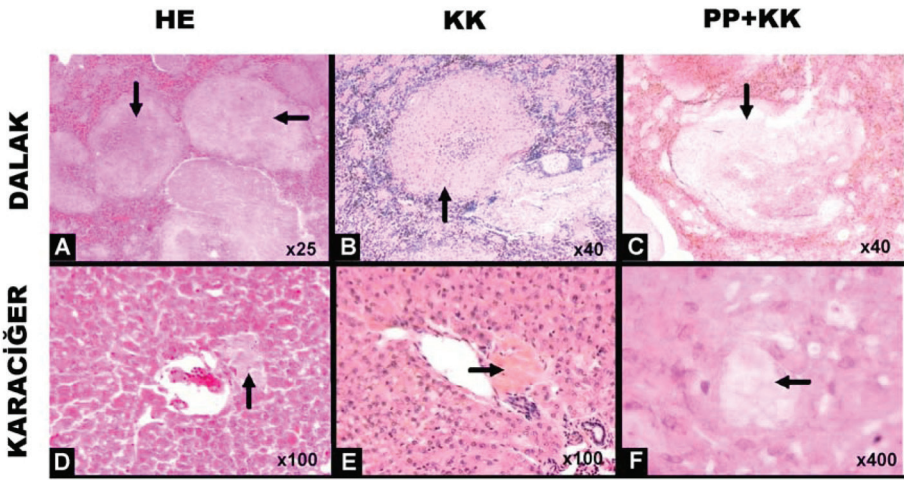
Şekil 1. Amiloid birikimleri, Lugol- sülfirik asit boyama. **A-** Böbrek *(ok)*, **B-** Dalak *(oklar)*, **C-** Karaciğer *(oklar)*

Fig 1. Amyloid deposits, Lugol-sulfuric acid staining. **A-** Kidney *(arrow)*, **B-** Spleen *(arrows)*, **C-** Liver *(arrows)*



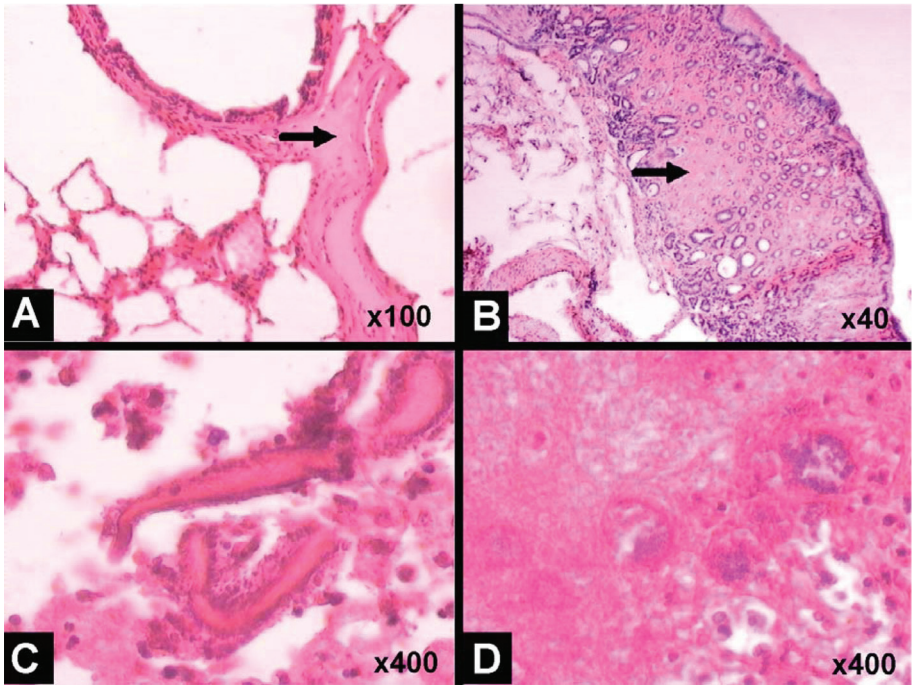
Şekil 2. Böbrekte amiloid birikimleri. **A,D-** Glomerulus *(ok)*, **B-** Tubulus lümenlerinde hiyalin silindiri *(okbaşı)* ve epitellerde hiyalin damlacıkları *(ok)*, **C,E,F-** İntertubuler interstisyumda amiloid birikimleri *(oklar)*. Hematoksilen-Eozin (HE), Kongo Kırmızısı (KK)

Fig 2. Amiloid deposits in kidney. **A,D-** glomeruli *(arrow)*, **B-** Hyaline cylinder in tubule lumens *(arrowhead)* and hyaline droplets in tubule epithelium *(arrow)*, **C,E,F-** Amyloid deposits in intertubular interstitium. Hematoxylin-eosin (HE), Congo Red (KK)



Şekil 3. Dalakta **(A,B)** ve karaciğerde **(D,E)** amiloid birikimleri. Sekonder amiloidozda dalak **(C)** ve karaciğerdeki **(F)** birikimlerin boya almaması. Potasyum Permanganat metodu [potasyum permanganat (PP) + Kongo Kırmızısı (KK)]

Fig 3. Amyloid deposits in spleen **(A,B)** and liver **(D,E)** Deposits in spleen **(C)** and liver **(F)** were not stained with potassium permanganate methode (PP + KK) on secondary amyloidosis



Şekil 4. A- Akciğerde interstisyumda amiloid birikimi *(ok)*, HE, **B-** Bağırsaklarda lamina propriyada amiloid birikimi *(ok)*, KK, **C,D-** Rumende epitelde nekroz, HE

Fig 4. A- Amyloid deposition in the lung interstitium *(arrow)*, HE, **B-** Amyloid deposits in the lamina propria in intestine *(arrow)*, Congo Red, **C,D-** Epithelial necrosis in rumen, HE

hemoajik pankreatitisle komplike generalize amiloidoz olgusunu tanımlamışlardır. Sunulan çalışmada, rumende geniş ülserle karakterize kronik yangının organlardaki amiloid birikiminin nedeni olabileceği düşünülmüştür.

Amiloidoz şüpheli dokuların nekropsi sonrası Lugol ve sülfirik asit ile muamele edildiğinde amiloid birikimlerinin mavi renkte görüldüğü bildirilmektedir [1]. Sunulan bu olguda amiloid birikimiyle ilgili organlarda makroskobik bir bulgu gözlenmemesi nedeniyle nekropsiden sonra bu uygulamaya gerek duyulmamıştır. Histopatolojik olarak amiloid birikimlerinin saptanması üzerine formaldehit solüsyonu içerisinde stok olarak bekletilen karaciğer, dalak ve böbrek dokularının kesit yüzlerine Lugol ve sülfirik asit damlatılması sonucu benzer şekilde mavi renkte amiloid birikimleri gözlenmiştir. Amiloidozlu dokuların uzun süre formolde bırakılması sonucu amiloidin boyanma özelliğinin azalabileceği, ancak kısa süreli tespitlerde iodinle yeniden boyanmanın mümkün olabileceği bildirilmiştir [18,19]. Bu sonuçla, formaldehit tespitinin amiloid birikiminin Lugol ve sülfirik asit muamelesiyle makroskopik teşhisini etkilemediği, nekropsi sonrası sadece taze dokularda değil formaldehitte tespit edilen dokularda da makroskobik olarak amiloidozun teşhis edilebileceğini göstermektedir.

Amiloidoza bağlı ölümlerin genellikle böbrek yetmezliği sonucu olduğu öne sürülmekte olup; Papendick ve ark.[3] amiloidozlu çitaların %74'ünde, Colegrove ve ark.[12] ise Kaliforniya denizaslanlarının çoğunda amiloidoza bağlı renal yetmezlik oluştuğunu bildirilmişledir. Bu çalışmada, her iki böbrekte korteks ve medulladaki amiloid birikimlerinin renal yetmezliğe neden olabileceğini düşündürmektedir.

Bu çalışmada bir dağ ceylanında generalize reaktif amiloidoz (AA-amiloidoz) olgusu makroskobik ve mikrokobik bulgularla tanımlanmış, amiloid birikimlerinin rumende

ülserli kronik yangıyla ilişkili olabileceği değerlendirilmiş ve amiloid birikimlerinin makroskobik olarak formol tespitinden sonra da gösterilebileceğine dikkat çekilmiştir.

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Kafkas Univ Vet Fak Derg
19 (4): 717-720, 2013
DOI: 10.9775/kvfd.2013.7598

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

CASE REPORT

Türkiye'de Köpeklerde *Babesia canis canis*'in Klinik ve Parazitolojik Olarak İlk Tespiti

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Makale Kodu (Article Code): KVFD-2013-7598

Özet

Bu makalede, Giemsa boyama ve Polimeraz Zincir Reaksiyonu (PZR) yöntemleri ile parazitolojik olarak *Babesia canis canis* tanısı konulan 3 köpekte klinik, biyokimyasal ve hematolojik bulgularının sunulması amaçlanmıştır. Olgu I ve II'de şiddetli ikterus, anemi, hemoglobinemi, hemoglobinüri, trombositopeni, taşipne, taşikardi ve miyozitis ile karaciğerde hasar geliştiği tespit edildi. Olgu I'de bu bulgulara ek olarak kolesistitis, Olgu II'de ise renal hasarın oluştuğu saptandı. Olgu III'te hafif anemi ve ikterus tablosuyla birlikte karaciğerde hasar ve miyozitis şekillendiği belirlendi. Tedavi sonucunda Olgu II ve III'de iyileşme sağlanırken Olgu I öldü. Sonuç olarak, köpeklerde *B. canis canis* klinik ve parazitolojik olarak Türkiye'de ilk kez bildirilmektedir.

Anahtar sözcükler: *Babesia canis canis, Babesiosis, Polimeraz Zincir Reaksiyonu, PZR, Köpek, Kars, Türkiye*

Clinical and Parasitological Detection of *Babesia canis canis* in Dogs: First Report from Turkey

Summary

This study was aimed to describe clinical, biochemical and haemathological findings in three dogs with *Babesia canis canis* diagnosed by Giemsa staining and Polymerase Chain Reaction (PCR) methods. In Case I and II severe icterus, anemia, haemoglobinemia, haemoglobinuria, thrombocytopenia, tachypnea, tachycardia, liver damage and myositis were determined. In addition in Case I cholecystitis and in Case II renal damage were developed. In Case III mild anemia and icterus along with liver damage and myositis were developed and clinical signs were mild. Following treatment Case II and III completely recovered while Case I died. In conclusion, *B. canis canis* has been determined clinically and parasitologically for first time in Turkey.

Keywords: *Babesia canis canis, Babesiosis, Polymerase Chain Reaction, PCR, Dog, Kars, Turkey*

GİRİŞ

Kanin babesiosis kenelerin naklettiği protozoal parazit olan *Babesia* türleri tarafından oluşturulan bir enfeksiyondur. Köpek *Babesia* türleri piroprasmik formlarının morfolojilerine göre, büyük (5 x 2.5 µm) ve küçük (2 x 1.5 µm) olarak iki grupta sınıflandırılmaktadır. Büyük türler *B. canis canis, B. canis vogeli, B. canis rossi* ve *Babesia* sp. (Coco), küçük türler ise *B. gibsoni, B. conradae* ve *B. microti-like (Theileria annae)*'dan oluşmaktadır [1-3]. Son yıllarda *B. c. canis, B. c. rossi ve B. c. vogeli*'nin, her ne kadar morfolojik olarak çok benzeseler de, klinik belirtilerinin değişkenlik göstermesi, ayrı yayılış alanlarına ve farklı vektörlere sahip olmaları nedeniyle, tek başlarına birer tür *(B. canis, B. vogeli, B. rossi)* olarak kabul edilmeleri eğilimi ağırlık kazanmıştır [2,4-6]. Perakut, akut, kronik ve subklinik seyir gösteren kanin babesiosis'de klinik belirtiler tür, vektör, ırk, yaş ve immun duruma göre değişebilmektedir [1-3,6-11]. *B. c. canis* orta derecede patojenik özellik gösterirken, *B. canis vogeli* daha hafif hastalık tablosuna neden olur. *B. c. canis* ve *B. gibsoni* ilerleyici hemolitik anemi yaparken, *B. canis rossi* dissemine intravasküler koagulopati (DIC) ile birlikte şok, sistemik yangı ve organ bozukluğuna yol açmaktadır [2,8,11]. Bilgilerimize göre Türkiye'de *B. c. canis*'in

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neden olduğu babesiosis vakası bildirilmemiştir [1,3,12,13]. Bu raporda, *B. c. canis*'in neden olduğu bebesiosis'in teşhis, klinik, biyokimyasal ve hematolojik bulgularının sunulması amaçlanmıştır.

OLGULARIN TANIMI

Olgu materyalini Kafkas Üniversitesi Veteriner Fakültesi İç Hastalıkları Anabilim Dalı kliniklerine iştahsızlık, durgunluk ve sarılık şikayetleriyle getirilen farklı yaş, cinsiyet ve ırkta 3 köpek oluşturdu. Köpeklerden tedavi öncesi ve tedaviden 15 gün sonra kan alınarak hemogram değerleri ve biyokimyasal parametrelere bakıldı. Hemogram değerleri MS4e marka hemogram cihazında, serumda biyokimyasal parametreler ise otoanalizörde (Kodak Vitros DT60II) ölçüldü. İdrar analizinde Cybow 10 M idrar stripi kullanıldı.

OLGU I

İki yaşında, dişi sokak köpeğinin incelendiği birinci olguda hastada 4 gün önce başlayan ve giderek şiddetini arttıran bir iştahsızlık olduğu öğrenildi. Hastada ayrıca ayakta durmakta zorlanma, çevreye karşı ilgisinin olmaması ile birlikte idrarının kırmızı renkte olduğu tespit edildi. Hayvanın üzerinde çok sayıda kene ile birlikte, klinik olarak apati, şiddetli ikterus, yüksek ateş (41.3ºC), hemoglobinuri, solunum ve nabız sayısının artış belirlendi. Hemogramda ise eritrosit (RBC), hemoglobin (Hb), hematokrit (HCT) değerlerinde ve trombosit sayısında şiddetli düşüş saptandı. Biyokimyasal analizlerde alkalen fosfataz (ALP), gamma gamma glutamiltransferaz (GGT), alanin aminotransferaz (ALT), aspartat aminotransferaz (AST), kreatin kinaz (CK), laktat dehidrogenaz (LDH), total bilirubin ve direkt bilirubin değerlerinin yükseldiği tespit edildi *(Tablo 1)*. İdrar muayenesinde bilirubinüriye rastlandı. Bu bulgulara göre olguda şiddetli anemi, trombositopeni ve myozitis şekillendiğinin belirlenmesinin yanında hastada kolangiohepatitis geliştiği belirlendi.

OLGU II

İkinci Olguyu 4 gündür halsizlik, iştahsızlık, durgunluk şikayetleri olan 3 yaşında erkek husky oluşturdu. Hastada ayrıca depresyon, sallantılı yürüyüş, çevreye karşı ilgisizlik, ikterus, solunum ve nabız sayısında artış, yüksek ateş (40.5ºC), hemoglobinuri belirlendi. Hayvanın derisinde farklı bölgelerde 8 adet keneye rastlandı. Hastanın RBC, Hb, HCT ve trombosit değerlerinde belirgin düşüş olduğu saptandı. Serum biyokimyasında ALP, CK, üre, kreatinin, total bilirubin ve direkt bilirubin değerlerinin yüksek olduğu belirlendi *(Tablo 1)*. Elde edilen verilere göre hayvanda şiddetli anemi, trombositopeni ve miyozitis şekillendiği belirlendi. İdrar analizinde ise bilirubinuri ve proteinüri tespit edildi. Ayrıca mevcut verilere göre hastada renal ve hepatik hasarın gelişmiş olabileceği düşünüldü.

OLGU III

İştahsızlık, durgunluk şikayetleriyle getirilen 5 yaşında erkek kangal ırkı köpeğin incelendiği III. Olguda inguinal bölgede 3 adet keneye rastlanırken, hastanın çevreye karşı ilgisinin azaldığı, mukozalarında hafif ikterusla birlikte solgunluk ve yüksek ateşi (40.6ºC) olduğu belirlendi. Hematolojik muayenede RBC, Hb, HCT değerlerinde hafif

Tablo 1. *Olgularda tedavi öncesi ve sonrası bazı hematolojik ve biyokimyasal parametrelerindeki değişiklikler*
Table 1. *Changes in some haematological and biochemical parameters in cases before and after treatment*

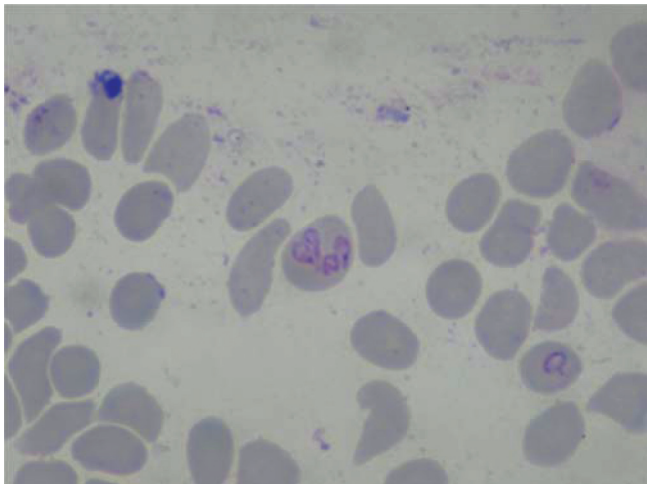
Parametreler		**Referans Değerler** [14]	**Olgu I*** **TÖ**	**Olgu II TÖ**	**Olgu II TS**	**Olgu III TÖ**	**Olgu III TS**
Hematolojik	WBC (x10³/µL)	5.5-16.9	6.01	5.33	10.53	13.15	9.05
	RBC (x10⁶/µL)	5.5-8.5	1.79	3.37	7.73	4.18	6.13
	HGB (g/dL)	12-18	4.4	8.7	11.9	10.9	15.9
	HCT (%)	37-55	12.3	23.1	36.4	31.6	46.9
	PLT (x10³/µL)	175-500	11	6	248	341	259
Biyokimyasal	ALP (U/L)	20-150	840	424	142	214	82
	GGT (U/L)	1-10	20	5	3	3	3
	ALT (U/L)	10-88	250	56	53	47	38
	AST (U/L)	10-88	188	61	52	63	42
	CK (U/L)	20-200	236	277	152	684	87
	LDH (U/L)	50-495	977	750	103	3779	128
	Üre (mg/dL)	25-55	52.5	107.1	50.2	41.9	42.2
	Kreatinin (mg/dL)	0.5-1.5	1.2	2.8	0.9	0.86	0.9
	Total Protein (g/dL)	5.4-7.7	5.6	4.3	7.4	5.2	5.4
	Albumin (g/dL)	2.3-3.8	2.2	1.8	3.5	2.7	2.8
	Total Bilirubin (mg/dL)	0.1-0.6	16.1	12.4	0.12	0.19	0.1
	Direkt Bilirubin (mg/dL)	0-0.3	>7	6.5	0.1	0.05	0.04

TÖ: *Tedavi öncesi,* ***TS:*** *Tedavi sonrası* *Olgu I eks olduğu için TS değerler bulunmamaktadır*

düşüş saptandı. Biyokimyasal analizlerde ise ALP, CK ve LDH değerlerinin yüksek olduğu belirlendi *(Tablo 1)*. Hematolojik ve biyokimyasal analizler sonucunda köpekte hepatik hasar gelişmiş olabileceği düşünüldü. Bunun dışında miyozitis ve hafif anemi şekillendiği anlaşıldı.

Her üç hayvanın üstünden toplanan kenelerin *Dermacentor reticulatus* olduğu anlaşılmış, yapılan sürme perifer kan frotilerinin Giemsa boyama sonuçlarında, mikroskopta eritrositler içinde büyük yapıda, çift veya çoklu piroplazmik formdaki *Babesia* etkenleri görülmüştür *(Şekil 1)*.

Klinik belirtiler ve parazitolojik laboratuar muayenesi (Giemsa boyama) sonucunda babesiosis teşhisi konulan olgular tedaviye alındı. Tedavide her üç köpeğe 6 mg/kg İM yolla tek doz imidokarb (İmicarp®, TeknoVet) uygulandı. Destekleyici tedavi amacıyla B kompleks ve C vitaminleri ve intravenöz yolla laktatlı ringer + dekstroz verildi. Olgu 1 tedavi başlandıktan bir gün sonra öldü. Hasta sahibinin istememesi nedeniyle nekropsi yapılamadı. İki ve üçüncü olgularda ise tedavi başlandıktan 15 gün sonra hastaların iyileştiği, hematolojik ve biyokimyasal değerlerin normale döndüğü belirlendi *(Tablo 1)*.



Şekil 1. Giemsa ile boyanmış sürme kan preparatında görülen tipik büyük *Babesia* etkenleri

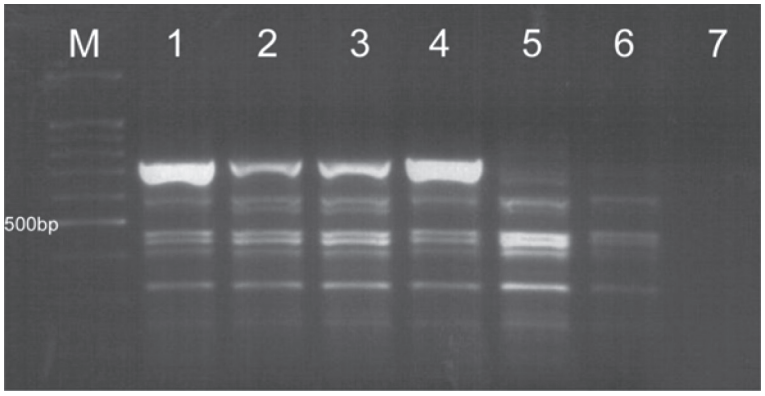
Fig 1. Typical large *Babesia* agents detected in blood smear with Giemsa stain

İncelenen olgularda etkenlerin tür düzeyinde doğrulaması, *B. canis canis*'e özgü primerler kullanılarak PZR testi ile yapıldı. Hayvanlardan EDTA'lı tüplere alınan kan örneklerinden ticari kit (High Pure Nucleic Acid Isolation Kit, Roche) kullanılarak üreticinin bildirdiği şekilde DNA ekstraksiyonu yapıldı. Polimeraz Zincir Reaksiyonu testinde Duarte ve ark.'nın [15] belirlediği ve 18S rRNA gen bölgesinden *B. c. canis*'e özgün 746 bp'lik bölgeyi çoğaltan Bab1 5'-GTG AAC CTT ATC ACT TAA AGG-3' ve Bab3 (5'-CTA CAC AGA GCA CAC AGC C-3') primerleri kullanıldı. Reaksiyon miktarı 25 µl olarak belirlendi ve her reaksiyon için 18.25 µl steril su, 2.5 µl 10x PCR buffer (Invitrogen), 1 µl 50 mM $MgCl_2$, 0.5 µl dNTP miks (Invitrogen), 20 pmol/µl konsantrasyondaki her primerden 0.5 µl, 0.25 µl Taq DNA polymerase 5U/µl (Invintrogen) ve 1.5 µl DNA örneği kullanıldı. Reaksiyon Biometra TGradient PCR cihazında toplam 35 döngü (94ºC'de 30 sn, 56ºC'de 30 sn, 72ºC'de 45 sn) üzerinden yapıldı. İlk döngüden önce 94ºC'de 2 dk denaturasyon ve en son döngüden sonra da 72ºC'de 10 dk ekstensiyon basamakları uygulandı. Reaksiyon sonuçları %1.5 Agaroz jelde yürütülerek UV ile görüntülendi. Türe özgü PZR sonucunda, her 3 olguda da özgün 746 bp'lik bantlar oluşurken, kontrol olarak kullanılan *B. c. rossi* ve *B. c. vogeli* DNA'larından benzer bant elde edilmemiştir *(Şekil 2)*.

TARTIŞMA ve SONUÇ

Köpeklerde *B. c. canis*'in neden olduğu babesiosis özellikle Orta Avrupa ve Rusya'da yaygın olarak görülmekte olup, son yıllarda kuzeye doğru bir yayılış göstererek Norveç ve Hollanda gibi ülkelerde de rapor edilmeye başlanmıştır [1,2,16,17]. Etken *D. reticulatus* tarafından nakledilmekte olup, hastalık bu kenenin yayılış alanları ile ilişkilidir [1,2]. Ülkemizde, günümüze kadar, genellikle *Rhipicephalus sanguineus* tarafından nakledilen *B. c. vogeli* ve *B. gibsoni* varlığı bildirilmiştir [3,12,13]. Öte yandan, çalışmamızda babesiosisli köpeklerde *B. c. canis*'in tespiti Türkiye için ilk rapor olup, gerek etkenin gerekse de *D. reticulatus*'un ülkemizde belli bölgelerde kalıcı popülasyon oluşturduğuna işaret eder niteliktedir.

Etkene ve komplikasyonlara göre değişmekle birlikte babesioisli köpeklerde genellikle anoreksi, depresyon, akut



Şekil 2. PZR sonuçları; M: 100bp DNA marker, **1-** Olgu-I, **2-** Olgu-II, **3-** Olgu-III, **4-** *B. canis canis* kontrol DNA, **5-** *B. canis rossi* kontrol DNA, **6-** *B. canis vogeli* kontrol DNA, **7-** Negatif kontrol (su)

Fig 2. PCR results; M: 100bp DNA marker, **1-** Case-I, **2-** Case-II, **3-** Case-III, **4-** *B. canis canis* control DNA, **5-** *B. canis rossi* control DNA, **6-** *B. canis vogeli* control DNA, **7-** Negative control (water)

hemoliz, hemoglobinüri, ikterus, taşipne, taşikardi, splenomegali değişen derecelerde hemolitik anemi ve trombositopeni görülmektedir [1,7,11,13]. Bu raporda klinik tablonun ağır seyrettiği olgularda (Olgu I ve II) anoreksi, apati, yüksek ateş, ikterus, hemoglobinuri, şiddetli anemi, trombositopeni, solunum ve nabız sayısında artış görüldü. Daha hafif klinik tabloya sahip olan köpekte ise (Olgu III) iştahsızlık, mukozalarda hafif ikterusla birlikte solgunluk ve ateşin yüksek olduğu belirlendi.

Kanin babesiosis'te en sık karşılaşılan hematolojik bulguları HCT değerin düşmesi, eritrositopeni ve hemoglobinemidir [1,2,9,10]. Sunulan raporda da II. Olguda hematokrit değerde belirgin düşüş, şiddetli eritrositopeni ve hemoglobinemi belirlenirken I. Olguda bu değerlerdeki düşüşün daha hafif olduğu görüldü. Kanin babesiosis'de en sık karşılaşılan bozukluklardan biri olan trombositopeninin [9-11] Olgu I ve II'de şiddetli olduğu belirlenirken Olgu III'te trombosit değerinin normal sınırlarda olduğu saptandı. Son yıllarda, *B. c. canis*'in kendi içinde farklı virulansa sahip iki genetik grup içerdiği ve bunların farklı hematolojik bulgulara, özellikle de farklı şiddette trombositopeniye neden olduğu bildirilmiştir [18]. Bizim olgularımızdaki farklılığın kesin olarak bundan kaynaklanıp kaynaklanmadığının ortaya konması için daha ayrıntılı çalışmaya ihtiyaç vardır. Yapılan bazı çalışmalarda *B. c. canis* enfeksiyonlarında lökopeni şekillendiği [7,11] bildirilmiş olsa da, bu çalışmada her üç olguda da lökosit sayısının referans değerler arasında olduğu görüldü.

Babesiosis'te böbrek yetmezliği, hepatopati ve miyozitis gibi çoklu organ bozukları gelişebilmektedir [9,11,19]. Olgu I'de ALT ve AST enzim değerlerinin yüksek olması, olgu II ve III'te ALP enzim değerinin yüksek bulunması üç olguda da karaciğerde olası bir hasar geliştiğine işaret etmektedir. Olgu I de ALP ve GGT enzimlerinin yüksek bulunması köpekte kolestazis'in hepatik hasara eşlik ettiği şeklinde yorumlanabilir. *B. c. canis* enfeksiyonlarında kolestazis şekillenmesi hakkında bir bilgiye rastlanmamıştır. Bu nedenle bu bulgunun önemli olduğu kanısındayız. Her üç olguda da CK ve LDH enzimlerinin yüksek bulunması miyozitis oluştuğunu göstermiştir. Yine literatürlerle uyumlu olarak [8,11,19] Olgu II'de üre ve kreatinin değerlerinin yüksek olması ve idrarda proteine rastlanması renal hasar gelişmiş olabileceği gösteren veriler olarak değerlendirilebilir.

Sunulan raporda *B. c. canis* enfeksiyonunun önemli biyokimyasal değişikliklerinden olan hiperbilirubinemi ve hemoglobinürinin [7,20], Olgu I ve II'de şiddetli geliştiği tespit edildi. Bu olgularda hemoglobinuri ve bilirubinüri gelişmesinin nedeni eritrositlerdeki aşırı hemolize bağlı olarak dolaşımda fazla miktarda Hb ve bilürübin bulunması, bu maddelerin renal eşiği aşmasından kaynaklanabilir.

Bu çalışma ile *B. c. canis* Türkiye'den ilk kez rapor edilmiştir. Olguların yerli hayvanlar olması ve bölgede de *D. reticulatus* popülasyonunun varlığı (yayınlanmamış bulgular), etkenin Kars gibi alpin iklime sahip yerlerde yerleşik hal aldığına işaret etmektedir. Bu çalışmada ayrıca, hastaların klinik ve biyokimyasal bulguları da izlenerek, hastalık konusunda ayrıntılı veriler elde edilmiştir.

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Kafkas Univ Vet Fak Derg
19 (4): 721-723, 2013
DOI: 10.9775/kvfd.2013.8648

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

CASE REPORT

A Case Report: Isolation of *Alysiella filiformis* from Pig's Lungs [1]

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[1] *This study was financially supported by a grant from the Ministry of Education and Science of the Republic of Serbia, under the project TR. 31079*

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Makale Kodu (Article Code): KVFD-2013-8648

Summary

Alysiella filiformis is considered a common resident in the oral cavities of many animals. All reports of *Alysiella* indicate that it is restricted to the oral cavity of warm-blooded vertebrates, where it apparently is nonpathogenic. However, increased losses of young pigs occured in one farm in Serbia. Spumous content in bronchia and partly clotted blood in blood vesels o f the lungs were present. Characteristic signs of oedema disease were present and *E. coli* serogroup 0139 was isolated. Furthermore, *Alysiella* filiformis was the single agent isolated from the lungs of diseased pigs. This is the first isolation of *Alysiella filiformis* from pig lungs.

Keywords: *Alysiella filiformis, Pig, Lungs*

Olgu Sunumu: Domuz Akciğerlerinden *Alysiella filiformis* İzolasyonu

Özet

Alysiella filiformis birçok hayvanın ağız boşlukları ortak bir sakini olarak kabul edilir. Literatüre göre etkenin sıcakkanlı hayvanların ağız boşluğu ile sınırlı ve patojenik olmadığı bildirilmiştir. Sırbistan bir çiftlikte genç domuz kayıpları arttışı gözlenmiştir. Postmortem incelemede akciğer kan damarları ve bronşlarda kısmen pıhtılaşmış kan ve köpüklü içerik gözlenmiştir. Ödem hastalığının karakteristik işaretler mevcut olduğunu ve *E. coli* serogrup 0139 izole edildi. Ayrıca, *Alysiella filiformis* hastalıklı domuz akciğerlerinden izole edilmiş tek bir ajan idi. Bu çalışmada hastalıklı domuz akciğerlerden *Alysiella filiformis* izolasyonu ilk kez bildirilmiştir.

Anahtar sözcükler: *Alysiella filiformis, Domuz, Akciğerler*

INTRODUCTION

Alysiella filiformis (A. filiformis) is considered a common resident in the oral cavities of many animals. All reports of *Alysiella* indicate that it is restricted to the oral cavity of warm-blooded vertebrates, where it apparently is nonpathogenic. *A. filiformis* is the solitary member of genus *Alysiella,* family *Neisseriaceae* [1,2]. Genus *Alysiella* comprises organisms that exist in characteristic flat, ribbon-like multicellular filaments. The cells within the filament are paired, and in axenic culture the filament often breaks up into groups of two or four cells. The width of an individual cell is about 2.0–3.0mm, and the length of a cell is about 0.6 mm [1,2]. *A. filiformis* has been isolated from the oral cavity of different animals including guinea pigs [3], sheep [4], and cows [5], and in Turkey it was isolated from hot spring water indicating its presence in the environment [6]. This paper presents a case of the isolation of *Alysiella filiformis* from the lungs of pig with oedema disease.

CASE HISTORY

During May 2009, one swine farm with 1.000 pigs situated in northwest of Serbia, reported increased losses of young pigs. According to anamnestic data, live affected pig showed a staggering gate, puffy eyelids giving a sleepy appearance and an abnormal high pitched squeak. During nine days, 14 pigs (weight around 15 kg) in very good condition died, and dispnea was the only clinical symptom present in diseased pigs. Post-mortem examinations showed

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Table 1. *Biochemical characteristics of Alysiella filiformis isolate*
Tablo 1. *Alysiella filiformis biyokimyasal özellikleri izole*

Medium	Reaction	Medium	Reaction
Catalase	positive	Inositol	acid not produced
Oxidase	positive	Xylose	acid not produced
Glucose	acid produced	Arabinose	acid not produced
Maltose	acid produced	Mannitol	acid not produced
Trehalose	acid produced	Gelatin	negative
Sucrose	acid produced	H_2S	not produced
Fructose	acid produced	MR	negative
Lactose	acid not produced	VP	negative
Cellobiose	acid not produced	Indole	negative
Raffinose	acid not produced	Urease	negative
Rhamnose	acid not produced	Aesculin	not hydrolyzed
Mannose	acid not produced	Nitrate	not reduced
Dulcitol	acid not produced	Nitrite	not reduced
Salicin	acid not produced	Simmon´s citrate	no growth

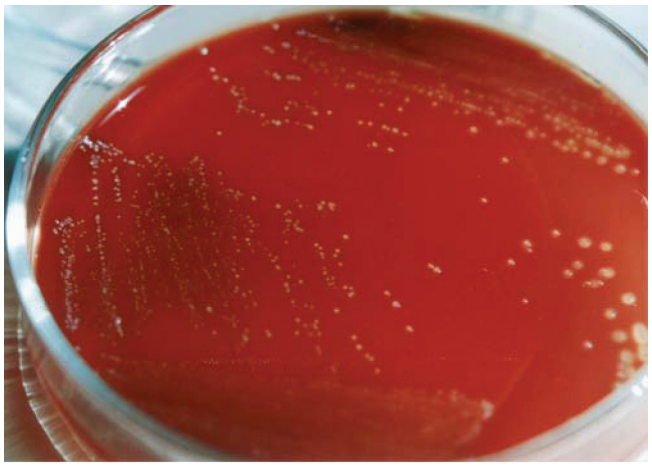

Fig 1. Colonies of *A. filiformis* on blood agar
Şekil 1. Kan agar *A. filiformis* kolonileri

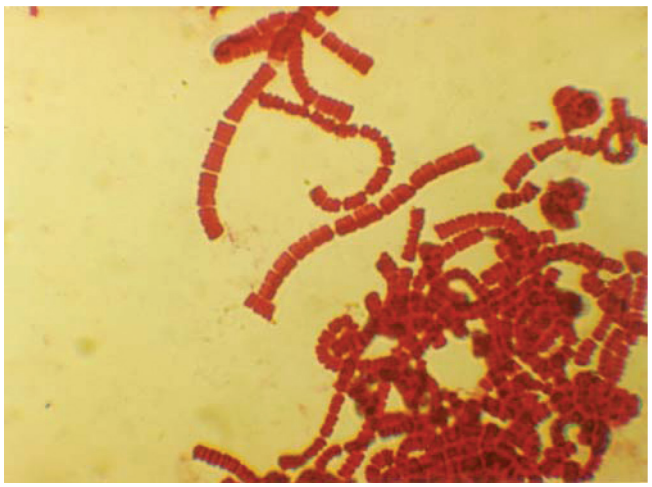

Fig 2. Ribbon-shaped filaments of *A. filiformis* (Gram stain, x1.000)
Şekil 2. *A. filiformis* şerit şeklindeki filamentler (Gram boyama, x1.000)

oedema of the greater curvature of the stomach wall, coiled colon and eyelids. Spumous content in bronchia and partly clotted blood in blood vesels of the lungs were present.

Samples of small intestines were inoculated on nutritient agar (HiMedia) containing 5% sheep blood and MacConkey agar (HiMedia), and incubated aerobically at 37°C for 24 h. Lung samples were also inoculated on 5% sheep blood agar and incubated aerobically at 37°C for 24 h. The cultural, biochemical and morphological characteristics of grown cultures were examined. Bacterial isolates were identified using standard methods for phenotypic characterization as previously described [1,2,7,8]. After 24 h of incubation, *E. coli* was grown on sheep blood agar and MacConkey agar from the small intestine samples. Using antisera (*E. coli* O antisera, HiMedia), isolate was identified to belong to serogroup O139. From lung samples, no growth was seen on MacConkey agar, but large numbers of smooth, convex, β-haemolytic, white-grayish colonies, about 1.5mm diameter, were present in pure culture on sheep blood agar.

After subcultivation on MacConkey agar at 37°C and 25°C, as well as on sheep blood agar at 25°C, culture was sterile, but bacterial growth occurred after subcultivation on sheep blood agar at 37°C. Gram staining of isolate revealed gram-negative cells, 0.6 x 2-3 µm, arranged side by side in pairs to form flat, ribbon-shaped filaments, with variable lenght.

Isolate was further tested by biochemical tests (bioMerieux, HiMedia).

On the basis of the cell morphology, cultural and biochemical characteristics, isolate was identified as *Alysiella filiformis*.

DISCUSSION

In difference from the other representatives of this family, cells of genera *Simonsiella* and *Alysiella* may exibit a characteristic multicellular micromorphology. Moreover, the unusual morphology of the *Alysiella* filaments serves to differentiate the genus from all other procaryotic organisms. *Alysiella* filaments differ from *Simonsiella* in consisting of continuous pairs of cells instead of being segmented into units of eight cells, and the terminal cells are not rounded, but square [2]. In this case, *A. filiformis* was isolated from lung tissue in pure culture, with ribbon-shaped filaments of with variable lenght, similar to other authors [2]. The biochemical properties of bacteria isolated from lung tissue in this study are very similar to those described in other studies [2,7,8].

The origin and significance of the presence of *A. filiformis* in the lung tissue is not clear. *A. filiformis* has been described as a comensal in oral cavity of animals [3-5], but

there is no report of its presence in other tissues or organs. Also, there is no known pathology as a consequence of presen ce of this bacterium. Because of this fact, it is hard to determine the role bacterium in described macroscopic lesions present in lung tissue of diseased pig. In the present case, suspicion of possible role of *A. filiformis* in lung pathology was based on fact that it was isolated from lung tissue in pure culture, with abundant growth and presence of clearly visible zone of beta-haemolysis around colonies.

The reasons for the colonization of lung tissue with *A. filiformis*, and its potential role in the etiology of pathoanatomical changes in lung tissue remain unclear. Further studies are needed to determine the pathogen potential of this bacterium.

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Kafkas Univ Vet Fak Derg
19 (4): 725-734, 2013
DOI: 10.9775/kvfd.2012.8415

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

REVIEW

Possible Natural Toxins in Organic Livestock Farming

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Makale Kodu (Article Code): KVFD-2012-8415

Summary

Public concern about food quality has intensified in recent years. In the last decades, the amount of farmland managed under certified organic practices has expanded dramatically and it is expected to increase in the future. There is a growing demand for organic foods driven primarily by consumers' perceptions of the quality and safety of these foods and to the positive environmental impact of organic agriculture practices. This growth in demand is expected to continue in the future. Obviously many factors in production systems have an impact on the health and welfare of the animals involved. Animals feed or forage may be the source of disorders for farm animals also could lead to human illness. The types of feeds administered do not differ significantly in organic farming and in this respect there are mostly quantitative differences observed. Pesticides, agricultural and industrial chemicals, heavy metals, radionuclides as well as natural pollutants as mycotoxins may pollute animal feed. The aim of this review was to find out the possible natural toxins of animals associated with organic farming.

Keywords: *Organic farming, Feeding, Natural toxins, Pollutants*

Organik Hayvancılıkta Olası Doğal Toksinler

Özet

Son yıllarda halkın gıda kalitesi ile ilgili endişeleri artmıştır. Son dönemlerde sertifikalı organik üretim uygulamaları yapılan alanlar önemli ölçüde genişlemiş ve gelecekte daha da artacağı tahmin edilmektedir. Organik ürün eldesiyle ilgili uygulamaların çevreye olan olumlu etkileri, elde edilen ürünlerin gıda kalitesi ve güvenliği ile ilgili tüketicilerin algıları bu ürünlere olan talebi giderek artırmaktadır. Organik ürünlere olan sözkonusu talebin gelecekte de artacağı tahmin edilmektedir. Organik üretim sistemindeki birçok uygulamanın hayvan sağlığı ve refahı üzerine olan etkileri açıktır. Hayvan yemlerinin çiftlik hayvanlarında bazı metabolik bozuklukların kaynağı olabilir, hatta insanlarda da hastalıklara yol açabilir. Organik hayvansal üretimde hayvanlara verilen yem çeşidinin niteliğinde belirgin bir fark yokken, genellikle miktarda nicelik farklılıklar gözlenmektedir. Pestisidler, tarımsal ve endüstriyel kirleticiler, ağır metaller, radyoetkin bileşikler ile doğal kirleticilerden mikotoksinler de dahil olmak üzere birçok etken hayvan yemini kirletebilirler. Bu derlemenin amacı, organik hayvancılıkta olası doğal toksinler hakkında bilgi sunmaktır.

Anahtar sözcükler: *Organik tarım, Beslenme, Doğal toksinler, Kirleticiler*

INTRODUCTION

The industrial agriculture system consumes fossil fuel, water, and topsoil at unsustainable rates [1]. The expansion of factory style animal agriculture creates environmental and public health concerns, including pollution from the high concentration of animal wastes and the extensive use of antibiotics, which may compromise their effectiveness in medical use. The type of agriculture that has become conventional throughout the industrialized world is, in historical terms, a new phenomenon [2-5]. Humans have practiced agriculture for more than 10000 years, but only in the past 50 years or so have farmers become heavily dependent on synthetic chemical fertilizers and pesticides and fossil fuel-powered farm machinery. In the last decades, however, industrial agriculture has increasingly separated animals from the land [6].

Organic farming is the result of theory and practice since the early years of the 20th century, involving various alternative methods of agricultural production, mainly in northern Europe [7-9]. Organic livestock farming is based on the principle of a close link between the animals and the soil. The need for a link with the soil requires animals

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to have free access to outside areas for exercise, and also implies that their feed should be not only organic, but preferably produced on the farm. This sector of organic farming is, also, strictly regulated by rules of animal welfare and veterinary care [10-13]. Organic farming was developed and driven by people searching for a sustainable way of farming. They often sold their products directly to consumers, bypassing the many marketing steps between the farm and the consumer. Today the situation is very different [14-18]. Organic farming is likely to receive a major boost in the European Union (EU) and most probably also worldwide since consumers have lost some trust in food derived from conventional production. However, this growth in organic farming is also expected to continue in the presumable future in Turkey [19]. This is as a result of recent crises and due to concerns apropos the use of pesticides in farming and antibiotics in livestock feed [11,20-23].

European Council Regulation (EEC) No 2092/91 on organic production of agricultural products and suggestions referring on agricultural products and foodstuffs was adopted on 24 June 1991. In 1999, the Council adopted Regulation (EC) No 1804/1999 of 19 July 1999, which lays down Community rules for producing organic livestock products; this completes the framework of Community legislation, which now covers both crop and animal products. Part B of Annex I to Regulation (EEC) No 2092/91, as amended on 19 July 1999 by Regulation No 1804/1999, lays down minimum rules for organic livestock production. The Member States may adopt stricter rules, under Article 12 of Regulation (EEC) No 2092/91, concerning the animals and animal products produced on their territory [13,24].

As the nutritional quality of organic foods for humans there seems to be no difference in nutritional quality between an organic diet and conventional diet, though the perception of consumers is that organically-produced crops and animal products are of higher nutritional quality and safety [25-28]. The small number of papers published is not surprising since the development of organic farming and its philosophy [29]. Therefore, this article is limited to review the possible natural toxins that represent significant risks to organic livestock.

1. ORGANIC FARMING AND ANIMAL FEEDING

To get insight into the different types of feeds used in organic and conventional production systems information was obtained from different sources on the internet. This because the information in scientific articles was too limited and fragmented [11,27,29]. From the information assessed it is clear that a major difference between organic and conventional production of feeds is that in organic production only limited crop production and other products are permitted. A remark should be made the natural source of the products is no guarantee for non-toxicity for humans [30-33].

International food safety standards and food hygiene requirements are equally valid for conventionally and organically produced food. The issue of other food quality characteristics is less clear-cut. There is a widely-held view that the food industry is best placed to make decisions about the quality of their products based on their understanding of market demands [9,18,34]. However, compulsory and optional quality standards do exist to ensure that essential product requirements are met and to protect consumers against fraudulent practices [35,36]. Most problems that occur in conventional agriculture may also be present in organic farming, such as erosion, nitrogen leaching, ammonia volatilisation from animal wastes, high levels of native soil cadmium, accumulation of trace metals in soil, and subsoil compaction caused by farm machinery [37-41]. Organic farming methods do not offer solutions to many of these problems. In contrast to conventional agriculture, organic farming without purchase of feed may result in a nutrient depletion of soils. Through the import of feeding stuff to farms, which means a net input of nutrients, depletion is normally avoided. As the feeding stuff may be produced elsewhere with inorganic fertilizers, organic farming indirectly depends on the soil fertility of conventional farming [42,43]. However, regulations about the conventionally grown feeding stuff to be used in organic farming differ between countries [24,34,44,45].

For cattle the types of feeding of the animals do not differ between organic and conventional systems. Feeding of organic cattle should however be derived from organic production systems [46-48]. Also the percentage of mixed feeds and raw feeds differ in organic cattle production. For example 60% of the feeds should be raw feeds for organic cows. For organic cows also no synthetic additives, antibiotics etc. are allowed. Rules for pigs and chickens are similar [15,20,49]. In conclusion the major differences in the quality of feeds between organic and conventional products systems seems to originate from differences in production of plants (such as limited use of crop production products) and the lack of certain additives in the feeds. The types of feeds administered do not differ significantly and there are mostly quantitative differences observed (more raw feeds) [13,14,27,50,51].

2. ENVIRONMENTAL CONTAMINANTS

One reason for the increase in organic agriculture in many countries in Europe today is we need to solve environmental problems. In such situations, we often tend to accept appealing solutions. Furthermore, the intensive propaganda by representatives of organic farming movements has had a strong influence on public opinion,

politicians, and scientists [38,44,52,53]. A wide range of organic and inorganic compounds may occur in feedstuffs, including pesticides, industrial pollutants, radionuclides and heavy metals. Pesticides that may contaminate feeds originate from most of the major groups, including organochlorine, organophosphate and pyrethroid compounds. Although pesticides are potentially toxic to farm livestock, the primary focus of concern centers on residues in animal products intended for human consumption [31,54-59]. It should be noted that organic producers are not prohibited from using all pesticides -certain pesticides from natural sources can be used. Natural pesticides like the chemically synthesised pesticides must be subject to safety evaluation. Natural pesticides used in organic management are usually restricted under certification schemes. International guidelines for organically produced foods include lists of substances that can be used for plant pest and disease control if the need for such is recognised by the certification body. In organic management, biological control is the preferred method of pest management [13,35,50].

Contaminants in animal feeds, such as pesticide residues, agricultural and industrial chemicals, heavy metals and radioactive nuclides, can result in safety hazards in foods of animal origin. As EC regulations (EC No 1804/1999) require that livestock, claimed to be produced organically, is fed on organically produced feed stuffs, the potential for contamination with pesticide residues and other agricultural chemicals is greatly reduced compared to conventional farming methods [3,39,60]. However, organic agriculture does not reduce the levels of persistent environmental pollutants in organically grown products. These may therefore be present in organic feedstuffs and hence in organic food of animal origin [13,32,35,61]. Conversely, excluding pesticides may result in increased concentrations of secondary plant metabolites and of mycotoxins of field fungi. Thus, to exclude pesticides does not necessarily mean that crop products do not contain unwanted substances [31,44].

3. POSSIBLE NATURAL TOXINS OF ANIMAL FEEDS

Animal products are a primary source of proteins, amino acids and fats, and when they are a major constituent of diets they contribute a significant part of total calories. They are, however, perishable products which require special attention to prevent their deterioration and contamination by various agents, biological as well as chemical. Some of these contaminants can be transferred by the feed [22,62-64]. Animal feeds are routinely subject to contamination from diverse sources, including environmental pollution and activities of insects and microbes. Animal feeds may also contain endogen toxins arising principally from specific primary and secondary substances produced by fodder plants [65-67]. Thus, feed toxins include compounds of both plant and microbial origin. Although these toxins are often considered separately, because of their different origins, they share several common underlying features. Therefore, particular compounds within both plant and microbial toxins may exert antinutritional effects or lessen reproductive performance in farm animals [23]. Also, the combined effects may be the result of additive or synergistic interactions between the two groups of compounds. The extent and impact of these interactions in practical livestock feeding remain to be quantified. Feed contaminants and toxins occur on a global scale but there are distinct geographical differences in the relative impact of individual compounds. The term “feed” is generally used in its widest context to include compound blends of straight ingredients as well as forages. With such a broad perspective, it is necessary and more instructive to introduce some focus [54,59,68].

3.1. Contamination from Natural Fertilisers

Variation in stocking rates of grazing ruminants can change the structure and composition of pastures with potential impacts on biodiversity and produce methane, a greenhouse gas [69,70]. The search for reducing agricultural surpluses led the EU to promote livestock production extensification and a decrease in stocking rates on grasslands. The value of species rich grasslands are also reinforced by the increased interest of consumers in site-specific and origin-labelled products, and the growing scientific evidence of the role of local grassland flora on various sensory characteristics of both cheese and meat products, such as colour and flavour of cheese and meat, respectively [34,71,72]. Animal manure and other organic waste are the main fertilisers used in organic farming. These natural fertilisers are also widely used in conventional agriculture with chemically synthesised fertilisers. Microbiological contamination arising from the use of natural fertilisers and measures needed to address it must focus on both organic and conventional agriculture. Untreated or improperly treated manure or biosolids used as fertilisers or soil nutrient agents, whether in organic or nonorganic agriculture, can lead to contamination of products water sources [42,53,73,74]. Animal and human faecal matters are known to contain human pathogens. Properly treated manure or biosolids are effective and safe fertilisers. Growers need to follow good agricultural practices for handling these natural fertilisers to minimise microbial hazards. Researches indicates that pathogenic organisms can survive up to 60 days under compost conditions. The Codex General Principles of Food Hygiene provide the basic rules for ensuring food safety for all foods. Organic production, as with all other types of food production, must follow the rules outlined in this international code of practice. However, for some, drift and runoff from near fields may result in microbial hazards. Growers may consider scheduling application of manure on near fields to maximise the time between manure application to those fields and harvest of fresh market products [5,35,50,51,53].

3.2. Bacterial Contamination

There is considerable interest in the occurrence of *Escherichia coli (E. coli)* in animal feeds following the association of the O157:H7 type of these bacteria with human illness [2]. The US Centre for Disease Control (CDC) identifies the main source of human infection with *E. coli* as meat contaminated during slaughter. Virulent strains of *E. coli*, such as *E. coli* 0157:H7 develop in the digestive tract of cattle, which is mainly fed with starchy grain. Replication of faecal *E. coli*, including the O157:H7 type was demonstrated in various feeds under conditions likely to occur on cattle farms in the summer months [2,8,49]. Since faecal contamination of feeds is widespread on farms, it is an important route for exposure of cattle to *E. coli* and other organisms. The potential for exposure to bacteria also exists when poultry litters are fed to cattle. Cows mainly fed with hay produce less than 1% of the *E. coli* found in the faeces of grain-fed animals. It is one of the most important goals of organic farming to keep the nutrient cycles closed. Therefore, ruminants like cattle and sheep are fed diets with a high proportion of grass, silage and hay. It can be concluded that organic farming potentially reduces the risk of *E. coli* infection [55,56,75-78]. *Listeria monocytogenes* occur in poor-quality silages and big-bale silage. When grass is ensiled under anaerobic conditions, the low pH regime ensures that *Listeria spp.* is excluded from the resulting silage. However, in big-bale silage a degree of aerobic fermentation may occur, raising pH levels and allowing the growth of *Listeria spp.* These bacteria also survive at low temperatures and in silages with high levels of dry matter. Contamination of silage with *Listeria spp.* is important as it causes abortion, meningitis, encephalitis and septicaemia in animals and humans. The incidence of various forms of listeriosis has been increasing in recent years [79-81].

3.3. Mycotoxins

There are consistent reports of worldwide contamination of feeds with fungi and their spores. In the tropics, *Aspergillus* is the predominant genus in dairy and other feeds [82,83]. Other species include *Penicillium*, *Fusarium* and *Alternaria*, which are also important contaminants of cereal grains. Fungal contamination is undesirable because of the potential for mycotoxin production. However, spores from mouldy hay, silage, brewers' grain and sugar beet pulp may be inhaled or consumed by animals with destructive effects termed "mycosis" [31,59,84-86]. Most important mycotoxins were resumed in *Table 1* [87].

Mycotoxins are metabolites produced by fungi of various genera while they grow on agricultural products before or after harvest or during transport or storage. Mycotoxin contamination of forages and cereals often occurs in the field following infection of plants with particular pathogenic fungi or with symbiotic endophytes. Contamination may also occur during processing and storage of harvested products and feed whenever environmental conditions are appropriate for spoilage fungi [88,89]. Mycotoxins are regularly found in animal feed ingredients such as maize, sorghum grain, rice meal, cottonseed meal, groundnuts and legumes, wheat, barley and others. Most are relatively stable compounds and are not destroyed by the processing of feed and may even be concentrated in screenings. Some fungi such as *Aspergillus spp*. and *Penicillium spp*. can invade grain after harvest and produce mycotoxins, while others, such as *Fusarium spp*., typically infest grains and produce mycotoxins before harvest [85,90-92]. In some circumstances *Aspergillus ssp.* can grow and produce mycotoxins before the crop is harvested. Both intrinsic and extrinsic factors influence fungal growth and mycotoxin production. The intrinsic factors include moisture content and acidity, whereas extrinsic factors also include appropriate substrates (rice, corn, nuts, wheat, and food and feeds originated from them) and production period for 3-6 days [35,57,93-95]. There is only limited information available on the occurrence of mycotoxin residues in animal products intended for human consumption. It is known that milk cows can convert Aflatoxin B_1 into

Table 1. *Common mycotoxins, commodity affected, and negative effects on health*
Tablo 1. *Sıklıkla karşılaşılan mikotoksinler, etkilenen ürünler ve sağlığa olan olumsuz etkileri*

Mycotoxin	Commodities	Fungal Source(s)	Effects of Ingestion
Aflatoxin B_1, B_2, G_1, G_2	Corn, peanuts, and many other commodities	*Aspergillus flavus* *Aspergillus parasiticus*	Aflatoxin B_1 identified as potent carcinogen by IARC [a] Adverse effects in various animals especially chickens
Deoxynivalenol Nivalenol (Vomitoxin)	Wheat, corn and barley	*Fusarium graminearum* *Fusarium crookwellense* *Fusarium culmorum*	Human toxicoses in India, China, Japan, and Korea Toxic to animals, especially pigs
Zearalenone	Corn, wheat	*Fusarium graminearum* *Fusarium culmorum* *Fusarium crookwellense*	Identified as possible carcinogen by IARC. Affects reproductive system in laboratory animals and pigs
Ochratoxin A	Barley, wheat and many other commodities	*Aspergillus ochraceus* *Penicilium verrucosum*	Suspected by IARC as human carcinogen Carcinogenic in laboratory animals and pigs
Fumonisin B_1	Corn	*Fusarium moniliforme* and several less common species	Suspected by IARC as human carcinogen. Toxic to pigs and poultry. Cause of equine eucoencephalomalacia (ELEM) a fatal disease of horses

a: *International Agency for Research on Cancer*

Aflatoxin M_1, which is found in milk. This has caused considerable trade problems when Aflatoxin M_1 is found in milk, and concern about the safety of such milk [35,57,96].

Mycotoxicoses are diseases caused by exposure to foods or feeds contaminated with mycotoxins. Mycotoxins show various biological effects in animals, such as liver and kidney toxicity, central nervous system effects or estrogenic effects. There are differences between animals on the susceptibility towards different mycotoxins [68,85,97]. Poultry secrete mycotoxins relatively fast because of a particular digesting system. The ingredients used for animal feeding should be checked to ensure that satisfactory quality standards are maintained and that mycotoxins are not present at higher than acceptable levels [96,98]. Further research is needed to study the metabolism of mycotoxins by animals and the residues of mycotoxins and their metabolites in animal tissues. However, in many instances the problems of mycotoxins in feeds have more direct effects that they can create illness in animals and prevent efficient growth or feed use [49,84,89].

Since fungicides are not allowed in organic production and given that mycotoxins constitute a major health hazard, their relative presence in foods produced organically or conventionally has been the subject of many studies. From these studies it cannot be concluded that organic farming leads to an increased risk of mycotoxin contamination. It is important to emphasise that good agricultural, handling and storage practices are required in organic as in conventional agriculture to minimise the risk of mould growth and mycotoxin contamination [35,91,98,99]. Several research teams in EU have carried out comparative surveys of the frequency and levels of mycotoxins in conventional and organic foods. The results are surprisingly consistent. Averaged across 24 direct comparisons of mycotoxins in conventional and organic foods in published studies, mycotoxins were detected in conventional food about 50 per cent more often than in corresponding organic food [11,31[. Mycotoxin levels in conventional food averaged a little over twice as high as in the corresponding organic foods. The probable explanations for the higher levels of mycotoxins in the conventional wheat crops grown in EU is the routine use of high levels of nitrogen fertilizer, and fungicide applications to prevent diseases and losses [31].

It has been suggested that organically produced food has higher levels of mycotoxin contamination because organic farming bans the use of fungicides. There is no evidence to support this claim [98,99]. In fact organic farmers would contend that their crops are less prone to fungal diseases because high doses of nitrogen increase the growth rate of crops leading to a thinning of the plant cell walls making the crop more vulnerable to fungal attack [49,99,100]. Good animal feeding practices also require that feed is stored in such a way to avoid contamination. As organically raised livestock are fed greater proportions of hay, grass and silage, there is reduced opportunity for mycotoxin contaminated feed to lead to mycotoxin contaminated milk [35,89,98].

3.4. Potential Plant Toxins

Many plant components have the potential to precipitate adverse effects on the productivity of farm livestock. These compounds are present in the foliage seeds of almost every plant that is used in practical feeding [101]. Typical concentrations of selected toxins were presented in *Table 2* [59].

Table 2. *Plant toxins: sources and concentrations*
Tablo 2. *Bitkisel toksinler: kaynakları ve konsantrasyonları*

Toxin	Principal Source(s)	Typical Concentrations
Lectins	Jackbean	73 units/mg protein
	Winged bean	40-320 units/mg
	Lima beans	59 units/mg protein
Trypsin inhibitors	Soybean	88 units/mg
Antigenic proteins	Soybean	-
Cyanogens	Cassava root	186 mg HCN/kg
Condensed tannins	Acacia spp.	65 g/kg
	Lotus spp.	30-40 g/kg
Quinolizidine alkaloids	Lupin	10-20 g/kg
Glusosinolates	Rapeseed	100 mmol/kg
Gossypol	Cottonseed	0.6-12 g/kg (free)
Saponins (steroidal)	*Brachiaria decumbens, Panicum spp.*	-
S-methyl cysteine sulphoxide	Kale	40-60 g/kg
Mimosine	*Leucaena leucocephala*	145 g/kg (seed)
		25 g/kg (leaf)
Phyto-oestrogens	Clover, lucerne, soybean	-

Plant toxins may be divided into heat-labile and heat stable groups. Heat-labil group comprising lectins, proteinase inhibitors and cyanogens, which are sensitive to standard processing temperatures. Heat-stable group includes many compounds such as antigenic proteins, condensed tannins, quinolizidine alkaloids, glucosinolates, gossypol, saponins, nonprotein amino acids (such as S-methyl cysteine sulphoxide and mimosine), and phyto-oestrogens [102-104]. The role of these substances as anti-nutritional factors has been considered in detail by D'Mello [59]. Contamination of animal feeds with weed seeds is also a major problem worldwide. The impact of weed seeds arises from the toxins they contain and from their diluent effects on nutrient density of feeds. Examples of weed seeds that are controlled by legislation in various countries include those of *Datura spp.*, common vetch, castor-oil plant and *Crotalaria spp.*[35,54,59].

4. CONTROL OF FEEDBORNE HAZARDS

Over many years feeding animals and preparing feed ingredients has not received adequate quality and safety attention. FAO organized in 1997 an Expert Consultation on Animal Feeding and Food Safety to determine how to better address the problems of feed ingredients and contaminated feed [54]. The primary purpose of this Expert Consultation was to discuss current animal feed problems, and to develop a draft Code of Practice for Good Animal Feeding for consideration by the Codex Alimentarius Commission, as advice to FAO member countries. This draft Code covers good animal feeding practices, and adherence to Good Manufacturing Practices (GMPs) in the procurement, handling, manufacturing, storage and distribution of commercially-produced feeds for food-producing animals. Feed and feed ingredients should be obtained and preserved in stable conditions to prevent hazardous effects due to contamination or deterioration. When received, feeds should be in good condition and meet generally accepted quality standards. GMPs should be followed always [54,84].

Preventive management of mycotoxin contamination of food and animal feed should be checked regularly in organic farming. Strategies to prevent mycotoxin contamination must be applied strictly. It is important for producers to realise that good agricultural practices (GAPs) represent the primary line of defence against contamination of cereals with mycotoxins, followed by the implementation of GMPs during the handling, storage, processing, and distribution of cereals for human food and animal feed [105]. Many strategies to prevent mycotoxin contamination of food and animal feed have been developed. Various crop genotypes resistant to fungus infection [106-108], pre-harvest control strategies [89,109], field and harvest management as well as post-harvest applications including improving drying and storage conditions [110-113], and the use of biological agents [114-116] have been shown important in the prevention of mycotoxigenic mould growth and mycotoxin formation. Preservation can be facilitated by low temperature storage, ensiling, dehydration or the addition of appropriate chemicals [54]. However, several natural plant extract and spice oils of eugenol, cinnamon, oregano, onions, lemongrass, turmeric and mint are known to prevent both mould growth and mycotoxin formation during post-harvest season [112,117-119]. Although activated charcoal, hydrated sodium calcium aluminosilicate, aluminosilicate, zeolite, and bentonite have shown good potential for use in the animal feed to help overcome aflatoxicosis. Recently, there has been an increasing interest in the use of bacteria, yeast, and fungi to help reduce the toxic effect of mycotoxins [114,120,121]. Another approach to the problem has been the use of mycotoxin-binding agents in the diet that sequester the mycotoxin in the gastrointestinal tract thus reducing their bio-availability [94,95,119,122]. The suitability of these applications may be questionable regarding the organic farming regulations. At the same time it should be noted that chemical treatment is not allowed within the EU for commodities destined for human consumption.

More information may be found in the first edition of the "Prevention and Reduction of Food and Feed Contamination" publication [105] that contains all the codes of practice related to the prevention and reduction of contaminants in foods and feeds adopted by the Codex Alimentarius Commission until 2011.

CONCLUSION

There is a growing demand for organic foods driven primarily by a consumer's perceptions of the quality and safety of these foods and to the positive environmental impact of organic agriculture practices. This growth in demand is expected to continue in the foreseeable future. Animal feed, including herbage, may be contaminated with organic and inorganic compounds. Main contamination sources are available in all farming systems and must be taken seriously. Mycotoxins holds many problems among the natural toxins. Naturally occurring toxicant contamination of feeds and foods with mycotoxins is inevitable and unpredictable and poses a unique challenge to organic farming as well as conventional farming. The best way to reduce the mycotoxin content in food and feed is the prevention of mycotoxin formation in the field, but this is often not sufficient, consequently other effective methods are required. A well maintained quality assurance system has to be set up based on the occurrence, detection and prevention. Good agricultural, handling, manufacturing and storage practices are required in both organic and conventional agriculture to minimize the risk of natural toxins. Residues transferred to edible animal products presents consumer health risks. Food safety is the shared responsibility of governments, academia, the food and feed

industry farmers and the consumer. In view of consumer expectations, it is important that governments, industry and consumer groups carefully examine issues related to organic food quality and safety and make whatever interventions may be necessary to ensure an appropriate level of consumer protection.

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YAZIM KURALLARI

1- Yılda 6 (Altı) sayı olarak yayımlanan Kafkas Üniversitesi Veteriner Fakültesi Dergisi'nde (Kısaltılmış adı: Kafkas Univ Vet Fak Derg) Veteriner Hekimlik ve Hayvancılıkla ilgili (klinik ve paraklinik bilimler, hayvancılıkla ilgili biyolojik ve temel bilimler, zoonozlar ve halk sağlığı, hayvan besleme ve beslenme hastalıkları, hayvan yetiştiriciliği ve genetik, hayvansal orijinli gıda hijyeni ve teknolojisi, egzotik hayvan bilimi) orijinal araştırma, kısa bildiri, ön rapor, gözlem, editöre mektup, derleme ve çeviri türünde yazılar yayımlanır. Dergide yayımlanmak üzere gönderilen makaleler Türkçe, İngilizce veya Almanca dillerinden biri ile yazılmış olmalıdır.

2- Dergide yayımlanması istenen yazılar Times New Roman yazı tipi ve 12 punto ile A4 formatında, 1.5 satır aralıklı ve sayfa kenar boşlukları 2.5 cm olacak şekilde hazırlanmalı ve resim, tablo, grafik gibi şekillerin metin içindeki yerlerine Türkçe ve yabancı dilde adları ve gerekli açıklamaları mutlaka yazılmalıdır.

Dergiye gönderilecek makale ve ekleri (şekil vs) *http://vetdergi.kafkas.edu.tr* adresindeki online makale gönderme sistemi kullanılarak yapılmalıdır.

Başvuru sırasında yazarlar yazıda yer alacak şekilleri (13 X 18 cm boyutlarından büyük olmamalı) online makale gönderme sistemine yüklemelidirler. Yazının kabul edilmesi durumunda tüm yazarlarca imzalanmış *Telif Hakkı Devir Sözleşmesi* editörlüğe gönderilmelidir.

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.

4- ***Makale Türleri***

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 oluşmalı ve metin, tablo, şekil vs dahil) 10 sayfayı aşmamalıdır. Yabancı dilde yazılmış makaleler yabancı dilde başlık, yabancı dilde özet ve anahtar sözcükler, Türkçe başlık, Türkçe özet ve anahtar sözcükler ile birlikte 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 4 sayfayı aşmamalıdır. Ayrıca, en fazla 4 şekil veya tablo içermelidir.

Ön Rapor, kısmen tamamlanmış, yorumlanabilecek aşamaya gelmiş orijinal bir araştırmanın kısa (en çok 6 sayfa) anlatımıdır. Bunlar orijinal araştırma makalesi formatında yazılmalıdır.

Gözlem, 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 kaynaklardan oluşmalı ve 4 sayfayı geçmemelidir.

Editöre Mektup, bilimsel veya pratik yararı olan bir konunun veya ilginç bir olgunun resimli ve kısa sunumudur ve 1 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 ve kaynaklardan oluşmalı ve 10 sayfayı geçmemelidir.

Çeviri, makalenin orijinal formatı dikkate alınarak hazırlanmalıdır.

Yazarla ilgili kişisel ve kuruma ait bilgiler ana metin dosyasına değil, on-line başvuru sırasında sistemdeki ilgili yerlere unvan belirtilmeksizin eklenmelidir.

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.

6- Kaynaklar, metin içinde ilk verilenden başlanarak numara almalı ve metin içindeki kaynağın atıf yapıldığı yerde parantez içinde yazılmalıdır.

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.

Örnek: **Gokce E, Erdogan HM:** An epidemiological study on neonatal lamb health. *Kafkas Univ Vet Fak Derg,* 15 (2): 225-236, 2009.

Kaynak kitap ise yazarların soyadları ile adlarının ilk harfleri, eserin adı, baskı sayısı, sayfa numarası, basımevi, basım yeri ve basım yılı olarak yazılmalıdır.

Editörlü ve çok yazarlı olarak yayınlanan kitaptan bir bölüm kaynak olarak kullanılmışsa, bölüm yazarları, bölüm adı, editör(ler), kitap adı, baskı sayısı, sayfa numarası, basımevi, basım yeri ve basım yılı sırası dikkate alınarak aşağıdaki örneğe göre yazılmalıdır.

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.

Online olarak ulaşılan kaynaklarda web adresi ve erişim tarihi kaynak bilgilerinin sonuna eklenmelidir.

Diğer kaynakların yazımında bilimsel yayın ilkelerine uyulmalıdır.

Kaynak listesinde "et al." ve "ve ark." gibi kısaltmalar yapılmaz.

7- Bakteri, virus, parazit ve mantar tür isimleri ve anatomik terimler gibi latince ifadeler orijinal şekliyle ve italik karakterle yazılmalıdır.

8- Editörlük, dergiye gönderilen yazılar üzerinde gerekli görülen kısaltma ve düzeltmeleri yapabileceği gibi önerilerini yazarlara iletebilir. Yazarlar, düzeltilmek üzere yollanan yazıları online sistemde belirtilen sürede gerekli düzeltmeleri yaparak editörlüğe iade etmelidirler. Editörlükçe ön incelemesi yapılan ve değerlendirmeye alınması uygun görülen makaleler ilgili bilim dalından bir yayın danışmanı ve iki raportörün olumlu görüşü alındığı takdirde yayımlanır.

9- Yayınlanan yazılardan dolayı doğabilecek her türlü sorumluluk yazarlara aittir.

10- Yazarlara telif ücreti ödenmez.

11- Resim ve baskı masrafları için yazarlardan ücret alınır. Ücret bilgileri *http://vetdergi.kafkas.edu.tr/* adresinden öğrenilebilir.

12- Yazarlara 50 adet ayrı baskı ücretsiz olarak yollanır.

INSTRUCTIONS FOR AUTHORS

1- Kafkas Üniversitesi Veteriner Fakültesi Dergisi (Journal of the Faculty of Veterinary Medicine, Kafkas University) (abbreviated title: Kafkas Univ Vet Fak Derg), published bi-monthly, covers original papers, short communication and preliminary scientific reports, case reports, observation, letter to the editor, review and translation on all aspects of veterinary medicine and animal science (clinical and paraclinical sciences, biological and basic sciences, zoonoses and public health, animal feeding and nutritional diseases, animal breeding and genetics, hygiene and technology of food from animal sources, exotic animal science). Manuscripts submitted for publication should be written in Turkish, English or German.

2- The manuscripts submitted for publication should be prepared in the format of Times New Roman style, font size 12, A4 paper size, 1.5 line spacing and 2.5 cm margins of all edges. The legend or caption of all illustrations such as figure, table and graphic must clearly be written in both Turkish and foreign language and their appropriate position should be indicated in the text.

The manuscript and its supplementary (figure etc.) should be submitted by using online manuscript submission system at the address of *http://vetdergi.kafkas.edu.tr/*

During the submission, the authors should upload the figures of the manuscript (the dimensions must not to exceed 13 X 18 cm) to the online manuscript submission system. If the manuscript is accepted for publication, the copyright transfer agreement form signed by all the authors should be send to the editorial office.

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