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Research Article

Genetic Variability of CAST Gene in Native Sheep Breeds of Turkey [1]

Kozet AVANUS [1]

[1] *This study was presented as an oral presentation in 3rd Turkish-Bosnian Scientific Days within the Framework of the Partnership between the Veterinary Faculties of Sarajevo University and Istanbul University*

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Abstract

The aim of this study is to determine the genetic variability of CAST gene in native sheep breeds of Turkey by PCR-RFLP method. Six different native sheep breeds; Kivircik, Imroz, Karayaka, Hemsin, Red Karaman and Karakul were used in this study. This study was the first report about CAST gene variation in Karayaka, Red Karaman and Hemsin sheep breeds. After DNA isolation and PCR amplification, RFLP was performed with *Msp*I enzyme. Two alleles M (336bp and 286bp) and N (622bp) were identified on 2% agarose gel electrophoresis. Allel and genotype frequencies, observed (Ho) and expected heterozygosity (He) and deviation from Hardy Weinberg Equilibrium were estimated by statistical analyses. The frequency of M allele was highest in Imroz (96%) and N allele was identified most frequently in Kivircik (30%) breed. Highest frequencies of MN genotype were identified in Kivircik (60%), MM in Imroz (92.6%) and NN in Red Karaman (7.1%) breeds respecitvely. Kivircik, Imroz, Karayaka and Karakul breeds were null from NN genotype. Kivircik sheep showed the highest heterozygosity (60%) and Imroz had the lowest (7.4%). The highest heterozygosity value was identified in Kıvırcık (60%), the lowest in Imroz (7.4%). All breeds except Kivircik and Hemsin were found in Hardy-Weinberg equilibrium. Absence of NN genotype in some breeds and high frequency of MN genotype in Kivircik breed might be resulted from the selection process of native sheep breeds in their breeding regions.

Keywords: *Calpastatin, Native sheep breeds, Genetic variation*

Türkiye Yerli Koyun Irklarında CAST Genine Ait Genetik Çeşitliliğin Belirlenmesi

Özet

Bu çalışmanın amacı, Türkiye yerli koyun ırklarında CAST genine ait genetik çeşitliliğin PCR-RFLP yöntemi ile belirlenmesidir. Çalışmada 6 farklı yerli koyun ırkı; Kıvırcık, İmroz, Karayaka, Hemsin, Morkaraman ve Karagül kullanılmıştır. Bu çalışma Karayaka, Morkaraman ve Hemsin koyun ırklarında CAST geni çeşitliliğini ortaya koyan ilk çalışmadır. DNA izolasyonu ve PZR ile yükseltgemenin ardından RFLP analizi *Msp*I enzimi ile gerçekleştirilmiştir. %2'lik agaroz jel elektroforezi ile M (336bç ve 286bç) ve N (622bç) olmak üzere iki allel saptanmıştır. İstatistiksel hesaplamalar ile allel ve genotip frekansları ile beklenen (Ho) ve gözlenen (Ho) heterozigotluk değerleri ile Hardy Weinberg dengesine uyum durumları saptanmıştır. En yüksek M allel frekansı İmroz (%96), N allel frekansı ise Kıvırcık ırkında tespit edilmiştir. MN, MM ve NN genotip frekansları en yüksek olarak sırasıyla Kıvırcık (%60), İmroz (%92.6) ve Morkaraman (%7.1) ırklarında belirlenmiştir. Kıvırcık, İmroz, Karayaka ve Karagül ırklarında NN genotipi bulunamamıştır. Heterozigotluk değeri Kıvırcık ırkında en yüksek (%60) iken, İmroz ırkında en düşük (%7.4) olarak belirlenmiştir. Kıvırcık ve Hemsin dışında tüm ırklar Hardy-Weinberg dengesine uyumlu bulunmuştur. Bazı ırkların NN genotipini bulundurmaması ve Kıvırcık ırkının MN genotipi için en yüksek genotip frekansına sahip olması; yerli koyun ırklarının yetiştirildikleri bölgelerdeki seleksiyon sürecinden kaynaklanmış olabileceği düşünülebilir.

Anahtar sözcükler: *Kalpastatin, Yerli koyun, Genetik çeşitlilik*

INTRODUCTION

Sheep is one of the most important red meat sources in Turkey [1]. Calpastatin (CAST) gene captures special attention for its major role in both meat tenderness and growth in animals. Therefore CAST is one of the most screened genes in livestock. Various studies have been performed to identify the CAST gene variation in goats [2] and its association with meat quality traits in pigs [3] and cattle [4-7]. CAST gene was first identified in sheep by

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Palmer et al.[8] and it was located on the 5th chromosome in sheep genome [9]. Calpastatin (CAST) enzyme is the specific inhibitor of calpain proteases which regulates the rate and extent of post mortem tenderization [10]. Calpain enzyme plays a key role in meat tenderness by degrading myofibrillar proteins after slaughter during the process of rigor mortis [11]. Calpain CAST system (CCS) is important in muscle growth. Reduction of calpain and increase in CAST activities may result to increase in growth rate of skeletal muscle. CAST gene was described as an important regulator on birth weight; its influence was shown on growth rate until weaning in Romney lambs [12]. Chung and Davis [13] reported that CAST gene has positive effect on both average daily gain and post weaning weight in Targhee sheep. However Dehnavi et al.[14] did not find any relation between CAST gene and yearling weight in Zel sheep. Variation of CAST locus in various sheep breeds were identified by using PCR-RFLP [10,14-26], PCR SSCP [9,27-29] and DNA sequencing [27,30-32] methods. A point mutation in intron 12 region of CAST gene causes the substitution of Guanin (G) nucleotide to Adenine (A) and diverges the CCGG nucleotides to CCAG sequence. Since CCGG is recognition site to *Msp*I enzyme, G-A substitution makes the site unrecognizable by the enzyme; therefor the mutation can be identified with PCR-RFLP method [31]. Two alleles (M and N) and three genotypes (MM, MN and NN) were described in CAST locus after PCR-RFLP analysis with *Msp*I enzyme [8].

The aim of this study was to identify CAST genotype variation in thin-tailed sheep breeds; Kivircik, Imroz and Karayaka, semi-fat tailed breed; Hemsin and fat-tailed sheep breeds; Red Karaman and Karakul by using PCR-RFLP method.

MATERIAL and METHODS

This study was approved by Ethic Committee of the Istanbul University Veterinary Faculty (Approval number: 2011/163).

Thin-tailed sheep breeds; Kivircik (n=25), Imroz (n=27) and Karayaka (n=22), semi-fat tailed breed; Hemsin (n=19) and fat-tailed sheep breeds; Red Karaman (n=14) and Karakul (n=15), in total 122 sheep were used as animal samples. Blood samples were taken from Vena jugularis into sterile vacuumed EDTA tubes in five different sheep breeds. Genomic DNA was isolated from blood by using ExiPrep™ 16Plus automated nucleic acid extraction system (Bioneer Company, Chonbuk, Chonju, South Korea). DNA isolation in Karayaka sheep breed was performed from raw meat samples. DNA from meat samples were obtained by PureLink DNA isolation kit (Invitrogen, Carlsbad,CA, USA).

The region of the ovine CAST gene was amplified by using PCR with the forward primer 5'TGGGGCCCAATGA CGCCATCGATG3' and the reverse primer 5'GGTGGAGCA GCACTTCTGATCACC3', which captured a 622bp sized fragment from intron 12 and exon 13 (AF016006.1) [8,31].

PCR was carried out in a final volume of 50 µl containing; 100 ng genomic DNA, 20 pmol each primer, 200 mM dNTPs each, 1.5 mM $MgCl_2$, 10X PCR Buffer and 0.25U Taq polymerase (MBI Fermentas). PCR was performed with the following conditions; denaturing at 95°C in 3 min, 35 cycles of 95°C in 30 sec, 63°C in 50 sec, 72°C in 1 min and final extension at 72°C in 10 min (Bio-Rad T100, Bio-Rad Laboratories Inc., CA, USA). PCR products were digested with 1 µl (10U) *Msp*I enzyme (MBI Fermentas). Samples were incubated at 37°C by overnight for *Msp*I digestion. After performing RFLP, band patterns were visualized by 2% agarose gel.

Samples showed polymorphic band pattern for *Msp*I enzyme were sequenced both forward and reverse directions by REFGEN gene research and biotechnology firm (www.refgen.com) with ABI 3100 avant automated DNA sequencer in order to confirm the haplotypes.

Allele and genotype frequency observed and expected heterozygosity and chi square test to analyze the deviation from Hardy-Weinberg equilibrium (HWE) were estimated by using PopGene32 software program version 1.31 [33].

The sequence results were compared with the reference sequence of ovine CAST gene (GenBank: AF016006.1) by using BioEdit sequence alignment editor *(http://www.mbio.ncsu.edu/BioEdit/bioedit.html)* software program.

RESULTS

Two alleles of CAST locus (M and N) were identified after digestion with *Msp*I enzyme. Band patterns of M allele (336bp and 286bp) and N allele (622bp), MM, MN and NN genotypes were viewed on 2% agarose gel stained with EtBr *(Fig. 1)*.

Allele frequencies, genotype frequencies, observed and expected heterozygosity, chi square and p values of CAST locus were given in *Table 1*.

Three different haplotypes; a (CCGGG), b (CCGGA) and c (CCAGA) were identified for CAST locus *(Fig. 2-A)*. Haplotypes that constitute MM (aa, ab), MN (ac) and NN (cc) genotypes were shown in *Fig. 2-B*. None of the MN genotype showed haplotype b. Only Hemsin and Red Karaman breed were carry haplotype b within their MM genotype.

DISCUSSION

The frequency of M allele was the highest in Imroz (96%) sheep. N allele was identified most frequently in Kivircik (30%) breed. The highest frequencies of MN genotype was observed in Kivircik (60%), MM in Imroz (92.6%) and NN in Red Karaman (7.1%) breeds respectively.

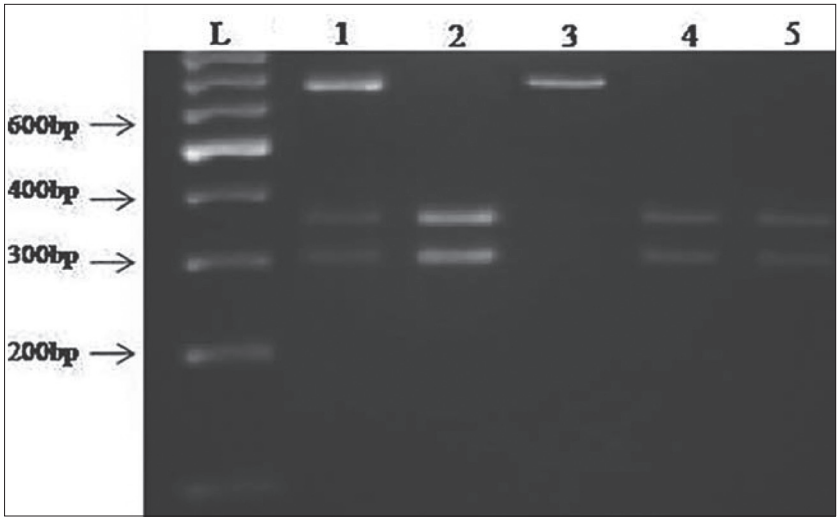

Fig 1. MM genotype (336bp and 286 bp in lane 2, 4, 5), MN genotype (622bp, 336bp and 286 bp in lane 1) and NN genotype (622 bp in lane 3) of CAST locus visualized on 2% agarose gel

Şekil 1. %2'lik agaroz jelde görüntülenen CAST lokusuna ait MN genotipi (622bç, 336bç ve 286 bç 1. kuyucukta) MM genotipi (336bç ve 286 bç 2., 4., 5. kuyucuklarda) ile NN genotipi (622 bç 3. kuyucukta)

Table 1. *Allele frequencies, genotype frequencies, observed and expected heterozygosity, chi square and p values for Hardy Weinberg Equation of CAST locus*

Tablo 1. *CAST lokusuna ait allel frekansları, genotip frekansları, gözlenen ve beklenen heterozigotluk, Hardy Weinberg Dengesi için ki kare ve p değeri*

Breed	n	Allele Frequency (%)		Genotype Frequency (%)			Heterozygosity			P
		M	*N*	*MM*	*MN*	*NN*	H_o	H_E	X^2	
Kivircik	25	70.0	30.0	40.0	60.0	0.0	0.600	0.429	4.235	0.040*
Imroz	27	96.3	3.7	92.6	7.4	0.0	0.074	0.073	0.020	0.889ns
Karayaka	22	90.9	9.1	81.8	18.2	0.0	0.182	0.169	0.162	0.688ns
Hemsin	19	89.5	10.5	84.2	10.5	5.3	0.105	0.194	5.139	0.023*
Karakul	15	73.3	26.7	46.7	53.3	0.0	0.533	0.405	1.697	0.193ns
Red Karaman	14	75.0	25.0	57.2	35.7	7.1	0.357	0.389	0.10	0.745ns

*ns = not significant P<0.05, * significant P<0.05*
*H_o = observed heterozygosity, H_E = expected heterozygosity, x^2 = Chi-square, **p** = probability*

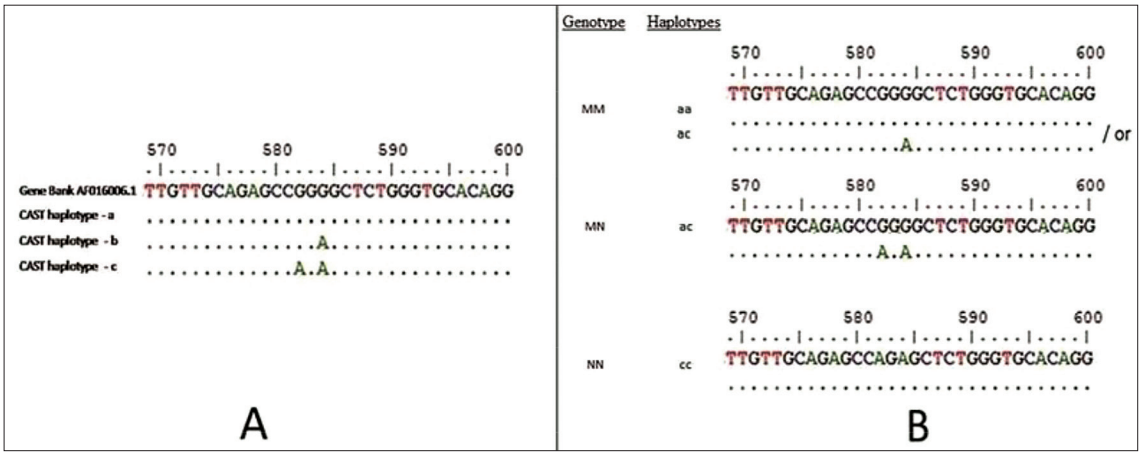

Fig 2. A- Three haplotypes (a,b and c) of CAST locus were identified in native sheep breeds of Turkey, **B**- Haplotypes constitute MM, MN and NN genotypes

Şekil 2. A- Türkiye yerli koyun ırklarında belirlenen CAST lokusuna ait üç haplotip (a,b ve c), **B**- MM, MN ve NN genotiplerini oluşturan haplotipler

Previous studies performed by other researchers for identifying genetic variation of CAST gene in different sheep breeds with RFLP method were summarized in *Table 2*.

According to the results of this study M and N allele frequencies and MM and MN genotype frequencies of Imroz sheep was found similar with Ile de France breed [22]. M and N allele frequencies of Red Karaman sheep were

Table 2. *Allele frequencies, genotype frequencies, heterozygosity values and compatibility of populations with HWE (p value) of CAST locus in different sheep populations reported by various researchers*

Tablo 2. *Çeşitli araştırmacıların allel frekansları, genotip frekansları, heterozigotluk değerleri, populasyonların HWE dengesine uyumları için ki kare ve p değeri*

Breed	N	Allele Frequency (%)		Genotype Frequency (%)			Heterozygosity			Reference
		M	*N*	*MM*	*MN*	*NN*	H_o	H_e	p	
Lori Sheep	100	63.8	36.2	40.7	46.2	13.1	ND	ND	ND	[15]
Kivircik	203	84.2	15.8	72.9	22.7	4.4	0.227	0.266	x	[16], [17]
	336	84.7	15.3	72.9	23.5	3.6	0.235	0.260	NS	
Karacabey Merino	248	80.0	19.9	66.9	26.2	6.9	0.262	0.320	xx	[17]
Chios (Sakız)	87	34.5	65.5	9.2	50.6	40.2	0.506	0.452	NS	
Imroz	49	98.9	1.02	97.9	2.04	0.0	0.020	0.020	NS	
Karya	90	54.4	45.6	54.3	38.8	6.9	0.577+	0.496*	NS	[26]
Cine Capari	97	73.7	26.3	29.6	49.6	20.8	0.423+	0.387*	NS	
Dalagh Sheep	110	55.5	44.5	36.0	38.0	26.0	0.380	0.490	x	[10]
	120	80.0	20.0	65.5	29.0	5.5	0.300	0.320	NS	[20]
Zel Sheep	200	85.5	14.4	75.0	21.0	4.0	0.210	0.250	x	[14]
Balkhi	300	88.0	12.0	74.0	24.0	0.0	0.240	0.210	NS	[18], [19]
Kajli	300	86.0	14.0	74.0	24.0	2.0	0.240	0.240	NS	
	100	90.0	10.0	80.0	2.0	0.0	0.100	0.310	NS	
Lohi	100	87.0	13.0	77.0	20.0	3.0	0.100	0.226	NS	[19]
Thalli	100	81.0	19.0	68.0	26.0	6.0	0.100	0.180	NS	
Atabi Sheep	120	81.0	19.0	67.5	27.5	5.0	0.275	0.306	ND	[21]
Polish Merino	82	76.2	23.8	56.1	40.2	3.7	ND	ND	ND	[22]
Berichon du Cher	41	92.7	7.3	85.4	14.6	0.0	ND	ND	ND	
Blackhead Mutton Sheep	59	81.4	18.6	71.2	20.3	8.5	ND	ND	ND	
Ile de France	30	95.0	5.0	90.0	10.0	0.0	ND	ND	ND	
Arabic Sheep	111	85.0	15.0	70.2	28.8	0.9	ND	ND	NS	[23]
Iranian Karakul Sheep	100	79.0	21.0	61.0	36.0	3.0	0.35	0.67	ND	[24]

x: *P<0.05,* ***xx:*** *P<0.01,* ***ND:*** *not determined,* ***NS:*** *not significant, * estimated from the referred article*
H_O *= observed heterozygosity,* H_E *= expected heterozygosity,* x^2 *= Chi-square,* ***p*** *= probability*

found similar with Cine Capari [26] and Polish Merino breed [22]. MM genotype frequency was also found similar with Polish Merino breed [22]. Observed heterozygosity and expected heterozygosity values of Red Karaman breed were found similar to both Iranian Karakul [24] and Cine Capari breed [26] respectively. M and N allele frequencies of Hemsin sheep breed were found similar with Balkhi [18] and Lohi [19] sheep breeds, on the other hand MM genotype frequency was found similar with Berichon du cher [22] sheep breed. NN genotype frequency of Hemsin breed was found similar with Dalagh [20] and Atabi sheep [21] breeds. Observed heterozygosity in Hemsin breed was found similar with Kajili, Lohi and Thalli [19] sheep breeds. Expected heterozgosity in Hemsin breed was also found similar with Thalli sheep breed. M and N allele frequencies of Karayaka sheep in this study were found similar with Kajili [18] and Berichon du cher [22] sheep breeds and MM and MN genotype frequencies were also similar to Kajili [18] and Blachead Mutton sheep breeds [22]. MM genotype frequency and observed heterozygosity of Kivircik breed were found similar with Lori sheep [10] and Karya sheep [26] respectively. M and N allele frequencies and MN genotype frequency of Karakul sheep were found similar with Cine Capari sheep breed [26]. Khan et al.[18] found that heterozygous (MN) genotype showed significantly higher weight gain from birth to eight months in Balkhi sheep and from birth to four months of age in Kajli sheep breeds respectively. Results of this study showed that Kivircik, Imroz, Karayaka and Karakul breeds were found null from NN genotype as; Balki [18], Kajli [19], Berichon du cher and Ile de France sheep [22] and Gokceada (Imroz) [17] sheep breeds were also reported. Yılmaz et al.[16] reported that animals with NN genotype showed lower average daily gain (ADG), back fat thickness (BT) and skin with back fat thickness (S+BT) values.

It can be concluded from the current study that, selection process of native sheep breeds in their breeding

regions may occurred negatively for NN genotype in Kivircik, Imroz, Karayaka and Karakul sheep breeds however it may occurred positively for MN genotype in Kivircik breed. Kivircik is the most popular sheep breed for red meat source in Turkey.

Imroz, Red Karaman, Karayaka and Karakul populations were found in HWE. However Kivircik and Hemsin were not in HWE being similar to Kivircik [16], Dalagh [10] and Zel sheep [14] populations. The highest and lowest heterozygosity values were identified in Kivircik (60%) and Imroz (7.4%) breeds respectively. Since Imroz breed is originated from Imroz Island, low heterozygosity value is an expected outcome for this breed.

CAST gene haplotypes obtained with sequencing, were found very similar with the findings of Gregula-Kania [31]; only CAST-b haplotype (CCGGA) was divergent than reported data. CAST b haplotype located in border of recognition site of *Msp*I enzyme, was identified only in MM genotype of Hemsin and Red Karaman breeds. However structure of M and N alleles were formed respectively by haplotype a and c which were localized in *Msp*I recognition site.

Understanding the effect and selection variation of CAST locus may help to improve marker assistant selection (MAS) studies in sheep breeding, particularly in meat tenderness and growth. This was the first report about CAST gene variation in Karayaka, Red Karaman and Hemsin sheep breeds of Turkey. Further studies on CAST locus should be performed in different native sheep breeds to enlighten the genotype structure and candidacy profile for MAS studies of sheep genetic resources of Turkey.

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REFERENCES

1. Ekiz B, Ekiz EE, Yalcıntan H, Yılmaz A, Kocak O, Gunes H: Effect of ram-ewe mixed transportation on certain welfare parameters in Red Karaman and Imroz sheep. *Istanbul Univ Vet Fak Derg*, 39 (2): 155-167, 2013.

2. Zhou H, Hickford JGH: Allelic polymorphism of the caprine calpastatin (CAST) gene identified by PCR-SSCP. *Meat Sci*, 79, 403-405, 2008. DOI: 10.1016/j.meatsci.2007.10.015

3. Ciobanu DC, Bastiaansen JWM, Lonergan SM, Thomsen H, Dekkers JCM, Rothschild MF: New alleles in calpastatin gene are associated with meat quality traits in pigs. *J Anim Sci*, 82, 2829-2839, 2004.

4. Juszczuk-Kubiak E, Rosochacki SJ: A novel RFLP/Alu I polymorphism of the bovine calpastatin (CAST) gene and its association with selected traits of beef. *Anim Sci Pap Rep*, 22 (2): 195-204, 2004.

5. Casas E, White SN, Wheeler TL, Shackelford SD, Koohmaraie, Riley DG, Smith TPL: Effects of calpastatin and μ-calpain markers in beef cattle on tenderness traits. *J Anim Sci*, 84, 520-525, 2006.

6. Schenkel FS, Miller SP, Jiang Z, Mandell IB, Ye X, Li H, Wilton JW: Association of a single nucleotide polymorphism in the calpastatin gene with carcass and meat quality traits of beef cattle. *J Anim Sci*, 84, 291-299, 2006.

7. Curi R, Chardulo LL, Mason MC, Arrigoni MDB, Silveira C, Oliveira HN: Effect of single nucleotide polymorphisms of CAPN1 and CAST genes on meat traits in Nellore beef cattle (Bos indicus) and in their crosses with *Bos taurus*. *Anim Genet*, 40, 456-62, 2009. DOI: 10.1111/j.1365-2052.2009.01859.x

8. Palmer BR, Roberts N, Hickford JG, Bickerstaffe R: PCR-RFLP for *Msp*I and NcoI in the ovine calpastatin gene. *J Anim Sci*, 76, 1499-1500, 1998.

9. Palmer BR, Su HY, Roberts N, Hickford JG, Bickerstaffe R: Single nucleotide polymorphisms in an intron of the ovine calpastatin gene. *Anim Biotech*, 11, 63-67, 2000. DOI: 10.1080/10495390009525948

10. Azari MA, Dehnavi E, Yousefi S, Shahmohamadi L: Polymorphism of calpastatin , calpain and myostatin genes in native Dalagh sheep in Iran. *Slovak J Anim Sci*, 12, 1-6, 2012. DOI: 10.1155/2012/472307

11. Page BT, Casas E, Heaton MP, Cullen NG, Hyndman DL, Morris CA, Smith TPL: Evaluation of single-nucleotide polymorphisms in CAPN1 for association with meat tenderness in cattle. *J Anim Sci*, 80, 3077-3085, 2002.

12. Byun SO, Zhou H, Forrest RHJ, Frampton CM, Hickford JGH: Association of the ovine calpastatin gene with birth weight and growth rate to weaning. *Anim Genet*, 39, 572-573, 2008. DOI: 10.1111/j.1365-2052.2008.01745.x

13. Chung H, Davis M: PCR-RFLP of the ovine calpastatin gene and its association with growth. *Asian J Anim Vet Adv*, 7, 641-652, 2012. DOI: 10.3923/ajava.2012.641.652

14. Dehnavi E, Ahani Azari M, Hasani S, Nassiry MR, Mohajer M, Khan Ahmadi AR Shahmohamadi L, Yousefi S: Association between yearling weight and calpastatin and calpain loci polymorphism in Iranian Zel sheep. *Iran J App Anim Sci*, 2 (2): 131-135, 2012.

15. Asadi N, Nanekarani S, Khederzadeh S: Genotypic frequency of calpastatin gene in lori sheep by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. *Afr J Biotechnol*, 13, 1952-1954, 2014. DOI: 10.1080/10495390009525948

16. Yılmaz O, Cemal İ, Karaca O, Ata N: Association of Calpastatin (CAST) gene polymorphism with weaning weight and ultrasonic measurements of loin eye muscle in Kivircik lambs. *Kafkas Univ Vet Fak Derg*, 20, 675-680, 2014. DOI: 10.9775/kvfd.2014.10816

17. Yılmaz O, Sezenler T, Ata N, Yaman Y, Cemal İ, Karaca O: Polymorphism of the ovine calpastatin gene in some Turkish sheep breeds. *Turk J Vet Ani Sci*, 38, 354-357, 2014. DOI: 10.3906/vet-1401-13

18. Khan S, Riaz MN, Ghaffar A, Ullah MF: Calpastatin (CAST) gene polymorphism and its association with average daily weight gain in Balkhi and Kajli sheep and Beetal goat breeds. *Pakistan J Zool*, 44 (2): 377-382, 2012.

19. Suleman M, Khan SU, Riaz MN, Yousaf M, Shah A, Ishaq R: Calpastatin (CAST) gene polymorphism in Kajli, Lohi and Thalli sheep breeds. *Afr J Biotechnol*, 4, 10655-10660, 2012. DOI: 10.5897/AJB11.2478

20. Khederzadeh S: Polymorphism of calpastatin gene in crossbreed Dalagh sheep using PCR-RFLP. *Afr J Biotechnol*, 10, 10839-10841, 2011. DOI: 10.5897/AJB11.265

21. Nanekarani S, Khederzadeh S, Kaftarkari AM: Genotypic frequency of calpastatin gene in Atabi sheep by PBR method. **In,** *Proceedings of the International Conference on Food Engineering and Biotechnology IPCBEE, Bangkok, Thailand*, 9, 189-192, 2011.

22. Szkudlarek-Kowalczyk M, Wiśniewska E, Mroczkowski S: Polymorphisms of calpastatin gene in sheep. *J Cen Eur Agri*, 12, 425-432, 2011. DOI: 10.5513/JCEA01/12.3.934

23. Mohammadi M, Nasiri MTB, Alami-saeid K, Fayazi J, Mamoee M,

Sadr AS: Polymorphism of calpastatin gene in Arabic sheep using PCR-RFLP. *Afr J Biotechnol*, 7 (15): 2682-2684, 2008.

24. Shahroudi FE, Nassiry MR, Valizadh R, Heravi A: Genetic polymorphism at MTNR1A, CAST and CAPN loci in Iranian Karakul sheep. *Iran J Biotech*, 4 (2): 117-122, 2006.

25. Nassiry MR, Tahmoorespour M, Javadmanesh A, Soltani M, Far SF: Calpastatin polymorphism and its association with daily gain in Kurdi sheep. *Iran J Biotech*, 4 (3): 188-192, 2006.

26. Ata N, Cemal I: Calpastatin gene polymorphism in Çine Çapari and Karya sheep. *Sci Pap Ser D Anim Sci*, LVI: 48–51, 2008.

27. Zhou H, Hickford JGH, Gong H: Polymorphism of the ovine calpastatin gene. *Mol Cell Probes*, 21, 242-244, 2007. DOI: 10.1016/j.mcp.2006.10.004

28. Dagong MI, Sumantri C, Noor RR, Herman R, Yamin M: Genetic polymorphisms of the coding region (exon 6) of calpastatin in Indonesian sheep. *Med Petern*, 34, 190-195, 2011. DOI: 10.5398/medpet.2011.34.3.190

29. Ranjbari M, Hashemi A, Mardani K, Darvishzadeh R: Allelic polymorphism of Makoei sheep calpastatin gene identified by polymerase chain reaction and single strand conformation polymorphism. *J Agri Sci Tech*, 14, 533-538, 2012.

30. Aali M, Moradi-Shahrbabak M, Sadeghi M: Detecting novel SNPs and breed-specific haplotypes at calpastatin gene in Iranian fat- and thin-tailed sheep breeds and their effects on protein structure. *Gene*, 537, 132-139, 2014. DOI: 10.1016/j.gene.2013.12.023

31. Greguła-Kania M: New allelic variant of the ovine calpastatin gene. *Afr J Biotechnol*, 10, 13082-13085, 2011. DOI: 10.5897/ajb11.1027

32. Djadid ND, Nikmard M, Zakeri S, Gholizadeh S: Characterization of calpastatin gene in Iranian Afshari sheep. *Iran J Biotech*, 9 (2): 145-149, 2011.

33. Yeh F, Yang RC, Boyle T: Popgene (v.1.32) Microsoft Windows-based freeware for Population Genetic Analysis. 2000. http://www.ualberta.ca/~fyeh/Pop32.exe, *Accessed:* 07.07.2014.

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Research Article

Long Term Investigations on Tick Infestations of Human

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Abstract

In this study, a total of 19866 samples which were collected from humans who applied to the hospitals with tick bites in the western part of Turkey (Bursa) between the years 2007 and 2011 (from February to November) were examined. Approximately 10% (1985) of samples were found as non-ticks like bee stings, lice, fleas and other arthropods. The ticks were identified as *Rhipicephalus* spp. (72.98%), *Ixodes* spp. (18.96%), *Hyalomma* spp. (7.18%), *Dermacentor marginatus* (0.027%) and *Haemaphysalis parva* (0.005%). Based on localities, majority of the tick samples were reported from the urbanized areas (81%). Especially, Ixodes spp. species were commonly found in highland and forestry areas of Bursa.

Keywords: *Tick infestation, Prevalence, Human*

İnsanlarda Kene Enfestasyonları Üzerine Uzun Süreli Araştırmalar

Özet

Bu çalışmada Türkiye'nin batısında (Bursa) 2007 Şubat - 2011 Kasım yılları arasında kene tutunması vakasıyla hastaneye başvuran insanlardan toplanan 19866 örnek incelenmiştir. Toplanan örneklerin yaklaşık %10 (1985) kadarının kene olmadığı; arı iğnesi, bit, pire ve diğer artropodlar olduğu görülmüştür. Tür teşhisi yapılan kenelerin %72.98'u *Rhipicephalus* spp., %18.96'sı *Ixodes* spp, %7.18'sı *Hyalomma* spp., %0.027'si *Dermacentor marginatus* ve %0.005'inin *Haemaphysalis parva* olduğu teşhis edildi. Kene örneklerinin %81'inin şehir kökenli olduğu ve özellikle Ixodes türü kenelerin Bursa'nın ormanlık alanlarında sıklıkla görüldüğü ortaya çıkarılmıştır.

Anahtar sözcükler: *Kene enfestasyonu, prevalans, İnsan*

INTRODUCTION

The tick fauna of Turkey consist of 46 species but there is very limited information about ticks infesting human. The seasonal activity, prevalence, and intensity of these ticks are not well known [1,2]. Twenty two tick species were identified on ruminants and other wild domestic animals in Marmara region [1]. In recent years with the increase of Crimean-congo hemorrhagic fever case, an increase was also observed in the number of humans who admitted to hospital with complaints of tick biting. In other words an increasing tick phobia has occurred in the society. So the tick bites of human became much more important [3,4].

Most tick species are lack of host-specificity and attacking several vertebrates during their life cycle. Knowledge of the tick diversity attacking humans facilitates are required to understand of epidemiological association between these hosts and tick-borne pathogens [4,5]. For this reason, to determine species of collected ticks from human is essential for epidemiology of ticks and tick-borne pathogens

The objective of this long term investigation was to determine tick bite cases in human, species of ticks and their seasonal activity in west part of Turkey, during a five year period (between February of 2007 and November of 2011).

MATERIAL and METHODS

Study Area

Bursa Province is located (40°E, 28-30°N) in the south-eastern part of the Marmara Region of Turkey. The altitude

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of the area is approximately 100 m above sea level. This region is characterized by hot and dry summers with some rainfall. The mean annual temperature in the area is 14-16°C with minimum and maximum averages of 5°C and 25°C.

Total precipitation averages are 686 mm/year, most of which is recorded in December; August has the least rainfall. The mean relative humidity is roughly 66%. The forest land is dominated by common oak, hornbeam, pine, beech, oak, linden and chestnut trees.

Collection and Identification of Ticks

Between February 2007 and November 2011, ticks were collected from human who applied to the city and town hospitals with complaints of tick bites in Bursa province, and date of tick bites, related patients data's were recorded. In the laboratory process, adult ticks were identified at the species basis, while larvae and nymphs at the genus basis. Identifications were performed by stereo microscope according to taxonomic key [6-8]. Additionally non-tick samples such as lice, fleas, nevus or papilloma have been determined and recorded in the data sheet.

RESULTS

A total of 17881 tick samples have been sent to our laboratory from different hospitals located at Bursa. As seen in the *Table 1*, 85% of ticks were sent from urban areas while 15% from rural areas. Approximately 10% of tick complaints of human admitted to hospital have been found as non-tick samples such as lice, fleas, bee stings, acne, skin reproduction and other insects *(Table 1)*.

Seven of 17881 (0.04%) ticks *(Argas percicus)* were identtified as Argasidae and all remaining ticks (99.96%) were identified as Ixodidae. The distribution of Ixodid ticks at the level of genus was found as follow; 72.98% *Rhipicephalus spp.* (11709 adult, 1083 nymph, and 258 larvae), 18.96% *Ixodes* spp. (2783 adult, 489 nymph and 119 larvae), 7.18% *Hyalomma spp.* (1371 adult and 56 nymph), 0.027% *Dermacentor marginatus* (5 adult) and 0.005% *Haemaphysalis parva* (1 adult). Adult of *R. sanguineus* (n=9125, 51.03%) was the most common species, followed by *I. ricinus, R. turanicus* and *H. aegyptium*. Ticks were found mainly attached to human extremities and followed by inguinal region, underarm, body and any other region with low infestation rate.

During the Human's complaint period between February and November, cases of tick bites were reported each month from 2007 to 2011. Most cases were observed between mid of April and late of July, reaching to a peak level with 6555 cases (36.65%) in June. Adults of *I. ricinus* was observed in winter but mid stages were found between late spring and summer. *Hyalomma marginatum* adults were found reaching to peak in June and *Hyalomma* nymphs were observed between late of March and mid of May *(Table 2)*.

Rhipicephalus sanguineus adults were mainly observed as dominant species between late of March and early November and reaching to a peak at late of May and June. Adults of R. *turanicus* were recorded between mid of February and early October, reaching to a peak in June. *Rhipicephalus spp.* larvae reached to highest level in March, the nymphs are most determined in early May. Human non-tick complaints were observed between late of March and late of August, reaching to peak in late of May up to mid of June *(Table 2)*. Human tick complaints decreased in following years *(Table 1)*.

DISCUSSION

Although Turkey's tick fauna consist of 46 species, 38 species within Ixodidae family, *Hyalomma, Haemaphysalis, Rhipicephalus, Dermacentor* and *Ixodes* genus have medical and veterinary importance [1,2]. Previous studies performed on ruminants indicated the existence of different tick species in six genus in Bursa province [6]. On the other hand, human infestation with ten ixodid tick species and two argasid species were recorded in Northern Marmara region [7]. In previous studies conducted at Thrace region and İstanbul, nine different tick species within six genus (5 Ixodidae, 1 Argasidae) biting humans have been determined [9,10]. Especially, *Rhipicephalus spp.* (larvae, nymph and adult) are the most dominant genus and *R. sanguineus* is found as main species biting humans. In this study, extensive presence of *Rhipicephalus spp.* ticks and most of the cases as recorded urban origin.

Table 1. *Status of prevalences of human tick complaints according to years*
Tablo 1. *İnsan kene tutunma şikayetlerinin yıllara göre dağılımı*

Years	Ticks	Non- Ticks	Total
2007	2.623	786	3.409
2008	3.150	512	3.662
2009	3.482	357	3.839
2010	4.051	218	4.269
2011	4.575	112	4.687
Total	17.881	1.985	19.866

Table 2. *Number of tick species infested human according to months*
Tablo 2. *İnsanlara tutunan kene türlerinin aylara göre dağılımı*

Tick Species	Months										Total
	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	
Ixodes ricinus	925	572	188	21	-	-	-	11	389	677	2783
Rhipicephalus sanguineus	-	21	926	2945	3753	845	408	172	48	7	9125
Hyalomma marginatum	-	-	2	146	282	48	39	-	-	-	517
Rhipicephalus turanicus	11	87	196	814	927	304	214	27	3	-	2583
Hyalomma aegyptium	-	-	15	216	482	105	32	4	-	-	854
Rhipicephalus annulatus	-	-	-	-	-	-	-	-	1	-	1
Dermacentor marginatus	1	1	-	-	-	-	-	1	-	2	5
Haemaphysalis parva	-	-	-	-	-	-	-	-	1	-	1
Argas percicus	-	-	-	3	4	-	-	-	-	-	7
Ixodes spp. Larvae	4	8	21	86	-	-	-	-	-	-	119
Ixodes spp. Nymph	-	28	98	168	106	89	-	-	-	-	489
Hyalomma spp. Nymph	-	21	16	19	-	-	-	-	-	-	56
Rhipicephalus spp. Larva	17	86	52	29	58	16	-	-	-	-	258
Rhipicephalus spp. Nymph	-	38	319	507	219	-	-	-	-	-	1083
Non- ticks	-	2	116	687	724	326	130	-	-	-	1985
Total	958	864	1949	5641	6555	1733	823	215	442	686	19866

Hyalomma marginatum is known as a tick species which is closely associated with wild animals [6,11]. Larvae-nymph stages of this tick prefer mainly reptiles and ground feeding birds [8,12]. Increasingly, we found human infestations with *H. marginatum* adults and immature forms appear mostly at mountain villages located in valley system areas covered partially with agricultural land and forestry areas of Bursa. It is known that *H. marginatum* is the main vector of Crimean-Congo virus and 8 cases were reported between 2005 and 2010 in Bursa province (Personal communication with health service). Human *H. aegyptium* infestations were recorded in southern Marmara region between May and September before whereas in Aydın region it was seen between March and December [13]. In this study it is determined between mid of April and beginning of September, urban cases were found in higher percentage. Although nymphs of *Hyalomma* spp. are found between March and May, number of these ticks was found less than other researches. Overall tick bites were found mostly from urban areas (85%). These results indicated that wild animals play an important role in this ticks biology.

Although adults of *I. ricinus* have been reported between February and May on human in Aydın region [13], this species was found between May and August in Marmara region [9-11,14]. In our previous study conducted at Bursa cooperatively with Uludag University Faculty of Medicine Pediatric Emergency department, ticks were collected from children and classified. We obtained similar results with the current study and tick species were identified as follow: *Rhipicephalus* spp. nymphs (42.3%), *Rhipicephalus* spp. larvae (22.1%), *Rhipicephalus saunguineus* adults (10.5%), *Rhipicephalus turanicus* adults (0.9 %), *Ixodes* spp. larvae (8.6 %), *Ixodes* spp. nymphs (6.7 %), *Hyalomma* spp. nymphs (4.8 %), *Hyalomma marginatum* adults (2.7%), *Hyalomma aegyptium* adults (0.9 %) [14].

Meanwhile in this study *I. ricinus* was not determined between July and August but has reached to peak level during winter. While larvae of *Ixodes spp*. were determined between February and May, nymphs were found between March and July. May and July are the most frequent time for larvae and nymphs. In a previous study in Bursa region *Ixodes* adults and immature forms were declared as the most common tick species of ruminants [6]. *Dermacentor marginatus* and *Haemaphysalis parva* were found at least grade. There is just one case of *R. annulatus* from Orhaneli district. Because of this tick species has one host life cycle this case can be considered as an accidental infestation.

Reducing numbers of non-tick cases during years should be thought as resulting from increased knowledge of society by media. Bursa region has heterogeneous geographic structure and climatic conditions. This features affect the activity of ticks should not be ignored. Especially people living in urban areas, veterinarians, soldiers and people visiting tick infested areas for picnic and camping have risk of tick bite whereas people living in rural areas panic because of info pollution.

In conclusion, tick bites occur more than estimated numbers in Bursa. High numbers of *Rhipicephalus* tick bites in central urban areas may be the result of high stray animal (dog and cat) numbers. Finally, results of this

study demonstrated that humans go to outdoor for picnic, camping, tracking, agriculture or any other reason should take necessary measures.

REFERENCES

1. Aydın L, Bakırcı S: Geographical distributions of ticks in Turkey. *Parasitol Res*, 101, 163-166, 2007. DOI: 10.1007/s00436-007-0694-5

2. Bursalı A, Keskin A, Tekin S: A review of the ticks (Acari: Ixodidae) of Turkey: species diversity, hosts and geographical distribution. *Exp App Acarol*, 57, 91-104, 2012. DOI: 10.1007/s10493-012-9530-4

3. Arıkan İ, Tıraş Ü, Saraçoğlu D, Taşar MA, Dallar Y: Evaluation of the cases appealed as Crimean-Congo hemorrhagic fever. *Ege J Med*, 48, 29-31, 2009.

4. Orkun Ö, Karaer, Çakmak A, Nalbantoğlu S: Identification of tick-borne pathogens in ticks feeding on humans in Turkey. *PLoS Neglected Trop Dis,* 8, e3067, 2014. DOI: 10.1371/journal.pntd.0003067

5. Sutherst RW: Global change and human vulnerability to vector-borne diseases. *Clin Microbiol Rew*, 17, 136-173, 2004. DOI: 10.1128/CMR.17.1.136-173.2004

6. Aydın L: GüneyMarmara Bölgesi Ruminantlarında Görülen Kene Türleri ve Dağılımı. *Doktora Tezi,* Uludağ Üniv. Sağlık Bil. Enst.,1994.

7. Karaer Z, Yukarı BA, Aydin L: Türkiye keneleri ve vektörlükleri. **In,** Ozcel MA, Daldal N (Eds): Parazitolojide Artropod Hastalıkları ve Vektörler. 363-434, Türkiye Parazitoloji Derneği, Yayın no: 13, İzmir, 1997.

8. Estrada Pena A, Bouattour A, Camicas JL, Walker AR: Ticks of Domestic Animals in the Mediterranean Region: A Guide to Identification of Species. Published by University of Zaragoza, Zaragoza, Spain, 2004.

9. Gargılı A, Kar S, Yılmazer N, Cerit Ç, Sönmez G, Şahin F, Alp HG, Vatansever Z: Evaluation ofticks biting humans in Thrace province, Turkey. *Kafkas Univ Vet Fak Derg* 16 (Suppl A): A141-A146, 2010. DOI: 10.9775/kvfd.2009.2555

10. Vatansever Z, Gargılı A, Aysul NS, Sengöz G, Estrada- Pena A: Ticks biting humans in the urban area of Istanbul. *Parasitol Res*, 102, 551-553, 2008.

11. Kar S, Dervis E, Akın A, Ergonul O, Gargili A: Preferences of different tick species for human hosts in Turkey. *Exp App Acarol*, 61, 349-355, 2013. DOI: 10.1007/s10493-013-9698-2

12. Turell MJ: Role of ticks in the transmission of Crimean-Congo hemorrhagic fever virus. **In,** Ergonil O, Whitehouse CA (Eds): Crimean-Congo Hemorrhagic Fever - A Global Perspective. 143-154. Springer, Dordrecht, The Netherlands, 2007.

13. Bakırcı S, Aysul N, Eren H, Ünlü AH, Karagenç T: Diversity of ticks biting humans in Aydın province of Turkey, *Ankara Üniv Vet Fak Derg*, 61 (2): 93-98, 2014.

14. Çelebi S, Aydın L, Hacımustafaoğlu M, Çelik U, Çakır D, Emir BR, Çetin M: Evaluation of children cases admitted for tick bite in Uludag University Faculty of Medicine. *J Ped Inf*, 4, 133-137, 2010. DOI: 10.5152/ced.2010.27

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Research Article

Genetic Parameter Estimates for Growth Traits in Saanen Kids

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Abstract

Genetic parameters of test day (from birth to sixth month) body weight and body size parameters of Saanen kids were estimated by random regression with third order Legendre polynomial. The analyses were applied to 2673 test day records of 382 Saanen kids (Twin = 328; Single = 54; Female = 204; Male = 178) in a private dairy goat farm in Samsun, at the black Sea region of Turkey. Permanent environmental variances were static for height at sacrum, height at withers and chest depth, increased for body weight and body length on time. Heritability values ranged from 0.2679 to 0.6135 for body weight and Height at withers. Genetic correlations changed between 0.725 and 0.979 in all traits. The positive high genetic correlations among traits suggested that selection for any one of these traits would result in considerable positive change in other traits.

Keywords: *Saanen kids, Heritability, Growth, Genetic parameters*

Saanen Oğlaklarında Büyüme Özellikleri İçin Genetik Parametre Tahminleri

Özet

Saanen oğlaklarının test günü canlı ağırlığı ve vücut ölçülerinin genetik parametreleri random regresyon ile üçüncü dereceden Legendre polinomu kullanılarak tahmin edilmiştir. Analizler, Türkiye'nin Karadeniz bölgesinde, Samsun'daki özel bir süt keçi çiftliğinde yetiştirilen 382 adet Saanen oğlağına (İkiz = 328; Tekiz = 54; Dişi = 204; Erkek =178) ait 2673 test günü (doğumdan altıncı aya kadar) kayıtlarına uygulanmıştır. Kalıcı çevre varyansları, sağrı yüksekliği, cidago yüksekliği ve göğüs derinliği için durağan iken vücut ağırlığı ve vücut uzunluğu için zamanla yükselmiştir. Kalıtım derecesi değerleri vücut ağırlığı ve cidago yüksekliği için 0.2679 ile 0.6135 arasında değişim göstermiştir. Genetik korelasyonlar tüm özelliklerde 0.725 ile 0.979 arasında değişmektedir. Özellikler arasındaki pozitif yüksek genetik korelasyonlar bu özelliklerden herhangi biri için yapılacak seleksiyonun diğer özellikler için de önemli bir ilerleme sağlanacağını göstermektedir.

Anahtar sözcükler: *Saanen oğlağı, Kalıtım derecesi, Büyüme, Genetik parametre*

INTRODUCTION

Goats become an alternative livestock animals for farmers in the rugged terrain of the Black Sea region in Turkey. Turkey's goat population is about 8.9 million head and 8.1 million of this population is the native hair goat [1]. That breed is characterized by low litter size, short lactation period and low milk yield [2]. To increase goat milk production in these areas, the Saanen breed was introduced as substitute to hair goat for the past 15 years.

Important factors affecting profitability for goat enterprises are early growth traits. The weight and size of kids at birth are determined not only by their genetic potential but also by environment and maternal effects [3]. When selecting goat breeding stock observing some growth characteristics of the kids may be useful [2]. Furthermore, recognizing genetic parameters of growth traits may also facilitate the breeding program. Growth traits are economically important for using early breeding of young animals.

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Traditionally, traits that are measured in time are analyzed with a multitrait model, defining the phenotypic values at distinct ages as different traits. One advantage of random regression models over multivariate models is that with random regression models it is possible to calculate (co)variances between or at every age or instance. Random regression models provide a method for analyzing independent components of variation that reveal specific patterns of change overtime [4].

Random regression (RR) models have become a popular choice for modeling of traits, which are measured repeatedly per individual, but change gradually and continually with time. The RR models have described general shape of growth curve by fixed regression for all dams and the individual genetic deviation from the fixed regressions. Regression coefficients from RR models are generally described by the orthogonal functions such as Legendre polynomials. Coefficients derived from fitting orthogonal functions are very useful for analyzing patterns of genetic variation in the trajectory. Legendre polynomials, a family of the orthogonal functions, are orthogonal and normalized, which results in better converge and more accurate results than the conventional polynomials. Applications in genetic evaluation schemes have been limited to the analysis of test-day records. Genetic merit for growth of meat producing animals is generally assessed treating records taken at different ages, or ranges of ages, as different traits [5,6].

Zhang et al.[7] reported that the mean values and standard errors of direct additive heritability estimates calculated with the optimum model were 0.17±0.07, 0.22±0.08, 0.07±0.07, 0.10±0.08, 0.30±0.12 and 0.08±0.10 for birth weight, body weight at 90 days, average daily gains from birth to 90 days, body weight at 300 days, average daily gains from birth to 300 days and average daily gains from 90 to 300 days, respectively for Boer goat. Janssens and Vandepitte [8] showed that heritability of body measurements were in the range of 0.26-0.57 and genetic correlations between these traits were high for Belgian Bleu du Maine, Suffolk and Texel sheep. Alade et al.[9] reported that heritability estimates from sire component were 0.39, 0.47, 0.39, 0.04, 0.03 and 0.22 for litter size, birth weight, pre-weaning gain, weaning weight, post-weaning gain and 9 month body weight, respectively. The corresponding values of repeatability estimates for mentioned study were 0.12, 0.61, 0.37, 0.52, 0.24 and 0.4. Zhang et al.[3] declared that the mean values and standard error of direct additive heritability estimates for body weight, body length, height and chest depth calculated with REML model were 0.19±0.08, 0.14±0.07, 0.24±0.09 and 0.25±0.10, respectively.

The objective of this study is to estimate the genetic parameters for weight and size including length, height at sacrum and withers and chest girth measures at birth to six months of age in Saanen goats raised in the Black Sea region of Turkey.

MATERIAL and METHODS

Material

Data was collected at a private dairy goat farm in Samsun, Turkey (40°31′N, 36°53′E and 650 m above the sea level), which consisted of 382 Saanen kids (Twin = 328; Single = 54; Female = 204; Male = 178) born from February to March 2013 from 278 does (only the data of lived animals during the experiment were used. Hence, 382 records were used for each time point). 11 bucks were used to impregnate the animals. For the analysis 2673 test day records were recorded. Body weight (BW) and body size parameters; body length (BL), height at sacrum (HS), height at withers (HW) and chest depth (CD) at birth were recorded within 24 h after the birth using the routine method as described by Chen [10]. These traits were measured monthly from birth to six months of age.

Kids were fed with dam's milk, had free access to alfalfa hay and concentrate after 2nd week under intensive system, Kids weaned at 65-70 days of age.

Methods

In this study, additive genetic and permanent environmental (co)variances and heritability values of the BW, BL, HS, HW and CD were estimated using random regression model with Legendre polynomials. These polynomials were used because they are orthogonal, normalized and resulted in a better convergence and more accurate results as compared to conventional polynomials [11] and they represent the coefficients most widely used to describe size-age relationships in animals [12]. Moreover, to model the (co)variance structure of the random components of the data for RRM, third order Legendre polynomial model was preferred due to the best fit [13-19]. Residual variance was also assumed to be constant. All data were analyzed with the DXMRR option of the DFREML statistical package [20]. The general random regression model used in this study is as follows:

$$y_{ijkl} = TB_i + S_j + \sum_{m=1}^{3} \beta_m \phi_m X_m(t_{ijk}) + \sum_{m=1}^{3} \alpha_{jm} \phi_m(t_{ij}) + \sum_{m=1}^{3} p_{jm} \phi_m(t_{ijk}) + e_{ijkl} \quad (1)$$

where y_{ijkl} is the i^{th} test day records of the kid k, TB_i is the i^{th} type of birth effect, S is the j^{th} sex effect, b_m is the m^{th} fixed regression coefficients, t_{ijk} is the test day of the kidk, $X_m(t_{ijk})$ is the m^{th} covariate evaluated at t_{ijk}, α_{jm} is the m^{th} additive genetic random regression coefficients for kid k, p_{jm} is the m^{th} permanent environmental random regression coefficients for kid k, ϕ_m is the m^{th} polynomial

evaluated for the age t_{ijk}, and e_{ijkl} is the random residual effect. To calculate phenotypic correlations, canonical correlation [21] was used because of repeated data for each time point belonged to 382 animals.

RESULTS

Means and standard deviations of BW, BL, HS, HW and CD for each time point for birth type and sex were given in *Table 1* and *Table 2*, respectively.

Third order Legendre polynomial model was used for random effects because of best fit. Other attempted models such as linear and quadratic were not argued in this study to avoid confusion. Estimated variance components for test day measurements were given in *Fig. 1*, *Fig. 2* and *Fig. 3* for additive genetic variance, permanent environmental variance and heritability estimates, respectively.

Estimates of genetic and canonical phenotypic correlations among BW, BL, HS, HW and CD traits were given in *Table 3*.

The estimates of additive genetic variances among traits were not similar. Genetic variances ranged from 0.1424 (BW) to 3.078 (HW), permanent environmental variances ranged from 0.1854 (CD) to 2.365 (HS) and heritability ranged from 0.2679 (BW) to 0.6135 (HW). The highest additive genetic variances were obtained for HS and HW, lowest was for CD. Permanent environmental variance was almost stationary over time for HS, HW, BL and CG, but tended to increase over time for BL. Changes in heritability estimates were compatible with changes in additive genetic variances for all traits. The heritability estimates of all traits were perpetually increased over time. Heritability for BW was the most increasing heritability over time. Strong positive phenotypic and genetic correlations were obtained among all traits showing values greater than 70 percent. All traits had insignificant genetic correlations with BW.

Table 1. *Mean and pooled standard deviation of traits on each time point for birth type (n=382)*
Tablo 1. *Her bir zaman noktasında doğum şekli için özelliklerin ortalaması ve bileşik standart sapması (n=382)*

Birth type	Traits	Birth	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	PSD
Single	BW	4.14	9.92	14.24	18.39	22.82	27.35	31.65	1.57
	BL	38.87	42.71	46.73	49.69	52.26	54.69	56.82	2.00
	HS	40.30	42.92	46.07	49.32	52.35	55.82	60.43	2.01
	HW	40.10	42.94	46.62	50.96	54.55	57.71	60.48	1.77
	CD	12.44	15.98	17.79	19.56	21.12	22.96	25.49	1.24
Twin	BW	2.98	8.24	11.88	15.71	19.43	23.80	27.87	1.29
	BL	37.19	40.83	45.02	47.54	50.08	52.31	54.37	1.86
	HS	38.90	41.50	44.17	47.44	50.54	53.85	57.79	1.88
	HW	38.92	41.44	44.85	48.94	52.50	55.56	58.17	1.44
	CD	11.42	14.87	16.44	18.23	19.58	21.69	24.35	0.90

BW: *body weight,* ***BL:*** *body length,* ***HS:*** *height at sacrum,* ***HW:*** *height at withers,* ***CD:*** *chest depth,* ***PSD:*** *pooled standard deviation*

Table 2. *Mean and pooled standard deviation of traits on each time point for sex (n=382)*
Tablo 2. *Her bir zaman noktasında cinsiyet için özelliklerin ortalaması ve bileşik standart sapması (n=382)*

Sex	Traits	Birth	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	PSD
Male	BW	4.03	10.30	14.88	19.26	23.26	27.65	31.85	3.12
	BL	38.89	42.68	46.34	50.13	52.58	55.10	57.42	2.18
	HS	39.89	42.76	45.79	49.37	52.47	55.84	60.87	2.41
	HW	39.68	43.00	47.16	50.79	54.61	58.08	61.37	2.53
	CD	12.34	16.24	17.97	20.03	21.42	23.29	25.45	1.42
Female	BW	3.55	8.59	12.29	16.06	20.44	24.99	29.34	2.85
	BL	37.85	41.63	46.06	48.03	50.69	52.92	54.85	2.01
	HS	39.83	42.22	45.19	48.17	51.17	54.68	58.49	2.17
	HW	39.76	42.59	46.33	50.19	52.99	56.03	58.53	2.22
	CD	11.92	15.10	16.83	18.38	19.94	21.92	24.85	1.38

BW: *body weight,* ***BL:*** *body length,* ***HS:*** *height at sacrum,* ***HW:*** *height at withers,* ***CD:*** *chest depth,* ***PSD:*** *pooled standard deviation*

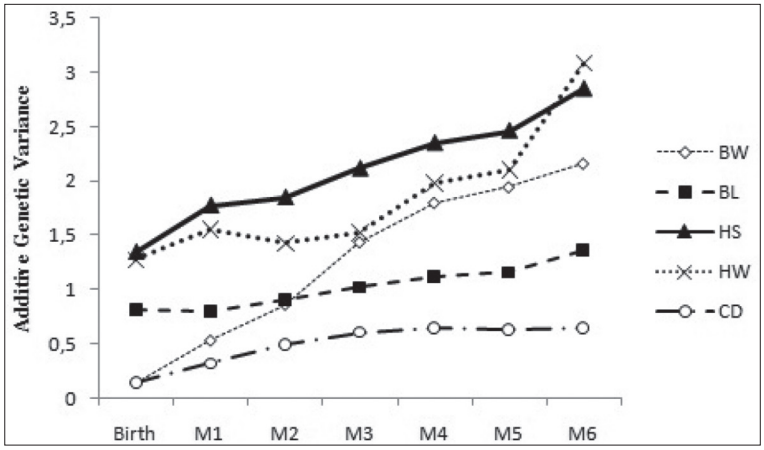
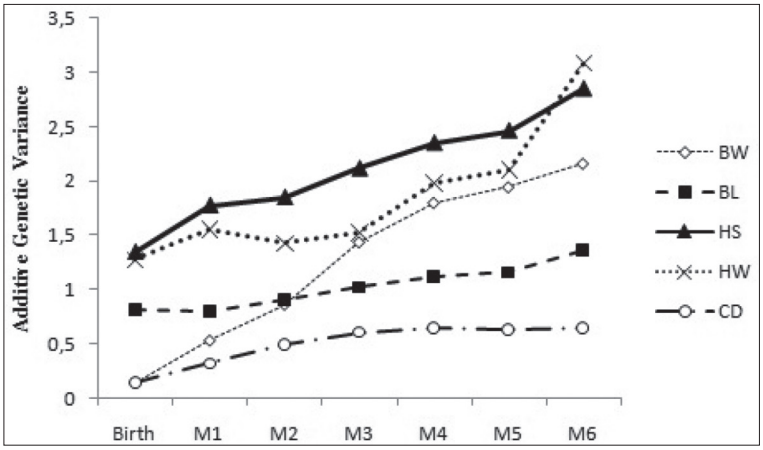


Fig 1. Additive genetic variances for traits of body weight, body length, height at sacrum, height at withers and chest depth of Saanen kids

Şekil 1. Saanen oğlaklarının vücut ağırlığı, vücut uzunluğu, cidago yüksekliği, sağrı yüksekliği ve göğüs derinliği özellikleri için eklemeli genetik varyansları

Fig 2. Permanent environmental variances for traits of body weight, body length, height at sacrum, height at withers and chest depth of Saanen kids

Şekil 2. Saanen oğlaklarının vücut ağırlığı, vücut uzunluğu, cidago yüksekliği, sağrı yüksekliği ve göğüs derinliği özellikleri için kalıcı çevre varyansları



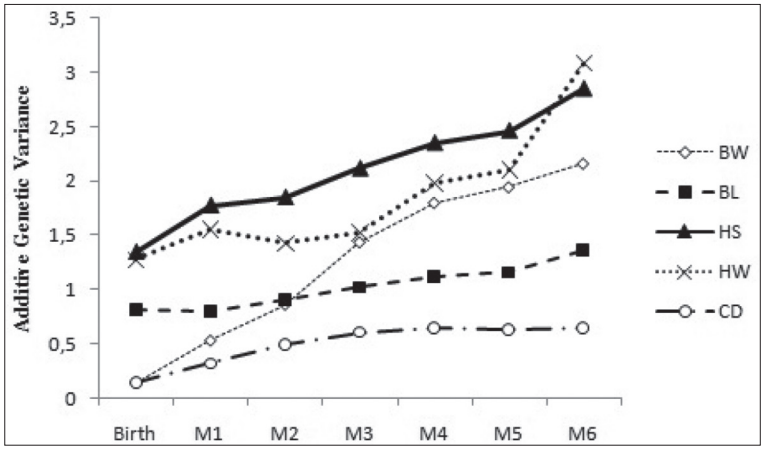


Fig 3. Heritability estimates for traits of body weight, body length, height at sacrum, height at withers and chest depth of Saanen kids

Şekil 3. Saanen oğlaklarının vücut ağırlığı, vücut uzunluğu, cidago yüksekliği, sağrı yüksekliği ve göğüs derinliği özellikleri için kalıtım derecesi tahminleri

DISCUSSION

The mean values of BW, BL, HS, HW and CD traits in Saanen kids raised in Black Sea region of Turkey were similar to values reported by Ocak et al.[2] for Saanens in the Cukurova sub-tropical region. Mean values of BW, BL and HS were greater than those reported by Bungsrisawat and Tumwasorn [22] and BW values were greater than those reported by Simsek et al.[23]. The higher values recorded for these traits may be the result of adaptation to environment or quality of environmental conditions.

For any production objective fixed effects are very important. In this study, fixed effects on growth traits were similar to those reported by Boujenane and El Hazzab [24], Otuma and Osakwe [25], Bedhane et al.[26] and Ocak et al.[2]. In all these studies as well as ours growth stages were highly affected by birth type and sex. But the effects of sex factor decreased with age and lost its importance as highlighted by Olfaz et al.[27].

Variance component estimations are more sensitive to sampling errors. For all interested traits, weight was the most variable trait with a coefficient of variation (CV) of

Table 3. *Estimates of genetic (above diagonal) and canonical phenotypic (below diagonal) correlations among traits*

Tablo 3. *Özellikler arasındaki genetik (köşegen üstü) ve kanonik fenotipik (köşegen altı) korelasyon tahminleri*

Traits	BW	BL	HS	HW	CD
BW		0.827**	0.971**	0.807**	0.979**
BL	0.951**		0.904**	0.936**	0.725**
HS	0.957**	0.953**		0.911**	0.919**
HW	0.973**	0.970**	0.978**		0.726**
CD	0.973**	0.947**	0.953**	0.959**	

BW: *body weight,* ***BL:*** *body length,* ***HS:*** *height at sacrum,* ***HW:*** *height at withers,* ***CD:*** *chest depth*

16%, for the other traits CV was around 5%.A considerable amount of the variation could be explained especially by fixed effect of birth type, similarly with the result of Janssens and Vandepitte [8] and Ocak et al.[2]. Estimates of additive genetic variance were higher than the values from previous studies Bedhane et al.[26], Zhang et al.[3]. Similar additive genetic variance estimates were presented by Schoeman et al.[28]. That was lower than the results of Abbasi and Ghafouri-Kesbi [29] except for HS and HW traits.

The estimates of permanent environmental variances were lower than the values from previous studies Bungsrisawat and Tumwasorn [22], Abbasi and Ghafouri-Kesbi [29] and Schoeman et al.[28]. Permanent environmental variances obtained in this study were higher than the study of Zhang et al.[3] with the exception of chest depth. Estimates of heritability were higher for BW, HS and HW than the results presented by Zhang et al.[3] and Bungsrisawat and Tumwasorn [22]. Similar heritability estimates obtained for BL and CD with the study of Zhang et al.[3]. Alike heritability estimates obtained for BL with the results presented by Bungsrisawat and Tumwasorn [22] Similar heritability estimates observed for all traits with results of Abbasi and Ghafouri-Kesbi [29]. Estimated genetic correlations for all traits were similar to the results of Zhang et al.[3], Abbasi and Ghafouri-Kesbi [29].

Estimated heritability values indicated that improvement in body weight and body measurements of Saanen kid is possible through selection procedures for using early breeding. The positive high correlations between body weight and body measurements indicate that these traits share a genetic component; therefore, selection for body measurements could possibly lead to improve in body weight and vice versa. The positive high genetic correlations among traits suggested that selection for any one of these traits would result in considerable positive change in other traits. Accurate estimates of genetic parameters are crucial for genetic improvement in livestock. The results obtained in this investigation can be applied for genetic improvement programme of Saanen goats.

REFERENCES

1. TSI: Turkish Statistical Institute report on livestock production of Turkey, 2013. www.turkstat.gov.tr/PreIstatistikTablo?istab_id=1568, *Accessed:* 27.06.2014.

2. Ocak S, Güney O, Önder H, Darcan N: Growth and development performances of CukurovaSaanen kids under tropical climate conditions. *J Anim Vet Adv,* 5 (11): 985-989, 2006.

3. Zhang C, Yang L, Shen Z: Variance components and genetic parameters for weight and size at birth in the Boer goat. *Livest Sci,* 115, 73-79, 2008. DOI: 10.1016/j.livsci.2007.06.008

4. Huisman AE, Veerkamp RF, Van Arendonk JAM: Genetic parameters for various random regression models to describe the weight data of pigs. *J AnimSci,* 80 (3): 575 - 582, 2002.

5. Takma Ç, Akbaş Y: Variance components and genetic parameter estimates using random regression models on test day milk yields of Holstein Friesians. *Kafkas Univ Vet Fak Derg*, 15, 547-551, 2009. DOI: 10.9775/kvfd.2009.055-A

6. Meyer K: Scope for a random regression model in genetic evaluation of beef cattle for growth. *Livest Prod Sci*, 86, 69-83, 2004. DOI: 10.1016/S0301-6226(03)00142-8

7. Zhang CY, Zhang Y, Xu D, Li X, Su J, Yang L: Genetic and phenotypic parameter estimates for growth traits in Boer goat. *Livest Sci*, 124, 66-71, 2009. DOI: 10.1016/j.livsci.2008.12.010

8. Janssens S, Vandepitte W: Genetic parameters for body measurements and linear type traitsin Belgian Bleu du Maine, Suffolk and Texel sheep. *Small Rumin Res,* 54, 13-24, 2004. DOI: 10.1016/j.smallrumres.2003.10.008

9. Alade NK, Dilala MA, Abdulyekeen AO: Phenotypic and genetic parameter estimates of litter size and body weights in goats. *IJSN*, 1, 262-266, 2010.

10. Chen D: The mensuration of body size and slaughter performance in Huainan Goat. *Herbivorous Livestock*, 102, 15-18, 1999.

11. Kirkpatrick M, Lofsvold D, Bulmer M: Analysis of inheritance, selection and evolution of growth trajectories. *Genetics*, 124 (4): 979-993, 1990.

12. Fitzhugh HA: Analysis of growth curves and strategies for altering their shape. *J AnimSci,* 42, 1036-1051, 1976.

13. Van Der Werf JHJ, Goddard ME, Meyer K: The use of covariance functions and random regressions for genetic evaluation of milk production based on test day records. *J Dairy Sci,* 81 (12): 3300-3308, 1998.

14. Pool MH, Meuwissen THE: Prediction of daily milk yields from a limited number of test days using test day models. *J Dairy Sci*, 82, 1555-1564, 1999. DOI: 10.3168/jds.S0022-0302(99)75383-X

15. Olori VE, Hill WG, Mcguirk BJ, Brotherstone S: Estimating variance components for test day milk records by Restricted Maximum Likelihood with a random regression animal model. *Livest Prod Sci*, 61, 53-63, 1999. DOI: 10.1016/S0301-6226(99)00052-4

16. Veerkamp RF, Thompson R: A covariance function for feed intake, live weight, and milk yield estimated using a random regression model. *J Dairy Sci,* 82, 1565-1573, 1999.

17. Kettunen A, Mantysaari EA, Pösö J: Estimation of genetic parameters for daily milk yield of primiparousAyshire cows by random regression test day models. *Livest Prod Sci,* 66 (3): 251-261, 2000. DOI: 10.1016/S0301-6226(00)00166-4

18. Strabel T, Szyda J, Ptak E, Jamrozik J: Comparison of random regression test-day models for production traits of dairy cattle in Poland. *Interbull Bulletin,* 31, 197-200, 2003.

19. Takma Ç, Akbaş Y: Estimates of genetic parameters for test day milk yields of a Holstein Friesian herd in Turkey with random regression models. *Arch Tierz*, 50 (4): 327-336, 2007.

20. Meyer K: DFREML 3.0α program package and user notes. Genetics and Breeding Unit, Univ. New England, Armidale, New South Wales, Australia, 1997.

21. Sarica M, Onder H, Yamak US: Determining the most effective variables for egg quality traits of five hen genotypes. *Int J Agric Biol,* 14,

235-240, 2013.

22. Bungsrisawat P, Tumwasorn S: Variance component and heritability of birth weight and body size at birth of a Saanen goat population in Thailand. The 3rd International Conference on Sustainable Animal Agriculture for Developing Countries (SAADC2011), July 26-29 2011, NakhonRatchasima, Thailand, 742-745, 2011.

23. Simsek UG, Bayraktar M, Gurses M: Investigation of growth and survivability characteristics in Saanen x pure Hair Goats crossbreeds F1 and B1. *FÜ Sağ Bil Vet Derg,* 21 (1): 21-26, 2007.

24. Boujenane I, El Hazzab A: Genetic parameters for direct and maternal effects on body weights of Draa goats. *Small Rumin Res,* 80, 16-21, 2008. DOI: 10.1016/j.smallrumres.2008.07.026

25. Otuma MO, Osakwe II: Estimation of genetic parameters of growth traits in Nigeria Sahelian goats. *J AnimVetAdv,* 7 (5): 535-538, 2008.

26. Bedhane M, Haile A, Dadi H, Alemu T: Estimates of genetic and phenotypic parameters for growth traits in Arsi-Bale goat in Ethiopia. *J Anim Sci Adv,* 3 (9): 439-448, 2013.

27. Olfaz M, Tozlu H, Onder H: Effect of hair color variation on milk production and kid growth in Turkish Hair goat. *J Anim Vet Adv,* 10 (8): 1037-1040, 2011. DOI: 10.3923/javaa.2011.1037.1040

28. Schoeman SJ, Els JF,Van Niekerk MM: Variance components of early growth traits in the Boer goat. *Small Rumin Res,* 26, 15-20, 1997. DOI: 10.1016/S0921-4488(95)00847-0

29. Abbasi MA, Ghafouri-KesbiF: Genetic co(variance) components for body weight and body measurements in Makooei sheep. *Asian-Aust J Anim Sci*, 24 (6): 739-743, 2011.

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Research Article

Erythropoietin Hormone and ACE Inhibitor Protect the Sperm Parameters of Adult Male Rats Against Doxorubicin Toxicity [1]

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Abstract

Doxorubicin (DXR) is used against the cancer but it has some adverse effects (gonadotoxicity, cardiotoxicity and nephrotoxicity). We aimed to determine the effect of DXR toxicity on reproduction and whether Darbepoetin (DP) and Ramipril (RAM) treatments play a protective role against this toxicity in male rats. Herein, adult male Sprague-Dawley rats (n=34) were divided randomly into five groups, as control (Group I; n=7, no medication) and four treatment groups (II to V). DXR was administered to all treatment groups (DXR, 2.5 mg/kg/w i.v. for 3 weeks) and they were Group II (DXR, n=6), Group III (n=7, DP 10 µg/kg/w i.p. for three weeks), Group IV (n=7, RAM 1 mg/kg/d p.o. for four weeks), and Group V (n=7, DP+RAM). Rats in all groups were sacrificed after four weeks of treatment. Spermatological parameters along with histopathological images and malondialdehyde levels of testicular tissue were evaluated. Weights of body, testicles, cauda epididymis and male accessory glands in DXR-treated groups were significantly different (P<0.05) from the control group. DXR toxicity adversely altered all the sperm parameters. But, DP plus RAM treatment in Group V improved the DXR-depressed motility and viable sperm rate. Sperm concentrations significantly decreased (P<0.05) in DXR-treated groups as compared to control group. In Group III and V, the increase in total abnormal sperm rate caused by the DXR injections was prevented. In conclusion, this study indicated that weekly DXR injections in adult rats depressed all the epididymal sperm parameters. Co-treatment by DP and RAM protected the sperm cells against the toxicity due to the DXR administration while single usages of the DP or RAM provided partial improvements.

Keywords: *Doxorubicin toxication, Erythropoietin, ACE inhibitor, Rat sperm*

Eritropoietin Hormonu ve ACE İnhibitörü, Erişkin Erkek Sıçanların Sperm Parametrelerini Doxorubicin Toksisitesine Karşı Korur

Özet

Kanser tedavisinde kullanılan Doxorubicin'in (DXR), kardiyotoksik ve nefrotoksik yan etkileri ile birlikte gonadotoksik özelliği de bulunmaktadır. Bu çalışmada, yetişkin erkek Sprague-Dawley sıçanlardaki sperm parametreleri üzerine DXR'nin toksik etkisi ile Darbepoetin (DP) ve Ramipril (RAM) tedavisinin bu toksikasyonu önleyici bir rolünün olup olmadığı araştırıldı. Yetişkin 34 adet erkek Sprague-Dawley sıçanlar rastgele 5 gruba ayrıldı. Kontrol grubunda bulunan sıçanlarda (Grup I; n=7) hiçbir ilaç kullanılmadı. Tüm tedavi gruplarında bulunan sıçanlara DXR uygulaması (DXR, 2.5 mg/kg/hafta i.v. 3 hafta, n=27) yapıldı ve bu gruplar Grup II (DXR, n=6), Grup III (DP 10 µg/kg/hafta i.p. 3 hafta, n=7), Grup IV (RAM 1 mg/kg/gün p.o. 4 hafta, n=7) ve Grup V'ten (DP+RAM, n=7) oluştu. Dört haftalık deneysel çalışma sonrasında tüm sıçanlara ötenazi uygulandı. Spermatolojik parametrelerle birlikte histopatolojik muayene ve testiküler dokulardaki malondialdehit (MDA) düzeyleri incelendi. DXR uygulanan gruplardaki ölçülen vücut, testis, kauda epididimis ve erkek eklenti bezlerinin ağırlıkları ile kontrol grubu parametreleri arasında önemli fark gözlendi (P<0.05). DXR toksisitesi tüm sperm parametrelerini olumsuz yönde etkiledi. Grup V'teki DP+RAM tedavisi, DXR'nin motilite ve canlı sperm oranı üzerine yaptığı toksik etkiyi önledi. Kontrol grubuna kıyasla, DXR uygulanan gruplardaki sperm yoğunluğu önemli derecede (P<0.05) düşüktü. Grup III ve V'te, DXR enjeksiyonuna bağlı total anormal sperm oranındaki artış önlendi. Sonuç olarak; bu çalışmada haftalık DXR enjeksiyonlarının yetişkin sıçanlarda tüm epididimal sperm parametrelerini olumsuz yönde etkilediği belirlendi. Birlikte kullanılan DP ve RAM tedavisi sperm hücresini DXR uygulamasına bağlı oluşan toksisiteye karşı korurken, DP ve RAM'ın tek başına kısmi iyileşme sağladı.

Anahtar sözcükler: *Doxorubicin toksisitesi, Eritropoietin hormonu, ACE inhibitörü, Sıçan spermi*

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INTRODUCTION

Doxorubicin (DXR), obtained from *Streptomyces peucetius* in 1970s, is an anthracycline antibiotic and used in cancer chemotherapy [1,2]. It inhibits the cancer cells and undesirably induces apoptosis in healthy cells, causing organ failures in heart, kidney, intestine, hair follicle, ovarium and testes [3,4]. Gonadotoxic effects of DXR disturb the spermatogenesis in testes leading to changes adversely affecting the male fertility [5,6]. Lipid peroxidation and oxidative stress result in malondialdehyde (MDA) production [7,8].

Erythropoietin (EPO) is a hormone required for proliferation, survival and differentiation of erythroid stem cells [9]. The EPO receptors (EPO-R) are found in many organs of both genders [10]. It has potential roles other than haematopoiesis and treatment of anaemia since the EPO and EPO-R are located in the tissues of brain, spinal cord, heart, gastrointestinal tract, lung, testes and leydig cells [11-13]. A new long-acting EPO analogue darbepoetin alpha (DP), stimulating erythropoiesis by the EPO-R, was used in the present study [14].

Angiotensin converting enzyme (ACE) converts the angiotensin I (A-I) to angiotensin II (A-II) and plays a role in the renin-angiotensin system (RAS) to regulate body fluids and electrolytes for maintaining blood pressure via the inactivation of bradykinin [15-17]. The ACE genes are encoded isoforms of both somatic (sACE) and testis-specific (tACE) genes [17,18]. The sACE, rather than the tACE, is responsible for male fertility. The ACE is involved in the regulation of fluid and electrolyte transport within the epididymis during the A-II activation and it is thus involved locally in the RAS of the epididymis [19,20]. The therapeutic effects of DP and RAM against the DXR-induced toxicity have been demonstrated recently [21].

Therefore, the aim of this study was to determine the protective effects of DP as an erythropoietin hormone and RAM as an ACE inhibitor treatment against the DXR toxicity on testicular and sperm parameters in adult male rats.

MATERIAL and METHODS

Thirty-four adult male Sprague-Dawley rats were supplied from Atatürk University Medical Experimental and Research Centre (Erzurum, Turkey). Rats were accommodated under 12 h light and 12 h darkness regime and provided standard pellet food and drinking water *ad libitum*.

Rats were divided into 5 groups as control and four treatment groups. Group I (Control) (n=7) had no medication and was given placebo (physiologic saline solution) into tail veins. Group II (DXR, n=6) received a dose of 2.5 mg/kg/w DXR (Doxorubicin hydrochloride, Adriblastina 50 mg, Deva, Pfizer Inc., USA) via tail veins weekly for three weeks. Group III (DP, n=7) had DXR plus 10 µ/kg/w i.p. DP (Darbepoetin alfa, Aranesp 10 µ, Eczacibasi, Amgen Inc., USA) weekly for three weeks. DXR and DP were administered weekly on days zero, 7, 14 and 21 relative to the initiation of the study. Group IV (RAM, n=7) received DXR plus 1 mg/kg/d p.o. RAM (Ramipril, Delix 5 mg, Sanofi, Pharma Vision, Turkey) daily for four weeks. Finally, Group V had DXR, DP and RAM at the same dose and duration, as above (groups II-IV). Following the animals being sacrificed under deep anaesthesia performed routinely (5 mg/kg Xylasine [Rompun, Bayer Inc., Germany] and 50 mg/kg Ketamine [Ketalar, Pfizer Inc., USA]), sampling was obtained at the 4th weeks of treatments. Treatment protocols for the groups are presented in *Table 1*.

The weights of body, testes, cauda epididymis and total male accessory gland (prostate, vesicular gland and coagulating gland) were measured in the groups. One testicle was kept at –20°C for biochemical analysis, while the other was placed into Bouin's solution for histopathological evaluation. The collections of cauda epididymal sperm and assessment of sperm parameters (namely motility, live-dead sperm and abnormal sperm rates) were performed [under the phase contrast microscope (Carl Zeiss Axio Scope.A1) on a warmed stage at 35.5±0.5°C] by modification of the method described by Akman and

Table 1. *Experimental protocols and drug administrations*
Tablo 1. *Çalışma protokolü ve ilaç uygulamaları*

Groups	n	Treatments			
		1. Week	2. Week	3. Week	4. Week
Group I (Control)	7	Physiologic saline i.v.	Physiologic saline i.v.	Physiologic saline i.v.	
Group II (DXR)	6	DXR 2.5 mg/kg/w i.v.	DXR 2.5 mg/kg/w i.v.	DXR 2.5 mg/kg/w i.v.	
Group III (DXR+DP)	7	DXR 2.5 mg/kg/w i.v. DP 10 µg/kg/w i.p.	DXR 2.5 mg/kg/w i.v. DP 10 µg/kg/w i.p.	DXR 2.5 mg/kg/w i.v. DP 10 µg/kg/w i.p.	
Group IV (DXR+RAM)	7	DXR 2.5 mg/kg/w i.v. RAM 1 mg/kg/d p.o.	DXR 2.5 mg/kg/w i.v. RAM 1 mg/kg/d p.o.	DXR 2.5 mg/kg/w i.v. RAM 1 mg/kg/d p.o.	RAM 1 mg/kg/d p.o.
Group V (DXR+DP+RAM)	7	DXR 2.5 mg/kg/w i.v. DP 10 µg/kg/w i.p. RAM 1 mg/kg/d p.o.	DXR 2.5 mg/kg/w i.v. DP 10 µg/kg/w i.p. RAM 1 mg/kg/d p.o.	DXR 2.5 mg/kg/w i.v. DP 10 µg/kg/w i.p. RAM 1 mg/kg/d p.o.	RAM 1 mg/kg/d p.o.

Note: *DXR and DP were administered via tail vein weekly on days zero, 7, 14 and 21. RAM was administered by oral route daily on days zero to 28.*
DXR: *doxorubicin,* ***DP:*** *darbepoetin,* ***RAM:*** *ramipril*

Aksoy [22]. The sperm concentration was determined with a Neubauer counting chamber, by using modified method of Türk et al.[23]. The MDA level was determined according to the method of Ohkawa et al.[24].

In histopathological examination of testicular tissue, the samples taken for histopathological preparations were fixed in Bouin's solution. After the routine follow-up procedures of alcohol-xylene, the sections of 5 µ paraffin blocks were stained with hematoxylin-eosin (HE) and examined under a light microscope (Olympus BX51 with DP72 camera, Japan) routinely. For statistical analyses, the number of normal spermatogenic cells (non-degenerative or non-necrotic) within a given tubulus seminiferus contortus was counted, as follows: For each slide selected from each group, the normal cell numbers were driven by considering randomly chosen cells observed in five different areas of 500±10 mm^2 found on each unit of the tubulus seminiferus contortus.

Atatürk University Local Board of Ethics Committee for Animal Experiments has approved the study protocol of this research (HADYEK decision no: 2013/61).

Statistical Analysis

All statistical analyses were carried out using SPSS statistical software programme (SPSS for MAC, version 20.0). Biochemical, histopathological and spermatological results were presented as means ± S.E.M. One-way analysis of variance (ANOVA) and post hoc Tukey test were used to determine differences in all the parameters between the groups. The values were considered significant when P<0.05.

RESULTS

The weights of body (P<0.05), total testicles (P<0.000), total cauda epididymis (P<0.01) and total male accessory gland (P<0.001) were significantly higher in the control group than those in all other (treatment) groups *(Table 2)*.

The MDA levels of testicular tissue were presented in *Table 3*. DXR administration increased the MDA level in Group II in comparison with that of control group (P<0.000). The DP administration prevented this increase in Group III. The MDA levels in Group IV and Group V increased as compared with that of control group. However, the RAM and DP plus RAM administrations only slightly decreased this level as compared with that of Group II.

In histopathological examinations, seminiferous tubule area, seminiferous tubule lumen area, germinative epithelial area, as well as vascular and necrotic changes were evaluated. The comparisons of histopathological changes were presented in *Fig. 1* and *Table 4*. Vascular changes such as oedema and hyperaemia were observed in Groups II-IV. The oedema and hyperaemia decreased in Group V. The area of tubulus seminiferous in the testes of Group II receiving the DXR was significantly lower than in the control group (P<0.005). Only the DP or RAM administrations did not prevent the damage induced by the DXR injection (P>0.05). However, the combined administration of DP+RAM prevented the DXR-induced damage (P<0.05) as comparable to that of the control group (P>0.05). The total area of tubulus seminiferous lumen in the testes of Group IV was significantly smaller than in the control group (P<0.05). Severe necrosis was seen in spermatogenic cells in Group II. The administration of DXR in Group II significantly decreased the area of the germinal epithelial cells as compared to that of the control group (P<0.01). Only the DP or RAM administrations could not prevent the damage caused by the DXR (P>0.05). However, the DP plus RAM administrations together

Table 3. *MDA levels in testicular tissue of DXR and treatment groups (Mean ± SEM)*
Tablo 3. *DXR ve tedavi gruplarının testiküler dokudaki MDA seviyeleri (Ortalama ± SEM)*

Groups	n	MDA Level (nmol/mg tissue)
Group I	7	13.33±1.15^{a}
Group II	6	26.14±3.93^{c}
Group III	7	15.16±1.17^{ab}
Group IV	7	19.29±1.42^{bc}
Group V	7	18.71±1.40^{bc}

$^{a-c}$ Means having different superscripts within a row are significantly different from each other (at least P<0.05)

Table 2. *The weights of body, testicles, cauda epididymis and male accessory glands (Mean ± SEM)*
Tablo 2. *Vücut, testis, cauda epididimis ve erkek eklenti bezlerinin ağırlıkları (Ortalama ± SEM)*

Group	n	Body Weight (g)	Total Testis Weight (mg)	Total Cauda Epididymal Weight (mg)	Male Accessory Glands Weight (mg)
Group I	7	314.00±12.33^{a}	3046.86±69.24^{a}	453.71±24.89^{a}	2564.43±172.69^{a}
Group II	6	259.33±12.64^{b}	2020.17±117.00^{b}	368.33±24.17^{b}	1585.67±153.13^{b}
Group III	7	238.29±10.167^{b}	1941.14±103.45^{b}	341.71±13.74^{b}	1358.71±179.35^{b}
Group IV	7	256.86±13.37^{b}	1963.14±52.14^{b}	363.57±21.05^{b}	1588.43±179.92^{b}
Group V	7	259.71±9.65^{b}	2028.43±74.10^{b}	362.14±24.72^{b}	1676.57±202.63^{b}

$^{a-b}$ Means having different superscripts within a row are significantly different from each other (at least P<0.05)

Table 4. *Histopathological evaluations of the effects of DXR and treatment groups on testicular tissue (Mean ± SEM)*
Tablo 4. *DXR ve tedavi gruplarının testiküler doku üzerine etkilerinin histopatolojik değerlendirmeleri (Ortalama ± SEM)*

Group	n	Histopathological Parameters			
		Seminiferous Tubule Area (μm²)	Seminiferous Tubule Lumen Area (μm²)	Germinative Epithelial Area (μm²)	Nucleated Cell Number (502.63±1.58 μm²)
Group I	7	21326.23± 816.65[a]	9889.12±536.14[a]	11437.11±1259.89[a]	71.00±4.98[a]
Group II	6	12568.13±1819.78[c]	7718.71±1035.36[ab]	4849.42±1191.30[b]	31.80±9.51[b]
Group III	7	14327.42±2007.27[bc]	8551.97±987.29[ab]	5702.20±556.44[b]	39.60±5.11[b]
Group IV	7	11665.80±485.11[c]	5963.60±278.36[b]	5775.45±1084.84[b]	48.75±4.77[ab]
Group V	7	18593.06±941.14[ab]	7410.76±263.85[ab]	11182.30±1118.10[a]	54.80±4.91[ab]

[a-c] Means having different superscripts within a row are significantly different from each other (at least P<0.05)

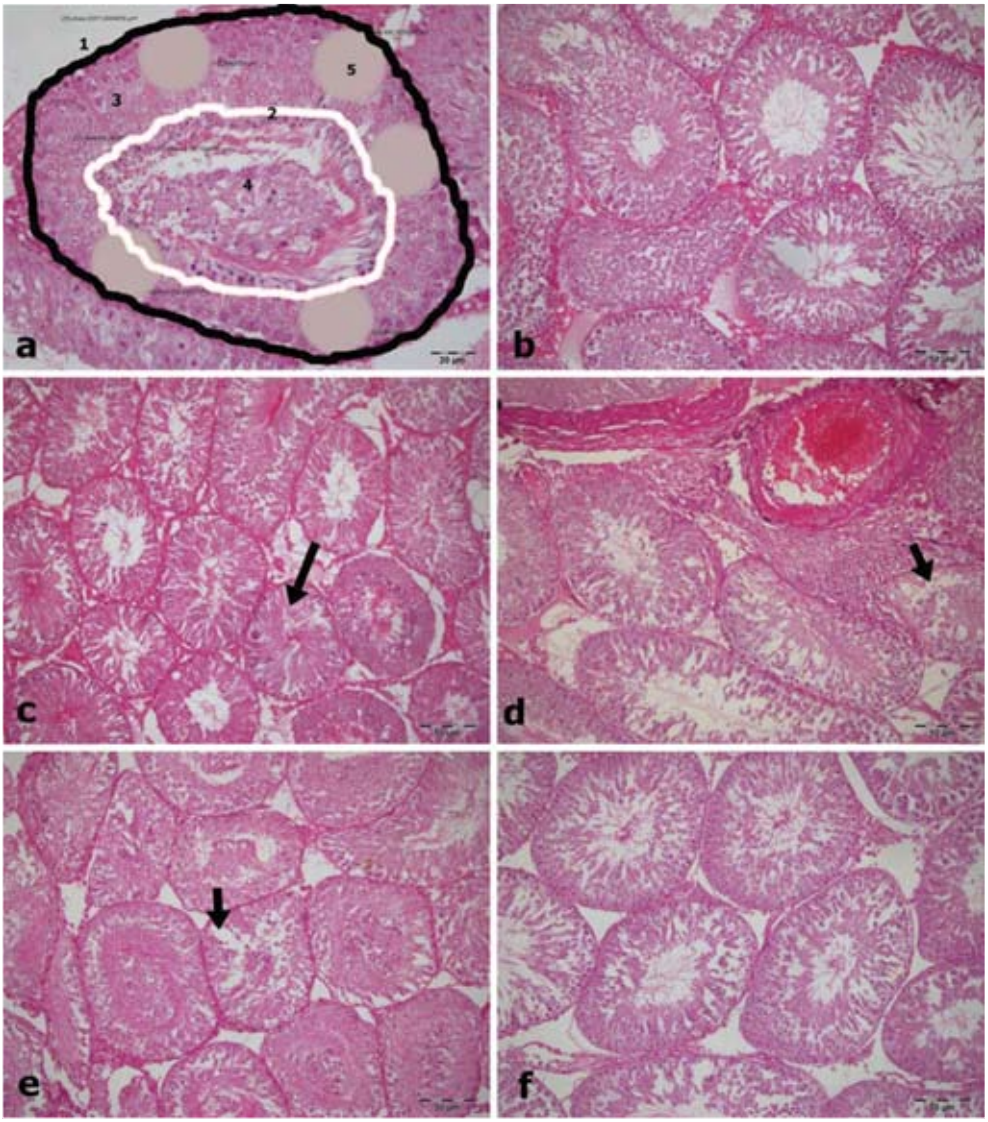

Fig 1. a: Measuring and counting methods of tubulus seminiferus contortus (1- seminiferous tubule area, 2- luminal space line of tubulus seminiferus contortus, 3- germinative epithelial area, 4- seminiferous tubule lumen area, 5- selected areas for spermatic cell counting), **b-f:** histopathologic comparison of tubulus seminiferus contortus and spermatic cell (**b:** Control group, **c:** Group II (DXR), **d:** Group III (DXR+DP), **e:** Group IV (DXR+RAM), **f:** Group V (DXR+DP+RAM), *arrows:* necrotic tubuli), (HE)

Şekil 1. a: Tubulus seminiferus kontortusun ölçüm ve sayım yöntemleri. (1- Seminifer tubul toplam alanı, 2- Seminifer tubul lumeninin alan sınırı, 3- Germinatif epitel alanı, 4- Seminifer tubulün lumen alanı, 5- spermatik hücre sayımı için rastgele seçilen alanlar), **b-f:** Tubulus seminiferus kontortus ve spermatik hücrelerin histopatolojik karşılaştırması (**b:** Kontrol grubu, **c:** Grup II (DXR), **d:** Grup III (DXR+DP), **e:** Grup IV (DXR+RAM), **f:** Grup V (DXR+DP+RAM), *oklar:* nekrotik tubuluslar ve spermatik hücreler), (HE)

ameliorated the DXR-induced damage as compared to that of the control group (P>0.05). The DXR administration significantly decreased the number of normal cells of the germinal epithelium in Group II as compared to that of the control group (P<0.01). Only numerical improvements were obtained by the administrations of RAM and DP plus RAM (P>0.05).

Epididymal sperm parameters obtained in this study were presented in *Table 5*. The DXR toxicity adversely affected all the percentages of epididymal sperm motility rate, live-dead sperm ratio, sperm density, head abnormality rate and total sperm abnormality rate (P<0.05). The DP plus RAM treatment prevented the depressive effects of DXR on the motility and live-dead sperm rate (P<0.05). However,

Table 5. *Effects of DXR and treatment groups on sperm parameters in adult male rats (Mean ± SEM)*
Tablo 5. *DXR ve tedavi gruplarının erişkin erkek sıçan sperm parametreleri üzerine etkileri (Ortalama ± SEM)*

Group	n	Sperm Parameters				
		Motility (%)	Dead Sperm (%)	Concentration (x10^6 sperm/ml)	Head Abnormality (%)	Total Abnormality (%)
Group I	7	49.59±1.56^{a}	38.26±1.62^{a}	60.27±6.49^{a}	9.44±1.16^{a}	13.99±1.22^{a}
Group II	6	23.23±1.64^{c}	62.43±3.17^{c}	29.95±3.40^{b}	14.07±1.22^{b}	21.53±1.13^{c}
Group III	7	30.23±4.56^{bc}	58.34±4.54^{bc}	33.93±3.88^{b}	12.51±0.98^{ab}	17.21±0.86^{ab}
Group IV	7	31.00±2.51^{bc}	59.16±2.7^{6c}	39.17±6.33^{b}	14.37±1.45^{b}	19.74±2.04^{bc}
Group V	7	42.51±1.17^{ab}	46.94±1.28^{ab}	29.58±3.66^{b}	11.73±0.72^{ab}	17.10±1.39^{ab}

$^{a\text{-}c}$ *Means having different superscripts within a row are significantly different from each other (at least P<0.05)*

the RAM or DP administrations as both given alone had no such protective effect on these parameters. Additionally, the DP and DP plus RAM administrations prevented the total sperm abnormalities induced by the DXR (P<0.01).

DISCUSSION

The effects of DXR on cancer cells are; i) the inhibition of topoisomerase-II that regulates the progress of DNA to interference with the DNA strand separation and helicase activity within the nucleus [2], ii) the generation of free radicals in cancer cells and cell membranes resulting in a damage to the DNA and protein structures [2,25], and iii) the respiratory disruption leading to the release of cytochrome-C initiating apoptosis within the mitochondria [26]. Thus, the DXR inhibits the synthesis of DNA, RNA and protein by triggering the apoptotic pathway of cell death and thus inducing the DNA damage [2,26,27]. The mechanism of this effect is not only for cancer cells, but it also occurs as a result of a general toxicity on normal cells [28]. The main reason for the limitation of the use of DXR is its cardiotoxic properties [3]. In addition to the cardiotoxicity and nephrotoxicity of DXR, its toxic efects on testes tissues, as also observed herein, have been shown previously in an experimental design in male rats [21]. Although the effects of DP and RAM are mainly related with the cardiovascular and circulatory system, therapeutic advantages of these drugs over various organs including testes warrant further investigation in patients under chemotherapy.

The EPO has anti-apoptotic, anti-inflammatory and anti-oxidant effects against the injuries by ischemia-reperfusion (IR) in various tissues [29]. It has been known that the EPO-R, being widespread area of the body, has a regulative role for the survival and proliferation of testes cells [12,14]. The EPO is produced within the layer of germ cells in humans and involved in reproductive function of the testis [10]. Sperm cell is under the influence of EPO through the fertilisation with regard to the EPO-R on sperm membrane before and after the ejaculation [10,30]. Lipid peroxidation (LPO) occurred in cell membrane changes the permeability or the integrity of the membrane resulting in DNA damage. Mammalian testes are very sensitive to this free radical damage [31,32]. The DXR produces the ROS in tissue that leads to increase in the LPO, oxidative stress and damages to the membrane and DNA. Thus, the DXR triggers the apoptotic pathway of cell death [27]. In the present study, the DXR administration (Group II) markedly increased the MDA levels in rat testicular tissue in comparison with those of other groups. This result was similar to the findings of Çeribaşı et al.[33], indicating the occurrence of LPO in the cellular membranes within the testes [31,32]. Researchers reported that the DP administration reduces the high MDA levels of testes subjected to the IR in rats [14]. Both the DP and DP+RAM treatments prevented the increase in the MDA level caused by the DXR injection herein. Furthermore, in our preliminary studies, the DP treatment increased the haematocrit (HCT) level in rats given DXR (data not shown herein).

Generally, the results of DXR administration in testes are; i) thickening of the basal lamina of seminiferous tubules [33], ii) vacuolisation and/or damages in the Sertoli cells [28,33,34], iii) degeneration of spermatogonia formed around the nuclei by nuclear condensation of spermatogonia with cytoplasmic content characterised by apoptosis [4], iv) prominent germ cell loss [28,35], v) reduction of seminiferous tubule size [35], vi) a mild inhibition of sperm release due to atrophy of the tubulus seminiferus [6,28,33], vii) necrosis, degeneration, desquamation, disorganisation, spermatogonial loss due to direct effect of interstitial oedema and congestion in the germ cells [33], and viii) eventually testicular atrophy allowing Sertoli cells survival only [28]. The findings in testicular tissue revealed that the DXR causes apoptosis due to testicular toxicity as accompanied by the enlargement and severe vacuolisation of interstitial tissue in the interstitial space and a severe vacuolisation and fibrinoid debris in the tubulus seminiferous [4,35].

In the present study, similar changes such as necrosis, degeneration, desquamation, spermatogenic cell loss, interstitial oedema and congestion in the interstitial tissue of testes were observed in a previous report [33].

Reduction of the area in the seminiferous tubules by the DXR was due to the reduction of the area in germinal epithelium. Furthermore, the DXR causes gonadotoxic necrosis resulting in a lower number of germ cells because of the reduction in the germinal epithelial area. The adverse interactions between the MDA level and the above-mentioned measurements explain the damage observed in the male reproductive organs. Also, researchers reported that the use of green tea as an antioxidant prevented some forms of testicular toxicity caused by the DXR in mice [35]. The EPO has marked anti-apoptotic and mitotic functions of various cells including in the endothelial cells, myoblasts, vascular smooth muscle cells, leydig cells, cortical neurons and both renal and non-erythroid cells [36]. In young cryptorchidic rats, the reason of how the EPO treatment before and after the IR protected the testicular morphology and maintained this protective mechanism has not been fully clarified [37,38]. Possible mechanism to explain the testicular damage could be the reduction of nourishments within the tissue caused by vascular changes, and therein, it might have been involved in the ischemia due to Sertoli cell dysfunction [39]. In spontaneously hypertensive rats (SHR), testicular histology reveals the reduction of germ cell, irregular shapes, and decrease in the diameter of the seminiferous tubules, as all due to the lack of expression of tACE in the germinal cells [17]. In stroke-prone spontaneously hypertensive rats (SP-SHR), the changes in different vascular beds and the structural hypertensive alterations within the inter-testicular structures in arterioles led to damages to spermatogenesis resulting in the reduced number of mature and/or immature gamete cells [40]. Not only serious and long-term hypertension but also mild and short-term hypertension may be a risk factor for male reproductive health [17].

Herein, neither the DP nor RAM alone did prevent the reduction in the area of seminiferous tubules and germinal epithelial layer caused by the DXR challenge. Single administration each of them provided only a partial recovery. However, when used together, the combination of DP plus RAM provided more effective protection against the gonadotoxicity by the single use of these drugs. When used alone, the RAM caused a marked reduction in the area of seminiferous tubule lumen as compared to that of the control rats. These results might be related with the effects of RAM on the vascular system. The DXR has been defined as the male reproductive toxicant leading to the reduction of sperm concentration in 4 weeks [28]. Herein, three times administrations of DXR, affecting the whole body up to 4 weeks, decreased the sperm density within the cauda epididymis.

In some researches, the DXR has reduced both the sperm concentration and motility [33,41]. In contrast to the findings of Plassman and Urwyler [28] and Imahie et al.[6], three times administrations decreased the rate of epididymal sperm motility. Any change in the function of ion channels on the sperm plasma membrane or the mitochondrial milieu may affect the sperm motility [42]. Sperm plasma membrane is quite sensitive in an environment containing excessive amounts of ROS, because the membrane has large amounts of long-chain unsaturated fatty acids and low concentration of scavenging enzymes [43]. In this study, considering the increased MDA levels in the testicular area, it has been considered that the DXR-induced production of free radicals may reduce the motility via an increase in the LPO by the side of the unsaturated fatty acids causing a damage to the tail as a propelling organ of the spermatozoa [33]. Furthermore, the DXR may impair the genes in the DNA responsible for the motility. The reduction in motility rate implies that the DXR has direct effects on the cauda epididymal sperm.

The DXR administration for 8 weeks has caused proportional increases in the head, tail and total abnormal sperm rate [33]. Herein, its administration for 4 weeks increased the dead sperm rate of cauda epididymis as well as the abnormal head ratio and total abnormal sperm rate. This increase in abnormal sperm rate may have occurred as a result of the disruption of epididymal function [44]. The EPO has been detected in human seminal plasma, without any correlation between the EPO levels in seminal plasma and the parameters studied such as sperm density, morphology, cytoplasmic droplets and the number of leukocytes [37]. On the contrary, the sperm cell carries the EPO-R, but its function has not been fully explored yet [30]. However, the EPO molecules to bind EPO-R on the plasma membrane allows a compatible modification that triggers the activation of an event resulting in the protection of cells from the ischemia, apoptosis and ROS [42]. Sperm cells require relatively hypoxic conditions for survival in *in vivo* and *in vitro* milieu. The presence of EPO activity within the epididymis indicates that it supports the sperm cell to remain viable in an hypoxic milieu [42]. Green tea administration improves the sperm motility, whereas melatonin prevents both the reduction of motility and density in the DXR-induced damages [35,41]. On the other hand, the administration of ellagic acid had no protective effect on the motility, concentration and abnormal sperm rate [33].

The enzymes having ACE activity in mammals are widely distributed among different tissues including those in the testis and epididymis where high levels of enzyme activity take place [45]. In stallions, the activity is greater in sperm membrane than in the seminal plasma [16]. All forms of the ACE respond similarly to variable substances, and are equally vulnerable to the specific inhibitors [46]. Captopril, an ACE inhibitor, is an example to reduce the ACE activity in the sperm plasma membrane [16]. However, there has been no effect on the sperm motility in long-term Captopril-treated humans [18]. On the other hand, the influence of ACE on the receptors localised on the sperm neck and tail to bind A-II may affect the sperm

thrusting movement [47], while the underlying mechanism of its effect on the sperm motility remains unclear. Herein, although some beneficial effects were observed with the DP treatment, its usage alone against the DXR-toxicity could not prevent the deterioration in sperm motility, live-dead sperm rate, density and head abnormalities, but it markedly prevented the increase in the total abnormal sperm rate. This fact may suggest the idea that the DP supports the sperm cells via its anti-oxidant properties due to an increase in the HCT level in the region via the removal of DXR-induced free radicals and to the prevention of the damage on the epididymal function [42].

The highest tACE concentration has been reported in spermatozoa with normal morphological features and motility [47]. The RAM, used as an ACE inhibitor herein, provided a partial beneficial contribution without preventing the depression of sperm parameters caused by the DXR. However, the administration of DP plus RAM together prevented depressions of motility, dead sperm and abnormal sperm rate caused by DXR.

In our study, the DXR administration for three times caused a general toxicity reducing the body weight, while the DP plus RAM could not prevent this decline, as indicated earlier [28]. The decrease in the body weight may indicate that the ACE activity has an effect on the body weight, since it has been shown earlier that the SHR animals has low body weights [17]. The reason for the ineffectiveness of the therapy to recover the body weight would be related with the dose and duration of DXR administration. In this respect, one can propose that the recovery from the DXR-induced gonadotoxicity may likely to be accelerated by the therapy after the cessation of DXR given. Therefore, it is important to note that the prevention be more vital than the recovery with regard to the general anthracycline toxicity during the chemotherapy.

The DXR reduces the testis weight as well as all male reproductive organ weights [33,35]. It has been proposed that this weight-decreasing effect is due to the parenchymal atrophy in the seminiferous tubules, the spermatogenic damage within the testicular tissue and the decrease in sperm density [33]. Being as a cytotoxic drug, the DXR inhibits the topoisomerase II activity by influencing the cell DNA resulting in apoptosis at the meiotic division of type A spermatogonia, the intermediate spermatogonia and spermatocytes in the rat testis [4]. Herein, the DP and RAM administrations could not prevent the weight depression of testicles, cauda epididymis, and male accessory glands in the treatment groups (receiving the DXR) because of short-term duration (4 *vs.* 8 weeks) of exposure of rats to the DP and RAM during the experimental period studied.

In conclusion, single usages of the DP or RAM could not prevent the entire depressive changes, although they achieved partial improvements, in epididymal sperm parameters due to the DXR administration in adult rats. However, the DP plus RAM treatments provided remarkable protective effects for motility, live-dead sperm rate and total abnormal sperm rate thereby. These favourable effects may involve both the oxygenation of the tissues via an erythropoietin hormone and vascular regulation and cardiovascular compensation with the help of an ACE inhibitor. Therefore, the disruption of male genital organs has been protected from DXR-induced gonadotoxicity by the combination therapy using these drugs. The anti-oxidant effects of the DP and RAM largely prevented the free radical formation, as the actual reason for the increase in the MDA level observed in all the DXR-treated animals. It was considered that these drugs might reduce the damages on Sertoli and germ cells in the testicles and plasma membrane of epididymal sperm.

REFERENCES

1. Arcamone F, Cassinelli G, Fantini G, Grein A, Orezzi P, Pol C, Spalla C: Adriamycin, 14-hydroxydaimomycin, a new antitumor antibiotic from S. Peucetius var. caesius. *Biotechnol Bioeng*, 11, 1101-1110, 1969. DOI: 10.1002/bit.260110607

2. Gewirtz DA: A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics adriamycin and daunorubicin. *Biochem Pharmacol*, 57, 727-741, 1999. DOI: 10.1016/S0006-2952(98)00307-4

3. Swain SM, Whaley FS, Ewer MS: Congestive heart failure in patients treated with doxorubicin. *Cancer*, 97, 2869-2879, 2003.

4. Shinoda K, Mitsumori K, Yasuhara K, Uneyama C, Onodera H, Hirose M, Uehara M: Doxorubicin induces male germ cell apoptosis in rats. *Arch Toxicol*, 73, 274-281, 1999.

5. Howell SJ, Shalet SM: Spermatogenesis after cancer treatment: Damage and recovery. *J Natl Cancer Inst Monogr,* 12-17, 2005. DOI: 10.1093/jncimonographs/lgi003

6. Imahie H, Adachi T, Nakagawa Y, Nagasaki T, Yamamura T, Hori M: Effects of adriamycin, an anticancer drug showing testicular toxicity, on fertility in male rats. *J Toxicol Sci*, 20, 183-183, 1995. DOI: 10.2131/jts.20.183

7. Ozkanlar S, Akcay F: Antioxidant vitamins in atherosclerosis–animal experiments and clinical studies. *Adv Clin Exp Med*, 21, 115-123, 2012.

8. Karapehlivan M, Uzlu E, Atakişi O, Erdoğan HM, Uzun M, Çitil M: Doksorubisin uygulanan tavşanlarda plazma sialik asit, malondialdehit ve redükte glutatyon düzeylerine L-karnitinin etkileri. *Kafkas Univ Vet Fak Derg*, 13, 155-160, 2007. DOI: 10.9775/kvfd.2007.29-A

9. Youssoufian H, Longmore G, Neumann D, Yoshimura A, Lodish H: Structure, function, and activation of the erythropoietin receptor. *Blood*, 81, 2223-2236, 1993.

10. Kobayashi T, Yanase H, Iwanaga T, Sasaki R, Nagao M: Epididymis is a novel site of erythropoietin production in mouse reproductive organs. *Biochem Biophys Res Commun*, 296, 145-151, 2002. DOI: 10.1016/S0006-291X(02)00832-X

11. Chikuma M, Masuda S, Kobayashi T, Nagao M, Sasaki R: Tissue-specific regulation of erythropoietin production in the murine kidney, brain, and uterus. *Am J Physiol Endocrinol Metab*, 279, E1242-E1248, 2000.

12. Magnanti M, Gandini O, Giuliani L, Gazzaniga P, Marti HH, Gradilone A, Frati L, Aglianò AM, Gassmann M: Erythropoietin expression in primary rat sertoli and peritubular myoid cells. *Blood*, 98, 2872-2874, 2001. DOI: 10.1182/blood.V98.9.2872

13. Lappin T: The cellular biology of erythropoietin receptors. *Oncologist*, 8, 15-18, 2003. DOI: 10.1634/theoncologist.8-suppl_1-15

14. Akcora B, Altug ME, Kontas T, Atik E: The protective effect of darbepoetin alfa on experimental testicular torsion and detorsion injury. *Int J Urol*, 14, 846-850, 2007. DOI: 10.1111/j.1442-2042.2007.01844.x

15. Corvol P, Eyries M, Soubrier F: Handbook of Proteolytic Enzymes. **In,** Barret A, Rawlings N, Woesser J (Eds): 332-349, Elsevier Academic Press, New York, 2004.

16. Ball B, Gravance C, Wessel M, Sabeur K: Activity of angiotensin-converting enzyme (ACE) in reproductive tissues of the stallion and effects of angiotensin II on sperm motility. *Theriogenology*, 59, 901-914, 2003. DOI: 10.1016/S0093-691X(02)01127-5

17. Atanassova N, Lakova E, Bratchkova Y, Krasteva G, Donchev M: Expression of testicular angiotensin-converting enzyme in adult spontaneously hypertensive rats. *Folia Histochem Cytobiol*, 47, 117-122, 2009. DOI: 10.2478/v10042-009-0002-6

18. Sabeur K, Vo A, Ball B: Effects of angiotensin II on the acrosome reaction in equine spermatozoa. *J Reprod Fertil*, 120, 135-142, 2000. DOI: 10.1530/jrf.0.1200135

19. Inoue N, Kasahara T, Ikawa M, Okabe M: Identification and disruption of sperm-specific angiotensin converting enzyme-3 (ACE3) in mouse. *PLoS One*, 5, e10301, 2010. DOI: 10.1371/journal.pone.0010301

20. Wong P, Uchendu C: The role of angiotensin-converting enzyme in the rat epididymis. *J Endocrinol*, 125, 457-465, 1990. DOI: 10.1677/joe.0.1250457

21. Ozkanlar Y, Aktas MS, Turkeli M, Erturk N, Oruc E, Ozkanlar S, Kirbas A, Erdemci B, Aksakal E: Effects of ramipril and darbepoetin on electromechanical activity of the heart in doxorubicin-induced cardiotoxicity. *Int J Cardiol*, 173, 519-521, 2014. DOI: 10.1016/j.ijcard.2014.03.044

22. Akman O, Aksoy M: Assessment of sperm quality by hypo-osmotic swelling test (HOSt) combined by supravital staining in mice. *J Anim Res*, 18, 1-6, 2008.

23. Türk G, Ateşşahin A, Sönmez M, Yüce A, Çeribaşi AO: Lycopene protects against cyclosporine A-induced testicular toxicity in rats. *Theriogenology*, 67, 778-785, 2007. DOI: 10.1016/j.theriogenology.2006.10.013

24. Ohkawa H, Ohishi N, Yagi K: Assay for lipid peroxides in animal-tissues by thiobarbituric acid reaction. *Anal Biochem*, 95, 351-358, 1979. DOI: 10.1016/0003-2697(79)90738-3

25. Quiles JL, Huertas JR, Battino M, Mataix J, Ramírez-Tortosa MC: Antioxidant nutrients and adriamycin toxicity. *Toxicology*, 180, 79-95, 2002. DOI: 10.1016/S0300-483X(02)00383-9

26. Thorn CF, Oshiro C, Marsh S, Hernandez-Boussard T, McLeod H, Klein TE, Altman RB: Doxorubicin pathways: Pharmacodynamics and adverse effects. *Pharmacogenet Genomics*, 21, 440, 2011. DOI: 10.1097/FPC.0b013e32833ffb56

27. Doroshow JH: Role of hydrogen peroxide and hydroxyl radical formation in the killing of Ehrlich tumor cells by anticancer quinones. *Proc Natl Acad Sci U S A*, 83, 4514-4518, 1986.

28. Plassmann S, Urwyler H: Improved risk assessment by screening sperm parameters. *Toxicol Lett*, 119, 157-171, 2001. DOI: 10.1016/S0378-4274(00)00314-3

29. Liu X, Shen J, Jin Y, Duan M, Xu J: Recombinant human erythropoietin (rhEPO) preconditioning on nuclear factor-kappa B (NF-kB) activation & proinflammatory cytokines induced by myocardial ischaemia-reperfusion. *Indian J Med Res*, 124, 343-354, 2006.

30. Tug N, Altunkaynak ME, Aktas RG, Kilic U, Yilmaz B, Cam C, Karateke A: Does erythropoietin affect motility of spermatozoa? *Arch Gynecol Obstet*, 281, 933-938, 2010. DOI: 10.1007/s00404-009-1289-4

31. Turner T, Brown K: Spermatic cord torsion: loss of spermatogenesis despite return of blood flow. *Biol Reprod*, 49, 401-407, 1993.

32. Wilhelm Filho D, Torres MA, Bordin AL, Crezcynski-Pasa TB, Boveris A: Spermatic cord torsion, reactive oxygen and nitrogen species and ischemia-reperfusion injury. *Mol Aspects Med*, 25, 199-210, 2004. DOI: 10.1016/j.mam.2004.02.020

33. Çeribaşı AO, Sakin F, Türk G, Sönmez M, Ateşşahin A: Impact of ellagic acid on adriamycin-induced testicular histopathological lesions, apoptosis, lipid peroxidation and sperm damages. *Exp Toxicol Pathol*, 64, 717-724, 2012. DOI: 10.1016/j.etp.2011.01.006

34. Hou M, Chrysis D, Nurmio M, Parvinen M, Eksborg S, Söder O, Jahnukainen K: Doxorubicin induces apoptosis in germ line stem cells in the immature rat testis and amifostine cannot protect against this cytotoxicity. *Cancer Res*, 65, 9999-10005, 2005. DOI: 10.1158/0008-5472.CAN-05-2004

35. Sato K, Sueoka K, Tanigaki R, Tajima H, Nakabayashi A, Yoshimura Y, Hosoi Y: Green tea extracts attenuate doxorubicin-induced spermatogenic disorders in conjunction with higher telomerase activity in mice. *J Assist Reprod Genet*, 27, 501-508, 2010. DOI: 10.1007/s10815-010-9438-z

36. Avlan D, Erdouğan K, Cimen B, Apa DD, Cinel I, Aksöyek S: The protective effect of selenium on ipsilateral and contralateral testes in testicular reperfusion injury. *Pediatr Surg Int*, 21, 274-278, 2005.

37. Temma K, Shimoya K, Hashimoto K, Zhang Q, Koyama M, Murata Y: Detection of erythropoietin in human seminal plasma. *Fertil Steril*, 81, 798-801, 2004. DOI: 10.1016/j.fertnstert.2003.07.039

38. Ergur BU, Kiray M, Pekcetin C, Bagriyanik HA, Erbil G: Protective effect of erythropoietin pretreatment in testicular ischemia-reperfusion injury in rats. *J Pediatr Surg*, 43, 722-728, 2008. DOI: 10.1016/j.jpedsurg.2007.11.028

39. Akagashi E, Kumamoto Y, Itoh N, Tsukamoto T, Suzuki T, Ohta Y: Manidipine improves spermatogenesis in the stroke-prone spontaneously hypertensive rat. *J Androl*, 18, 210-216, 1997. DOI: 10.1002/j.1939-4640.1997.tb01902.x

40. Akagashi K, Itoh N, Kumamoto Y, Tsukamoto T, Suzuki T, Ohta Y: Hypertensive changes in intratesticular arteries impair spermatogenesis of the stroke-prone spontaneously hypertensive rat. *J Androl*, 17, 367-374, 1996. DOI: 10.1002/j.1939-4640.1996.tb01802.x

41. Lee K, Lee I, Kim S, Moon C, Park S, Shin D, Park S, Kim H, Kim J: Melatonin attenuates doxorubicin-induced testicular toxicity in rats. *Andrologia*, 44, 796-803, 2012. DOI: 10.1111/j.1439-0272.2011.01269.x

42. Tug N, Kilic U, Karateke A, Yilmaz B, Ugur M, Kilic E: Erythropoietin receptor-like immunostaining on human spermatozoa. *Reprod Biomed Online*, 21, 718-720, 2010. DOI: 10.1016/j.rbmo.2010.05.022

43. Aitken R, McLaughlin E: Molecular mechanisms of sperm capacitation: progesterone-induced secondary calcium oscillations reflect the attainment of a capacitated state. *Soc Reprod Fertil Suppl*, 63, 273-293, 2007.

44. Cooper T, Yeung C: Sperm Maturation in Human Epididymes. **In,** Christopher JDJ, Barratt CL (Eds): Sperm Cell: Production, Maturation, Fertilization, Regeneration. 72-107, Cambridge University Press, New York, USA, 2006.

45. Hohlbrugger G, Schweisfurth H, Dahlheim H: Angiotensin I converting enzyme in rat testis, epididymis and vas deferens under different conditions. *J Reprod Fertil*, 65, 97-103, 1982. DOI: 10.1530/jrf.0.0650097

46. Vanha-Perttula T, Mather J, Bardin C, Moss S, Bellve A: Localization of the antigotensin-converting enzyme activity in testis and epididymis. *Biol Reprod*, 33, 870-877, 1985. DOI: 10.1095/biolreprod33.4.870

47. Aleksinskaya M, Nikolaeva M, Danilov S, Kuz'michyov L, Levchuk T, Yelistratova O, Sukhikh G: Relationship between spermatozoon movement velocity and expression of testicular isoform of angiotensin-converting enzyme on their surface. *Bull Exp Biol Med*, 141, 236-239, 2006.

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Research Article

SIRT2 - JAK1 Interaction Decreases IL-6 Induced Inflammatory Response in Cancer Cells

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Abstract

Interleukin-6 (IL-6) is a pro-inflammatory cytokine critical in immunoregulation. Aberrant IL-6 signaling has been implicated in various diseases including inflammation-associated cancer. Sirtuin-2 (SIRT2) is a cytoplasmic deacetylase and is generally considered a tumor suppressor. SIRT2 gene removed mouse develops tumors in many organs, primarily in the liver and breast tissues as the mouse ages. The purpose of the study was to investigate the roles of SIRT2 on IL-6 induced inflammatory response in mouse embryonic fibroblasts (MEFs) with SIRT2 gene removed and HeLa cervical cancer cell lines that overexpress SIRT2 gene. Immunoprecipitation and immunoblotting techniques were utilized in the study. SIRT2 interacts with JAK1, which is one of the downstream proteins of IL-6. We also found that overexpression of SIRT2 in HeLa cervical cancer cells decreases IL-6 induced inflammatory response by decreasing the activity of JAK1. These results suggest that SIRT2 has a protective role against chronic inflammatory diseases including inflammation-associated cancer.

Keywords: *Sirtuin, Inflammation, STAT3, Cervical cancer, HeLa cells, Deacetylation*

SIRT2 - JAK1 Etkileşimi Kanser Hücrelerindeki IL-6'in Neden Olduğu İnflamatuar Tepkiyi Azaltır

Özet

İnterlökin-6 (IL-6), bağışıklık sisteminin düzenlenmesinde kritik bir pro-inflamatuar sitokindir. Anormal IL-6 sinyalinin, inflamatuara bağlı kanser de dahil olmak üzere çeşitli hastalıklarda rol oynadığı gösterilmiştir. Sirtuin-2 (SIRT2), sitoplazmik bir diasetilaz olup, genellikle tümör baskılayıcı olarak kabul edilir. SIRT2 geni çıkarılmış fare yaşlandıkça, başlica karaciğer ve meme dokusunda, ve pek çok organında tümörler gelişir. Bu çalışmanın amacı, SIRT2 geni çıkarılmış embriyonik fare fibroblastlarda (MEF'ler) ve SIRT2 genini aşırı eksprese eden HeLa servikal kanser hücrelerinde SIRT2'nin, IL-6 tarafından tetiklenen inflamatuar yanıtı üzerindeki rollerini araştırmaktı. Bu çalışmada immunolojik çökeltme ve immüno-inkube teknikleri kullanılmıştır. SIRT2, IL-6'nın aşağı tarafındaki takım proteinlerinden biri olan JAK1 ile etkileşime girer. HeLa servikal kanser hücrelerinde, SIRT2'nin aşırı ekspresyonu, JAK1'in aktivitesini azaltarak, IL-6'in neden olduğu inflamatuar yanıtı azalır. Bu sonuçlar, SIRT2 proteinin, kronik inflamatuar hastalıklara ve inflamatuara-bağlı kansere karşı koruyucu bir rol oynadığını önermektedir.

Anahtar sözcükler: *Sirtuin, İnflamasyon, STAT3, serviks kanseri, HeLa hücreleri, Diasetilasyon*

INTRODUCTION

Interleukin 6 (IL-6) is an immune response protein which is secreted primarily by T-cells and macrophages to fight with infection during trauma, burns, and other tissue damages. IL-6 is an important mediator for setting the body temperature up and acute-phase response [1]. IL-6 interacts with IL-6 receptor, and binding of IL-6 to its receptor triggers the interaction between IL-6 receptor and the signal-transducing membrane protein gp130. This dimerization induces the activation of the tyrosine kinases of the Janus family (JAK1, JAK2, and Tyk2). Activated JAK1 phosphorylates the transcription factor Signal Transducer and Activator of Transcription 3 (STAT3) proteins at tyrosine 705 and triggers their homodimerization. Dimerized STAT3 complexes translocate into the nucleus where they bind enhancer elements of IL-6 inducible genes, such as c-Myc, which plays many cellular processes [2,3]. IL-6 activates the inflammatory process in many diseases, such as diabetes, obesity, cancer, and atherosclerosis [4-6]. In the blood of metastatic cancer patients at advanced stages, higher levels of IL-6 were detected, suggesting that IL-6 enhances

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tumor cell survival [7,8]. Therefore, anti-IL-6 agents have been sought as therapeutics for many cancer types.

Sirtuin (SIRT) deacetylase enzymes play important roles in the regulation of stress response, metabolism, aging, gene silencing, and cancer. SIRT2 is a cytoplasmic member of SIRT family. SIRT2 gene removed mouse does not display any major phenotype; however, as mice age, they display increased incidence of tumor formation especially in the liver and breast tissues; and at a lesser extent in the lung, pancreas, stomach, intestine and other organs [9]. Previous studies supports the idea of SIRT2 enzyme may have some anti-aging, anti-tumorigenic and anti-inflammatory functions [10-13]. SIRT2 has been shown to deacetylate numerous substrates and the mechanisms how SIRT2 decreases inflammation and inflammation-associated disorders has been poorly understood up to date. Here, the role of SIRT2 on the regulation of immune response was investigated.

MATERIAL and METHODS

Cell Lines

SIRT2 wild type (WT) and knockout (KO) MEF cells were maintained in a 37°C incubator with 5% CO_2 and 6% oxygen in Dulbecco's Modified Eagle's medium (DMEM) containing 15% fetal bovine serum (FBS) as described previously [9,14]. HeLa cells were maintained in 37°C incubator with 5% CO_2 and 21% oxygen in DMEM containing 10% FBS.

Immunoprecipitation (IP) of SIRT2 and JAK1

HeLa cells were lysed with IP buffer (10 mM HEPES, pH 7.9, 180 mM KCl, 1.5 mM $MgCl_2$, 0.1% NP-40, 1 mM EDTA, 0.1 mM PMSF), including protease and phosphatase inhibitors. Total cell extracts were incubated with anti-SIRT2 (Sigma, St. Louis, MO) or anti-JAK1 (BD Biosciences, San Jose, CA) antibody for 16 h at 4°C, followed by the incubation with magnetic beads (Dynabeads; Life Technologies Carlsbad, CA) for 2 h. After washing three times with IP buffer, bound proteins were extracted by boiling the samples in the loading buffer for 5 min, and isolated proteins were resolved via 10% SDS PAGE.

Western Blotting

Western blotting on nitrocellulose membrane was performed using iBlot transfer system (Life Technologies). PVDF membranes were incubated with anti-STAT3 (Cell Signaling, Danvers, MA), anti-P-STAT3 (Cell Signaling), anti-SIRT2 (Sigma), anti-a-tubulin (Cell Signaling) primary antibodies for 16 h at 4°C.

Immunofluorescence

Cells seeded on glass coverslips were fixed in 4% paraformaldehyde in PBS and then blocked with 1% BSA in PBS. Cells were incubated with anti-JAK1 (Cell Signaling) or anti-SIRT2 (Sigma) antibodies in PBS followed by incubation with goat-rabbit IgG conjugated with Alexa Fluor 488 and 594 secondary antibodies (Life Technologies) in PBS. Cells were washed in PBS, stained with the nuclear marker DAPI, mounted, and imaged on a fluorescence microscope.

RESULTS

SIRT2 Interacts with JAK1 Protein

To determine if there is a physical interaction between SIRT2 and JAK1 proteins, immunoprecipitation (IP) technique with anti-JAK1 and anti-SIRT2 antibodies along with magnetic beads were used. After IP of SIRT2, samples were subsequently immunoblotted with anti-JAK1 antibody *(Fig. 1)*. Presence of JAK1 protein in the immunoprecipitated SIRT2 protein complexes showed a physical interaction between SIRT2 and JAK1. In addition, immunofluorescence was used to determine whether these two proteins colocalized in cancer cells. Immunofluorescence staining showed that these proteins colocalized mainly in the cytoplasm *(Fig. 2)*.

Removal of SIRT2 Protein in Mouse Embryonic Fibroblast Cells Increases the Activity of JAK1 in Response to IL-6

JAK1 phosphorylates the transcription factor STAT3 at

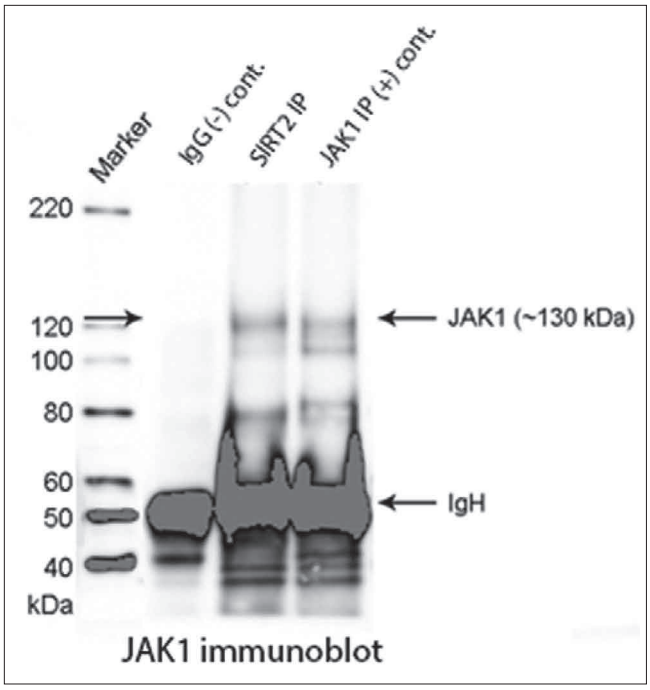

Fig 1. SIRT2 physically interacts with JAK1. Cell extracts were immunoprecipitated with anti-SIRT2, anti-JAK1, and IgG antibodies, and subsequently immunoblotted with anti-JAK1 antibody. IgG was used as a negative control and JAK1 immunoprecipitation was used as a positive control

Şekil 1. SIRT2 ile JAK1 fiziksel etkileşim gösterir. Hücre ekstreleri anti-SIRT2, anti-JAK1, ve IgG antikorları ile çökeltilir, ve daha sonra, anti-JAK1 antikoru ile immun-inkübe edilmiştir. IgG, negatif kontrol ve JAK1 immüno-çökeltme pozitif kontrol olarak kullanıldı

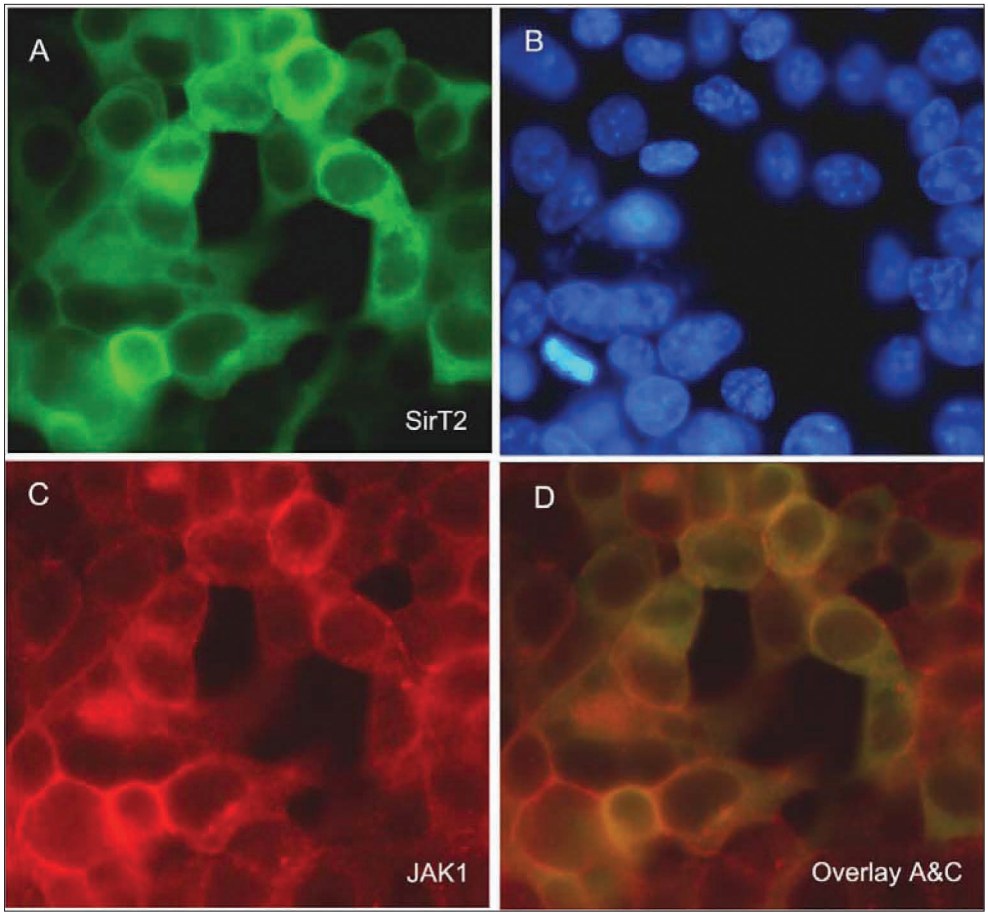

Fig 2. SIRT2 and JAK1 are colocalized in the cytoplasm. HeLa cervical cancer cells were stained with (A) anti-SIRT2 *(green staining)* (B) DAPI nuclear marker *(purple staining)* or (C) anti-JAK1 *(red staining)*, and (D) anti-SIRT2 and anti-JAK1 staining were subsequently merged

Şekil 2. SIRT2 ile JAK1 sitoplazmada birlikte lokalize halde bulunmaktadır. HeLa servikal kanser hücreleri (A) anti-SIRT2 ile boyandı *(yeşil boyama),* (B) DAPI nükleer işaretleyici *(mor boyama),* veya (C) anti-JAK1 *(kırmızı boyama),* ve (D) anti-SIRT2 ve anti-JAK1 boyama sonradan birleştirildi

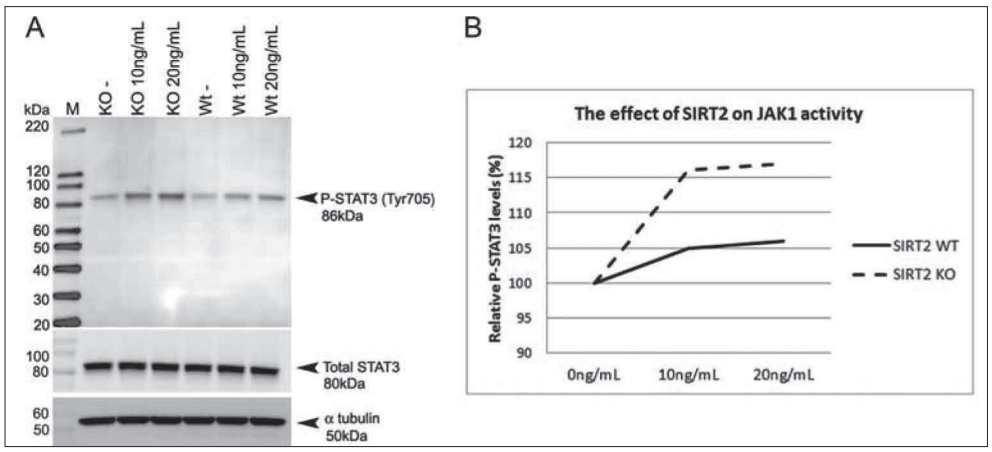

Fig 3. Removal of SIRT2 protein increases the activity of JAK1. (A) WT and SIRT2 KO MEFs were serum starved for 24 h. IL-6 was added into the cell culture media at 10 and 20 ng/mL concentrations and incubated for 30 min. Immediately after IL-6 exposure, cells were harvested, and lysed using cell lysis buffer including 1% NP-40, protease and phosphatase inhibitors. Proteins were resolved using 10% SDS PAGE, and immunoblotted with anti-P-STAT3, total anti-STAT3, and anti-a-tubulin. Images are representative of three independent experiments. (B) The intensity of protein bands in the representative western blot in *Fig. 3A* was quantified using the ImageJ software

Şekil 3. SIRT2 proteininin uzaklastirilmasi JAK1 aktivitesini artırır. (A) WT ve SIRT2 KO MEF'ler 24 saat boyunca serumdan mahrum bırakıldı. IL-6, 10 ve 20 ng/ml konsantrasyonlarda, hücre kültür ortamına ilave edildi ve 30 dakika süre ile inkübe edildi. Hemen IL-6'le maruz kaldıktan sonra, hücreler toplandi ve %1 NP-40, proteaz ve fosfataz inhibitörleri de dahil olmak üzere hücre liziz tamponu kullanılarak lize edildi. Proteinler %10 SDS PAGE kullanılarak çözüldü ve anti-p-STAT3, toplam anti-STAT3 ve anti-a-tubulin ile immun-inkübe edilmistir. Temsili western fotoğraflari, üç bağımsız deneyden elde edilmiştir. (B) *Şekil 3A*'daki temsili western blottaki protein bantlarının yoğunluğu ImageJ programı kullanılarak hesaplanmıştır

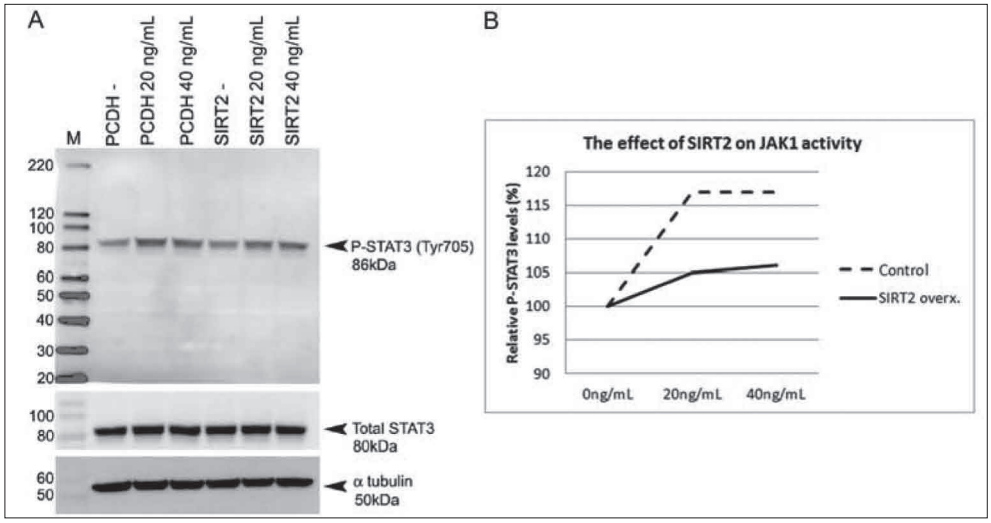

Fig 4. Overexpression of SIRT2 in HeLa cells decreases the IL-6 mediated STAT3 phosphorylation. (A) SIRT2 and control vector (PCDH) overexpressing stable HeLa cells were serum starved for 24 h. IL-6 were added into the cell culture media at 20 and 40 ng/mL concentrations and incubated for 30 min. Cells were harvested, and lysed using lysis buffer. Proteins were resolved using 10% SDS PAGE, and immunoblotted with P-STAT3, total STAT3, and a-tubulin. Images are representative of three independent experiments. (B) The intensity of protein bands in the representative western blot in *Fig. 4A* was quantified using the ImageJ software

Şekil 4. SIRT2 proteininin aşırı eksprese edilmesi JAK1 aktivitesini azaltir. (A) SIRT2 ve kontrol vektörü (PCDH) 24 saat boyunca serumdan mahrum bırakıldı. IL-6, 20 ve 40 ng/ml konsantrasyonlarda, hücre kültür ortamına ilave edildi ve 30 dakika inkübe edildi. Hücreler toplandi ve hücre liziz tamponu kullanılarak lize edildi. Proteinler %10 SDS PAGE kullanılarak çözüldü ve anti-p-STAT3, toplam anti-STAT3 ve anti-a-tubulin ile immun-inkübe edildi. Temsili western fotoğraflari, üç bağımsız deneyden elde edilmiştir. (B) *Şekil 4A*'daki temsili western blottaki protein bantlarının yoğunluğu ImageJ programı kullanılarak hesaplanmıştır

tyrosine 705. Phosphorylation of STAT3 induces formation of STAT3 dimers and leads to translocation to the nucleus. We investigated if the removal of SIRT2 protein changes the activity of JAK1 by measuring the phosphorylation of STAT3 at tyrosine 705 *(Fig. 3)*. In SIRT2 KO MEFs, phosphorylation of STAT3 was higher than that of in WT MEFs in response to IL-6, indicating SIRT2 decreased the kinase activity of JAK1 *(Fig. 3A, 3B)*.

Overexpression of SIRT2 in HeLa Cells Decreases the IL-6 Mediated STAT3 Phosphorylation

Next, we investigated if the overexpression of SIRT2 decreases JAK1 activity, and consequently reduces the phosphorylation of STAT3 in HeLa cervical cancer cell line. Consistent with the findings in SIRT2 WT and KO MEFs, when SIRT2 was overexpressed, phosphorylation of STAT3 at tyrosine 705 decreased *(Fig. 4A, 4B)*.

In summary, in this study, we showed that SIRT2 enzyme interacted with JAK1 protein. IL-6 mediated inflammation response was higher in SIRT2 gene removed mouse embryonic fibroblasts (MEFs). Moreover, when SIRT2 was overexpressed in cervical cancer cell line (HeLa), it decreased the activity of JAK1 and the phosphorylation of STAT3.

DISCUSSION

IL-6 has been associated with cell proliferation, immunomodulation, inflammation, and tumorigenesis. Activation of IL-6 signaling has been associated with advanced stages of cancer progression, such as in multiple myeloma, non-small cell lung carcinoma, colorectal cancer, renal cell carcinoma, prostate cancer, breast cancer, and cervical cancer. IL-6 which is a growth signal, inhibits apoptosis and enhances angiogenesis [16,17]. Chronic inflammation may lead to cancer development [5]. Inhibition of the activity of IL-6 may be therapeutically beneficial against chronic inflammation and cancer formation [18,19].

SIRTs have been associated with aging and longevity [20]. SIRTs are class III histone deacetylases and ADP-ribosyltransferases. These enzymes have a variety of substrates within the cells and are not restricted only to histones. In mammals, there are 7 SIRTs which are located in different compartments of the cells [21]. SIRT2 removed mouse model suggests that these proteins have various anti-aging, anti carcinogenic, and immune regulatory functions. Since these enzymes include numerous substrates, the anti tumorigenic effects of SIRTs are complex to elucidate. According to our results, JAK1 is one of the substrates of SIRT2. SIRT2 is a mainly cytoplasmic protein; however, in the G2/M transition and during mitosis, it translocates into the nucleus [15]. Like SIRT2, JAK1 is a mainly cytoplasmic protein and localized in the cytoplasmic side of plasma membrane. Consistently, our results also indicated that these proteins colocalized mainly in the cytoplasm.

Deacetylation of JAK1 by SIRT2 may be one of the contributors for the anti-tumorigenic effects of this SIRT protein. SIRT2 require NAD^+ as a co-factor, and its dependence on NAD^+ may provide a molecular link between nutrient/energy availability and the regulation of immune response. Likewise, inflammation is highly associated with aging and metabolism. Metabolic disorders, such as obesity, or aging may be functionally linked to the immunomodulatory roles of SIRT2 [22].

It has been reported that SIRT2 suppresses inflammatory response in arthritis autoimmune disease [12]. SIRT2 deacetylates nuclear factor-kappa B (NF-kB); and accordingly, the expression of NF-kB dependent genes including IL-6 decreases [12]. Consistent to this report, in this study, we found that SIRT2 interacts with JAK1 protein and decreases IL-6 mediated JAK1/STAT3 pathway; consequently, SIRT2 has a protection function against chronic inflammatory diseases and inflammation-associated carcinogenesis.

To conclude, our results indicate that SIRT2 interacts and decreases the activity of JAK1 kinase; and ultimately, it decreases the activity of IL-6 induced JAK/STAT3 signaling cascade, suggesting that SIRT2 have an immune response regulatory function through IL-6 in cancer cells. The activity of SIRT2 can be enhanced by calorie restriction and various chemicals, such as resveratrol [20]. Resveratrol has been reported to have beneficial effects for anti-cancer, anti-viral, anti-aging, and neuroprotective. Calorie restriction, resveratrol or other small molecules to increase the activity of SIRT2 may be beneficial to regulation of the immune response, and consequently, to prevent against chronic inflammatory diseases and inflammation-associated carcinogenesis. A sirtuin-based anti-inflammatory therapy should be further investigated with respect to both clinical and veterinary standpoints.

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REFERENCES

1. Kopf M, Baumann H, Freer G, Freudenberg M, Lamers M, Kishimoto T, Zinkernagel R, Bluethmann H, Kohler G: Impaired immune and acute-phase responses in interleukin-6-deficient mice. *Nature*, 368, 339-342, 1994. DOI: 10.1038/368339a0

2. Akira S, Nishio Y, Inoue M, Wang XJ, Wei S, Matsusaka T, Yoshida K, Sudo T, Naruto M, Kishimoto T: Molecular cloning of APRF, a novel IFN-stimulated gene factor 3 p91-related transcription factor involved in the gp130-mediated signaling pathway. *Cell*, 77, 63-71, 1994. DOI: 10.1016/0092-8674(94)90235-6

3. Rath T, Billmeier U, Waldner MJ, Atreya R, Neurath MF: From physiology to disease and targeted therapy: Interleukin-6 in inflammation and inflammation-associated carcinogenesis. *Arch Toxicol*, 89, 541-554, 2015. DOI: 10.1007/s00204-015-1461-5

4. Bastard JP, Jardel C, Bruckert E, Blondy P, Capeau J, Laville M, Vidal H, Hainque B: Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. *J Clin Endocrinol Metab*, 85, 3338-3342, 2000.

5. Balkwill F, Mantovani A: Inflammation and cancer: Back to Virchow? *Lancet*, 357, 539-545, 2001. DOI: 10.1016/S0140-6736(00)04046-0

6. Mauer J, Denson JL, Bruning JC: Versatile functions for IL-6 in metabolism and cancer. *Trends Immunol*, 36, 92-101, 2015. DOI: 10.1016/j.it.2014.12.008

7. Nakashima J, Tachibana M, Horiguchi Y, Oya M, Ohigashi T, Asakura H, Murai M: Serum interleukin 6 as a prognostic factor in patients with prostate cancer. Clin *Cancer Res*, 6, 2702-2706, 2000.

8. Neurath MF, Finotto S: IL-6 signaling in autoimmunity, chronic inflammation and inflammation-associated cancer. *Cytokine Growth Factor Rev*, 22, 83-89, 2011. DOI: 10.1016/j.cytogfr.2011.02.003

9. Kim HS, Vassilopoulos A, Wang RH, Lahusen T, Xiao Z, Xu X, Li C, Veenstra TD, Li B, Yu H, Ji J, Wang XW, Park SH, Cha YI, Gius D, Deng CX: SIRT2 maintains genome integrity and suppresses tumorigenesis through regulating APC/C activity. *Cancer Cell*, 20, 487-499, 2011. DOI: 10.1016/j.ccr.2011.09.004

10. Park SH, Zhu Y, Ozden O, Kim HS, Jiang H, Deng CX, Gius D, Vassilopoulos A: SIRT2 is a tumor suppressor that connects aging, acetylome, cell cycle signaling, and carcinogenesis. *Transl Cancer Res*, 1, 15-21, 2012.

11. Kim MJ, Kim DW, Park JH, Kim SJ, Lee CH, Yong JI, Ryu EJ, Cho SB, Yeo HJ, Hyeon J, Cho SW, Kim DS, Son O, Park J, Han KH, Cho YS, Eum WS, Choi SY: PEP-1-SIRT2 inhibits inflammatory response and oxidative stress-induced cell death via expression of antioxidant enzymes in murine macrophages. *Free Radic Biol Med*, 63, 432-445, 2013. DOI: 10.1016/j.freeradbiomed.2013.06.005

12. Lin J, Sun B, Jiang C, Hong H, Zheng Y: Sirt2 suppresses inflammatory responses in collagen-induced arthritis. *Biochem Biophys Res Commun*, 441, 897-903, 2013. DOI: 10.1016/j.bbrc.2013.10.153

13. Lee AS, Jung YJ, Kim D, Nguyen-Thanh T, Kang KP, Lee S, Park SK, Kim W: SIRT2 ameliorates lipopolysaccharide-induced inflammation in macrophages. *Biochem Biophys Res Commun*, 450, 1363-1369, 2014. DOI: 10.1016/j.bbrc.2014.06.135

14. Xu J: Preparation, culture, and immortalization of mouse embryonic fibroblasts. *Curr Protoc Mol Biol*, Chapter 28, Unit 28 1, 2005. DOI: 10.1002/0471142727.mb2801s70

15. Inoue T, Hiratsuka M, Osaki M, Oshimura M: The molecular biology of mammalian SIRT proteins: SIRT2 in cell cycle regulation. *Cell Cycle*, 6, 1011-1018, 2007. DOI: 10.4161/cc.6.9.4219

16. Liu Y, Li PK, Li C, Lin J: Inhibition of STAT3 signaling blocks the anti-apoptotic activity of IL-6 in human liver cancer cells. *J Biol Chem*, 285, 27429-27439, 2010. DOI: 10.1074/jbc.M110.142752

17. Middleton K, Jones J, Lwin Z, Coward JI: Interleukin-6: an angiogenic target in solid tumours. *Crit Rev Oncol Hematol*, 89, 129-139, 2014. DOI: 10.1016/j.critrevonc.2013.08.004

18. Wei LH, Kuo ML, Chen CA, Chou CH, Cheng WF, Chang MC, Su JL, Hsieh CY: The anti-apoptotic role of interleukin-6 in human cervical cancer is mediated by up-regulation of Mcl-1 through a PI3-K/Akt pathway. *Oncogene*, 20, 5799-5809, 2001.

19. Neiva KG, Warner KA, Campos MS, Zhang ZC, Moren J, Danciu TE, Nor JE: Endothelial cell-derived interleukin-6 regulates tumor growth. *Bmc Cancer*, 14, 99, 2014. DOI: 10.1186/1471-2407-14-99

20. Guarente L: Calorie restriction and sirtuins revisited. *Genes Dev*, 27, 2072-2085, 2013. DOI: 10.1101/gad.227439.113

21. Ozden O, Park SH, Kim HS, Jiang H, Coleman MC, Spitz DR, Gius D: Acetylation of MnSOD directs enzymatic activity responding to cellular nutrient status or oxidative stress. *Aging (Albany NY)*, 3, 102-107, 2011.

22. Galli M, Van Gool F, Leo O: Sirtuins and inflammation: Friends or foes? *Biochemical Pharmacology*, 81, 569-576, 2011. DOI: 10.1016/j.bcp.2010.12.010

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Research Article

Association of Single Nucleotide Polymorphism in Bone Morphogenetic Protein Receptor 1B (BMPR-1B) Gene with Growth Traits in Chicken

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Abstract

Growth traits are under the control of multiple genes. Understanding the genetic information of related genes is helpful for the selection and breeding course through marker assisted selection. The aim of the current study was to investigate the association of A287G SNP in BMPR-1B gene with growth traits in chicken. A single nucleotide polymorphism was identified in 240 individuals using the PCR-RFLP technique and confirmed by sequencing. The allelic and genotypic frequencies were compared, using the Chi-squared test. Associations between the genotype of each polymorphism and the traits were analyzed using the General Linear Model of statistical software SAS. Three genotypes (AA, AG and GG) were detected in Fayoumi and Rhold Island Red chicken. Sequencing revealed one mutation (287 A→G) in the genotype AA in comparison to the genotype GG. The A287G SNP of BMPR-1B gene was associated significantly with body weight at 2nd (*P*=0.022), 3rd (*P*=0.034), 4th (*P*=0.011), 5th (*P*=0.035), 6th (*P*=0.001), 7th (*P*=0.008) and 8th (*P*=0.016) week of age. In conclusion, BMPR-1B gene may be associated with body weight in chicken and may be considered in Marker Assisted Selection program to improve chicken growth performance.

Keywords: *Chicken, BMPR-1B, SNP, Growth traits*

Tavuklarda Kemik Morfogenetik Protein Reseptörü 1B (BMPR-1B) Genindeki Tek Nükleotid Polimorfizmi ile Büyüme Özellikleri Arasındaki İlişki

Özet

Büyüme özellikleri çok sayıda genin kontrolü altındadır. İlgili genlerdeki genetik bilgiyi anlamak, marker-destekli seleksiyon yoluyla seçim ve üreme sürecinde yardımcı olur. Bu çalışmanın amacı, tavuklarda BMPR-1B genindeki A287G SNP ile büyüme özellikleri arasındaki ilişkiyi araştırmaktır. PCR-RFLP tekniği kullanılarak, 240 bireyde bir tek nükleotid polimorfizmi tespit edildi ve sekanslama ile doğrulandı. Allellik ve genotipik frekanslar Ki-kare testi kullanılarak karşılaştırıldı. Her polimorfizm genotipi ve özellikler arasındaki ilişkiler SAS istatistiksel yazılımının Genel Lineer Modeli kullanılarak analiz edildi. Fayoumi ve Rhold Adası Kırmızı tavuklarında üç genotipi (AA, AG ve GG) saptandı. Sıralama, genotip GG'ye kıyasla genotip AA'da bir mutasyon (287 A→G) olduğunu gösterdi. BMPR-1B geninin A287G SNP'si, vücut ağırlığı ile 2. (*P*=0.022), 3. (*P*=0.034), 4. (*P*=0.011), 5. (*P*=0.035), 6. (*P*=0.001), 7. (*P*=0.008) ve 8. haftalarda (*P*=0.016) önemli ölçüde ilişkili idi. Sonuç olarak, BMPR-1B geni tavuklarda vücut ağırlığı ile ilişkili olabilir ve Markör Destekli Seçim programında tavukların büyüme performansını artırmak için dikkate alınabilir.

Anahtar sözcükler: *Tavuk, BMPR-1B, SNP, Büyüme özellikleri*

INTRODUCTION

Growth and egg production traits of chicken are controlled by a series of major genes and/or quantitative trait loci (QTL). Analyses of genetic markers in animals could lead to discernment of the genetic architecture of quantitative traits. There are two basic methods of QTLs identification: approach of the candidate gene and whole-genome scanning [1,2]. The candidate gene approach is an effective method for finding QTLs responsible for genetic variation in the traits of interest in agricultural animal species and calibrating whether specific genes are associated to economic traits in farm animals [3]. Several trials have been concerned in the fields of association

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analysis between candidate gene SNPs with animal growth and body composition traits [3-7].

Fayoumi is a native chicken breed originated in Egypt, reared for meat and egg production. It is adapted to subtropical environmental conditions and performed well under intensive management conditions [8]. Rhode Island Red (RIR) is a dual-purpose breed of American class. It is well adapted to the local environmental conditions [9]. Genetic polymorphisms are playing an increment important role as genetic markers in many sectors of animal breeding. As the molecular genetic techniques developed, it has become possible to obtain a new class of gene markers based upon the variability at DNA sequence level. Application of these molecular genetic markers potentially will greatly enhance the intensity of selection and will most efficiently uncover the productive potential of birds. Additionally, marker-associated selection (MAS) based on the studies concerned candidate genes and their effect on the phenotypic manifestations. An important intent of modern breeding in the poultry industry is to synthesis high-performance poultry lines and breeds in two main directions of productivity, meat and eggs [10].

The bone morphogenetic proteins (BMPs) are members of the transforming growth factor b (TGFb) superfamily. They are multifunctional proteins that regularize growth and differentiation in many cell types and play fundamental functions during embryogenesis and the fertility in mammals [11,12]. BMPs are potent inducers of cartilage and bone formation and has an important role in the bone healing process and in improving therapeutic efficacy [13,14], In multipotential mesenchymal cells derived from equine adipose cells, BMP-2 increase under magnetic field conditions which affect the osteogenic properties of the cells and enhance vascularization process [15]. A non-conservative substitution (Q249R) in the *BMPR-1B* sequence was related with the proliferation characterstics of some ewe breeds [16]. In the chicken ovary, granulosa cells are major target for BMPs and it was proposed that mRNA levels for *BMPR-1B* in granulosa cells are higher than in theca cells [17].

The objectives of the present study were to detect single nucleotide polymorphisms (SNPs) of *BMPR-1B* gene in Fayoumi and RIR chicken by PCR-RFLP and sequencing. In addition, investigating the association between these SNP and growth traits in chicken.

MATERIAL and METHODS

This study was carried out in accordance with the Zagazig University Animal Ethics Committee guidelines (ANWD-206), at the Biotechnology unit belonging to Department of Animal Wealth Development, Faculty of Veterinary Medicine, Zagazig University.

Experimental Flock and Management

A total of 300 day old chicks, Fayoumi and RIR breeds, were used in this study. Chicks were physically examined and wing banded. Brooding and growing requirements were provided using conventional floor system. All chicks were subjected to the same managerial, hygienic and climatic conditions. A standard diet was provided *ad libitum* at rearing period (8 weeks), including 22.5% crude protein and 2975.8 K.cal ME/kg. body weight to the nearest gram was recorded at hatch, 1st, 2nd, 3rd, 4th, 5th, 6th, 7th and 8th week of age.

DNA Extraction

A total of 240 blood samples were collected from plumage veins of Fayiomi and RIR chickens and conserved in tubes containing EDTA as an anticoagulant. DNA was isolated using Gene JET whole blood genomic DNA purification mini kit (Fermentas, Thermo Fisher Scientific, USA), following the manufacturer protocol. The quality of DNA was checked by running on 0.8% agarose gel and was quantified by reading absorbance at A260/A280 nm in a UV spectrophotometer.

PCR Amplification

A 581 bp fragment of *BMPR-1B* gene covering exon 6, intron 6 and exon 7 was amplified by polymerase chain reaction using Primers suggested by Zhang et al.[18] and DreamTaq Green PCR Master Mix (Thermo Scientific, fermentas, USA). PCR was carried out in a total volume of 25 µl reaction mixture containing 12.5 µl of Master Mix, 2 µL DNA, 1 µL each primer (10 µM), 8.5 µl deionized water and used T-professional thermal cycler (Biometra, Germany) according to the following program: initial denaturation at 95°C for 5 min; 40 cycles consisting of 30 s at 95°C, 30 s at 56°C and 30 s at 72°C for denaturation, annealing and extension steps, respectively; and a final extension at 72°C for 7 min. The PCR products were checked by electrophoresis on 1.5% agarose gel in 1×TAE buffer. The amplified product was visualized under UV transilluminator.

PCR-RFLP Analysis

A 20 µl of digestion mixture, consisting of 15 µl of the PCR product, 1×of recommended buffer, 2 µl of deionised water, and 6 units of restriction enzyme *Hind* III was placed in a 0.5 ml microcentifuge tube. The digestion mixture was mixed thoroughly in a vortex mixer and incubated at 37°C for 15 min. The digested product was run on a 2% agarose gel in TAE buffer along with 100 bp plus DNA ladder as molecular size marker. Gels were visualized under UV light and the genotype patterns were screened in gel documentation system.

Nucleotide Sequencing

The desired PCR product band was excised using a clean, sterile razor blade or scalpel and excised quickly to

minimize exposure of the DNA to UV light. The minimum agarose slice was transferred to a 1.5 ml micro centrifuge or screw cap tube and then purified by using a commercially available gel extraction kit (Fermentas, Thermo Fisher Scientific, USA). Samples were labeled and sent for sequencing. Sequencing was done by European Custom Sequencing Centre (GATC Biotech AG, Germany) using both forward and reverse primers of PCR amplification. The obtained sequences were edited manually using ChromasLiteVer. 2.01, *(http://www.tech nelysium.com.au/chromas.html)* and aligned with Clustal Omega software to identify nucleotide polymorphism.

Statistical Analysis

All statistical procedures were performed using SAS statistical system package V9.1 [19]. Allelic and genotypic frequencies of the single nucleotide polymorphism (SNP) were calculated and Chi-Square test was performed to examine Hardy-Weinberg equilibrium. Marker-trait association analysis was conducted using the one-way analysis of variance *(ANOVA)* through the general linear models (GLM) procedure. The comparison of means was carried out with Duncan's multiple range tests.

RESULTS

PCR Amplification

Genomic DNA of the two chicken breeds was amplified using specific primers for *BMPR-1B* gene. PCR products were detected by running a 1.5% agarose gel electrophoresis *(Fig. 1)*. The amplified products (581 bp)

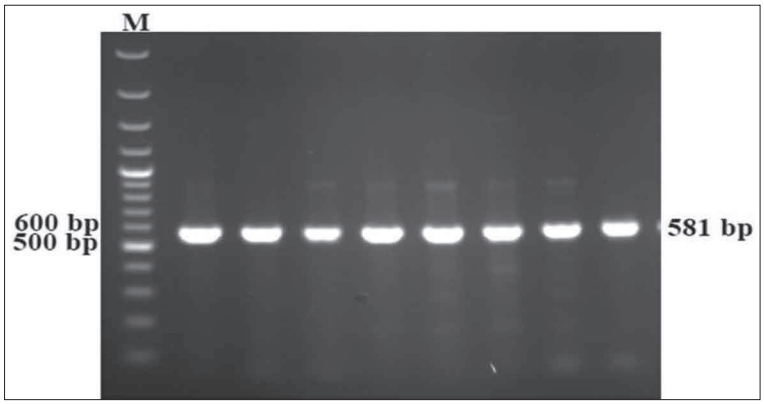

Fig 1. PCR Amplification of chicken *BMPR-1B* gene exon 6 to exon 7. M: 100 bp plus ladder

Şekil 1. Tavuk BMPR-1B geni ekson 6'dan 7. M ekson'a kadar PCR Amplifikasyonu: 100 bp artı merdiveni

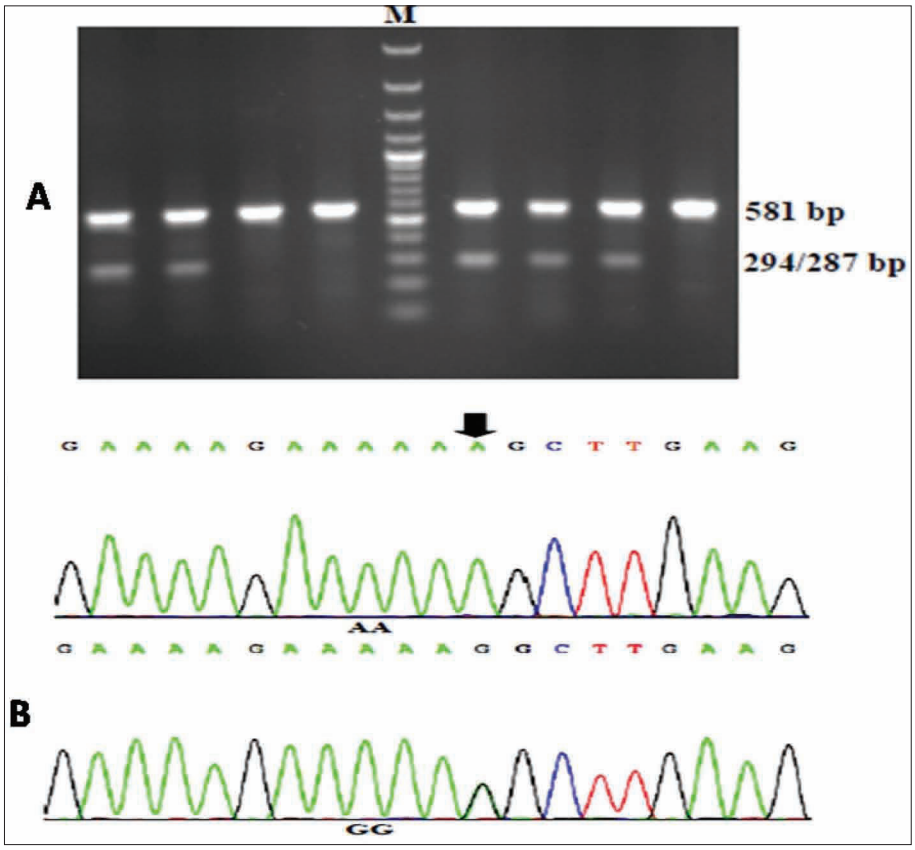

Fig 2. Genotyping of the A287G SNP in chicken *BMPR-1B* gene by (A) PCR-RFLP and (B) sequencing. *Arrow* indicates A/G substitution

Şekil 2. Tavuk BMPR-1B genindeki A287G SNP'nin (A) PCR-RFLP ve (B) sıralaması (sekanslaması) ile genotiplendirilmesi. *Ok,* A/G ikamesini belirtir

Table 1. *Frequency of genotypes and alleles of BMPR-1B gene in Fayoumi and RIR chicken breeds*
Tablo 1. *Fayoumi ve RIR tavuk ırklarındaki BMPR-1B geninin genotip ve allel sıklığı*

Breed	Genotype Frequency (n)			Allele Frequency		x^2
	AA	AG	GG	A	G	
Fayoumi	0.09 (11)	0.55 (66)	0.36 (43)	0.37	0.63	4.07 [1]
RIR	0.12 (14)	0.60 (72)	0.28 (34)	0.42	0.58	6.59 [2]

[1] Significant at level (P<0.05), [2] Significant at level (P<0.01)

Table 2. *Least squares means of body weight (g) according to genotypes at the SNP A287G of BMPR-1B gene*
Tablo 2. *BMPR-1B geninin SNP A287G'sindeki genotiplere göre vücut ağırlığının (g) en küçük kareleri*

Traits	Genotypes				
	AA	AG	GG	RSD	*P*-value
BW Day old (g)	28.7	28.6	27.2	2.7	0.652
BW 1st wk (g)	71.3	73.9	68.3	8.1	0.559
BW 2nd wk (g)	150.1^{ab}	156.8^{a}	126.6^{b}	12.9	0.022
BW 3rd wk (g)	235.2^{ab}	242.9^{a}	200.5^{b}	22.7	0.034
BW 4th wk (g)	320.4^{a}	328.7^{a}	266.6^{b}	24.6	0.011
BW 5th wk (g)	382.5^{ab}	398.6^{a}	327.2^{b}	43.1	0.035
BW 6th wk (g)	477.4^{a}	499.2^{a}	412.7^{b}	45.7	0.001
BW 7th wk (g)	571.2^{b}	677.4^{a}	518.3^{b}	62.6	0.008
BW 8th wk (g)	673.3^{b}	773.8^{a}	606.7^{b}	67.4	0.016

RSD: *residual standard deviation*
^{a,b} Values within a row with different superscripts differ significantly

were consistent with the target fragments and had a good specificity, which could be directly analyzed by RFLP and sequencing.

RFLP Analysis

The PCR-RFLP method was developed successfully for genotyping the A287G SNP in intron 6 of the chicken *BMPR-1B* gene, where all individuals have been screened. Three genotypes of AA, AG, and GG were detected and confirmed by sequencing *(Fig. 2 A,B)*. The fragment sizes of 581 bp for the GG genotype, 294/287 for the AA genotype, and a combination of 581, 294 and 287 bp for AG genotype.

Genotyping and Frequencies

Allele and genotype frequencies of *BMPR-1B* gene were calculated within each breed *(Table 1)*. The frequencies of GG genotype in Fayoumi and RIR chickens were 0.36 and 0.28, respectively; which obviously greater than AA frequencies. Therefore, the allele G was predominant in the populations (0.63 and 0.58, respectively). Chi-test showed that two chicken breeds were not in Hardy-Weinberg equilibrium, in which genotype frequencies had been distorted by recent selection, mutation, or migration.

Association of the BMPR-1B Genotypes with Growth Traits

The least squares means of body weight according to different genotypes of *BMPR-1B* gene in chicken populations were presented in *Table 2*. The A287G SNP of *BMPR-1B* gene is associated significantly with body weight at 2nd (*P*=0.022), 3rd (*p*=0.034), 4th (*P*=0.011), 5th (*P*=0.035), 6th (*P*=0.001), 7th (*P*=0.008) and 8th (*P*=0.016) week of age. Heterozygous genotype AG had a higher body weight than GG genotype over the whole experimental period. The clear significant differences between heterozygous AG genotype and AA genotype were detected ultimately at 7th and 8th week of age.

DISCUSSION

The primary objectives of the current study were to detect single nucleotide polymorphisms (SNPs) of *BMPR-1B* gene in Fayoumi and RIR chicken by PCR-RFLP and sequencing. Growth and body composition are an inclusive reflection of development of various parts of the chicken body and its final expression is the result of interaction among genetic, nutritional and environmental factors [20]. Growth is under complex genetic monitoring, and uncovering the molecular mechanism of growth

results in more efficient selection for growth in broiler chickens [21]. Identifying the QTL will facilitate poultry breeding programs for the economic important traits. Molecular genetic information is required to be used to consolidate genetic improvement of animal species. The candidate gene approach is a very powerful method to examine associations of gene polymorphisms with economically important traits in farm animals [22]. Application of breeding programs that utilize marker-assisted selection requires advances in some areas like detection and estimation of associations of identified genes and their genetic markers with economic traits. Phenotypic evaluation is critical to establish marker-assisted associations or carry out the candidate gene validations required to conduct MAS [23]. Up to now, majority of association studies, especially in chicken, have been performed using phenotypic information.

The A/G transition at the base position of 287 in *BMPR-1B* gene was investigated in two chicken breeds. The allele frequency of *G* was higher than that of *A* in those two breeds and was in the range that reported by Zhang et al.[18] in Zang chickens. While the allele frequency of *A* was higher than that of *G* in three Chinese native chickens, a synthetic broiler line [18] and Mazandaran native chicken [24]. Our results showed that A287G SNP of chicken *BMPR-1B* gene is associated significantly with body weight from the 2nd till the 8th week of age. On the contrary, Zhang et al.[18] and Niknafs et al.[24] recorded non-significant association between *BMPR-1B* gene and growth traits. Zhang et al.[18] stated that A287G SNP of chicken *BMPR-1B* is associated with egg production from 47 to 56 weeks. Previous studies showed that *BMPR-1B* gene as a well known effective gene for reproductive traits [16,25].

Chicken *BMPR-1B* mRNA sequences were first identified by Sumitomo et al.[26] and Lim et al.[27]. Lim et al.[27] reported that BMP signaling, including *BMPR-1B*, is involved in chick diencephalic development, and the expression level of BMPR-1B reduced in the theca of chicken ovary from F1 to F3 follicles. Onagbesan et al.[17] proposed that *BMPR-1B* is possibly concerned in follicular differentiation and maintenance of the follicular hierarchy. Therefore, the expression level or the activity of *BMPR-1B* in the granulosa and/or theca of chicken ovary may be associated with oocyte maturation.

BMPR-1B as a member of the transforming growth factor β (TGF-β) receptor superfamily played the important actions in signal transduction. The current model of induction of signalling responses is at the cell surface, the ligand binds a complex of transmembrane receptor serine/threonine kinases (types I and II) and incites transphosphorylation of the Gly-Ser (GS) segments in the type I receptor by the type II receptor kinases. The consequently activated type I receptors phosphorylate selected Smads at C-terminal serines, and these receptor activated Smads (R-Smads) then form a complex with a common Smad4. Energetic Smad complexes translocate into the nucleus, where they regularize transcription of target genes, through physical interaction, CBP or p300 coactivators and functional cooperation with DNA-binding transcription factors (X) [28].

In conclusion, the broiler chickens have been subjected to intensive breeding with so many objectives that should be simultaneously considered to reduce costs, improve health and product quality. So, several traits such as growth and body composition have been included in selection policies. In addition to difficulty of measurement of these traits, the correlations among them are complex. MAS can be a perfect option to improve selection programs. The results from the current study indicated that a SNP marker in the *BMPR-1B* gene was associated with growth traits in chickens, therefore, a potential marker for molecular MAS programs in chicken. However, the conclusion was only preliminary; it was worth increasing the number of chicken breeds, and expanding the number of samples to make in-depth study.

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Conflict of Interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Ikeobi CON, Woolliams JA, Morrice DR, Law A, Windso D, Burt DW, Hocking PM: Quantitative trait loci affecting fatness in the chicken. *Anim Genet*, 33, 428-435, 2002. DOI: 10.1046/j.1365-2052.2002.00911.x

2. Kim TH, Choi BH, Lee HK, Park HS, Lee HY, Yoon DH, Lee JW, Jeon GJ, Cheong IC, Oh SJ, Han JY: Identification of quantitative traits loci (QTL) affecting growth traits in pigs. *Asian Australas J Anim Sci,* 18, 1524-1528, 2005. DOI: 10.5713/ajas.2005.1524

3. Li H, Deeb N, Zhou H, Mitchell AD, Ashwell CM, Lamont SJ: Chicken quantitative trait loci for growth and body composition associated with transforming growth factor-β genes. *Poult Sci*, 82, 347-356, 2003. DOI: 10.1093/ps/82.3.347

4. Jiang YL, Fan XZ, Lu ZX, Tang H, Xu JQ, Du LX: Detection of 881A→881G mutation in tyrosinase gene and associations with the Black ear Coat Color in rabbits. *Asian-Australas J Anim Sci*, 15, 1395-1397, 2002. DOI: 10.5713/ajas.2002.1395

5. Yan B, Deng X, Fei J, Hu X, Wu C, Li N: Single nucleotide polymorphism analysis in chicken growth hormone gene and its associations with growth and carcass traits. *Chinese Sci Bull*, 48, 1561-1564, 2003. DOI: 10.1007/BF03183961

6. Zhang BZ, Lei MG, Deng CY, Xiong YH, Zuo B, Li FE: Association between PCR-RFLP polymorphism of the fifth intron in lipoprotein lipase gene and productive traits in pig resource family. *Asian Australas J Anim Sci*, 18, 458-462, 2005. DOI: 10.5713/ajas.2005.458

7. Meng H, Zhao JG, Li ZH, Li H: Single nucleotide polymorphism on peroxisome proliferators-activated receptor genes associated with fatness traits in chicken. *Asian-Australas J Anim Sci*, 18, 1221-1225, 2005. DOI: 10.5713/ajas.2005.1221

8. Hossary MA, Galal ESE: Improvement and adaptation of the Fayoumi chickens. *Anim Genet Resour*, 14, 33-39, 1994. DOI: 10.1017/

S1014233900000328

9. Ashraf M, Mahmood S, Ahmed F: Comparative reproductive efficiency and egg characteristics of Lyallpur silver Black and Rhode Island Red breeds of poultry. *Int J Agric Biol*, 5, 449-451, 2003.

10. Kulibaba RA, Podstreshnyi AP: Prolactin and growth hormone gene polymorphisms in chicken lines of Ukrainian selection. *Cytol Genet*, 46, 390-395, 2012.

11. Fang X, Xu H, Zhang C, Chen H, Hu X, Gao X, Gu C, Yue W: Polymorphism in BMP4 gene and its association with growth traits in goats. *Mol Biol Rep*, 36, 1339-1344, 2009. DOI: 10.1007/s11033-008-9317-1

12. Monget P, Fabre S, Mulsant P, Lecerf F, Elsen JM, Mazerbourg S, Pisselet C, Monniaux D: Regulation of ovarian folliculogenesis by IGF and BMP system in domestic animals. *Domest Anim Endocrinol,* 23, 139-154, 2002. DOI: 10.1016/S0739-7240(02)00152-2

13. Nishii N, Arai M, Yanai N, Togari A, Nakabayashi T: Effect of bone morphogenetic protein-2 (BMP-2) or troglitazone, as an inducer of osteogenic cells or adipocytes, on differentiation of a bone marrow mesenchymal progenitor cell line established from temperature-sensitive (ts) simian virus (SV) 40 T-antigen gene transgenic mice. *Biol Pharm Bull*, 32,10-17, 2009.

14. Seol YJ, Kim KH, Park YJ, Lee YM, Ku Y, Rhyu IC, Lee SJ, Han SB, Chung CP: Osteogenic effects of bone-morphogenetic-protein-2 plasmid gene transfer. *Biotechnol Appl Biochem*, 49, 85-96, 2008. DOI: 10.1042/BA20060247

15. Monika M, Krzysztof M, Daniel L, Anna S, Agnieszka Ś: Static magnetic field enhances synthesis and secretion of membrane-derived microvesicles (MVs) rich in VEGF and BMP-2 in equine adipose-derived stromal cells (EqASCs) - A new approach in veterinary regenerative medicine. *In Vitro Cell Dev Biol Anim*, 51, 230-240, 2015. DOI: 10.1007/s11626-014-9828-0

16. Souza CJH, MacDougall C, Campbell BK, McNeilly AS, Baird DT: The Booroola (FecB) phenotype is associated with a mutation in the bone morphogenetic receptor type 1B (BMPR1B) gene. *J Endocrinol*, 169, R1-R6, 2001.

17. Onagbesan OM, Bruggeman V, Van As P, Tona K, Williams J, Decuypere E: BMPs and BMPRs in chicken ovary and effects of BMP-4 and -7 on granulosa cell proliferation and progesterone production *in vitro*. *Am J Physiol Endocrinol Metabol*, 285, 973-83, 2003.

18. Zhang NB, Tang H, Kang L, Ma YH, Cao DG, Lu Y, Hou M, Jiang YL: Associations of single nucleotide polymorphisms in BMPR-1B gene with egg production in a synthetic broiler line. *Asian Australas J Anim Sci*, 21, 628-632, 2008.

19. SAS Institute: SAS/STAT users guide. SAS Institute Inc., Cary, NC, USA. 2003.

20. Scanes CG, Harvey S, Marsh JA, King DB: Hormones and growth in poultry. *Poult Sci*, 63, 2062-2074, 1984. DOI: 10.3382/ps.0632062

21. Deeb N, Lamont SJ: Genetic architecture of growth and body composition in unique chicken population. *J Hered*, 93, 107-118, 2002. DOI: 10.1093/jhered/93.2.107

22. Rothschild MF, Soller M: Candidate gene analysis to detect genes controlling traits of economic importance in domestic livestock. *Prob Newslet Agric Genom*, 8, 13-20, 1997.

23. Guimarães EP, Ruane J, Scherf BD, Sonnino A, Dargie JD: Marker-Assisted Selection. Food and Agricultural Organization of the United Nations, Rome, Italy, 427-440, 2007.

24. Niknafs S, Javaremi AN, Sadeghi M: Single nucleotide polymorphisms in BMPR-1B and STAT5B genes and their association with growth and reproductive traits in chicken. *Songklanakarin J Sci Technol*, 36, 137-142, 2014.

25. Wilson T, Wu XY, Juengel JL, Ross IK, Lumsden JM, Lord EA, Dodds KG, Walling GA, McEwan JC, O'Connell AR, McNatty KP, Montgomery GW: Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. *Biol Reprod*, 64, 1225-1235, 2001.

26. Sumitomo S, Saito T, Nohno T: A new receptor protein kinase from chick embryo related to type II receptor for TGF-beta. *DNA Seq*, 3, 297-302, 1993.

27. Lim Y, Cho G, Minarcik J, Golden J: Altered BMP signaling disrupts chick diencephalic development. *Mech Dev*, 122, 603-620, 2005. DOI: 10.1016/j.mod.2004.12.001

28. Derynck R, Zhang YE: Smad-dependent and Smad independent pathways in TGF-beta family signaling. *Nature*, 425, 577-584, 2003. DOI: 10.1038/nature02006

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Research Article

Study of Vaccinal Properties of *Clostridium chauvoei* Strains Isolated During a Blackleg Outbreak in Cattle in Algeria

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Abstract

The aim of this study was to investigate the vaccine properties of three Algerian strains of *Clostridium chauvoei*. Batch culture in laboratory fermenter was performed on CAM medium and Vaccine was obtained by formalin inactivation of whole stationary-phase culture. *In vitro* and *in vivo* toxicity of strains were evaluated, respectively, by the hemolytic activity and lethal effect on mice and guinea pigs of the culture supernatant. Pathogenicity of strains was assessed by injection of a culture to guinea pigs and mice. The passive protection assay was performed on mice inoculated with anti-serum and challenged with a virulent strain. Vaccine potency was tested on guinea pigs inoculated with vaccine and challenged with a virulent strain. The study of characteristics of growth distinguished the strain ALG2 from other strains by its greater growth rate (0.85 h^{-1}) and structural integrity. Pathogenicity and toxicity were observed for ALG2, while pathogenicity was lower and *in vivo* toxicity was absent in other strains. Passive protection tests showed broader protective effect of immune sera from strain ALG2. Active protection testing with the vaccine prepared from strain ALG2 showed that all vaccinated guinea pigs challenged with five wild strains have survived. This study highlighted the immunogenicity and protective capacity of *C. chauvoei* strain ALG2 isolated in Algeria, which may be a good candidate for vaccine production.

Keywords: *Clostridium chauvoei, Blackleg, Local strain, Vaccine, Immunoprotection*

Cezayir'de Sığırlarda Yanıkara Salgınında İzole Edilen *Clostridium chauvoei* Suşlarının Aşısal Özelliklerinin Araştırılması

Özet

Bu çalışmanın amacı üç farklı *Clostridium chauvoei* Cezayir suşunun aşı özelliklerini araştırmaktır. Laboratuvar fermenterinde bir parça kültürü CAM medyumda yapıldı. Durağan-faz kültürün tümü formalinde inaktive edilere aşı üretildi. Suşların *in vitro* ve *in vivo* toksisiteleri, kültür spernatantlarının fare ve kobaylarda hemolitik aktivite ve letal etkileri ile değerlendirildi. Suşların patojeniteleri kültürün kobay ve farelere enjeksiyonu ile değerlendirildi. Pasif koruma deneyi anti-serum inoküle edilen ve virulent suş uygulanan fareler üzerinde yapıldı. Aşı kapasitesi, aşı inoküle edilen ve virulent suş uygulanan kobaylarda test edildi. ALG2 suşu daha büyük büyüme oranı (0.85 h^{-1}) ve yapısal özellikleri ile diğer suşlardan ayırt edildi. ALG2 için patojenite ve toksisite gözlemlendi. Diğer suşlar için patojenite daha düşük ve *in vivo* toksisite yoktu. Pasif koruma testleri ALG2 den elde edilen immun serum için daha geniş koruyucu etki gösterdi. ALG2 suşundan hazırlanan aşı ile yapılan aktif koruma testi, 5 saha suşu ile muamele edilen aşılı kobayların hayatta kaldıklarını gösterdi. Bu çalışma Cezayir'de izole edilen *C. chauvoei* ALG2 suşunun immunojenik ve koruyucu kapasitelerini incelemiş ve aşı üretimi için uygun bir aday olabileceğini göstermiştir.

Anahtar sözcükler: *Clostridium chauvoei, Yanıkara, Lokal suş, Aşı, İmmunkoruma*

INTRODUCTION

Blackleg is an acute disease that mainly affects young cattle and sheep, characterized by myositis and toxemia, and is often fatal. *Clostridium chauvoei*, the causative agent of the disease, is a Gram positive, rode shaped, strict anaerobic, gas producing and endospore forming bacterium. The spores have high toughness, they can survive in the soil and food for many years, and they contaminate animals if swallowed. The geographical distribution of blackleg is global, but with regional concentrations. This disease is common in Algeria and responsible for economic losses in livestock. Because of the acute nature of the disease, treatment is not always effective. The most appropriate

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measure of control of this disease is vaccination; the formalin-killed whole bacterial culture is the commonly used [1]. The complex antigenic composition of *C. chauvoei* may be responsible for the variability in the protective effect of the vaccine according to strains used; some strains have a broader spectrum of protection than others [2]. Therefore, to ensure maximum effectiveness of a vaccine, it is recommended to make it from local strains [2]. The objective of this study was to test the vaccine properties of three Algerian local strains of *C. chauvoei*.

MATERIAL and METHODS

Bacterial Strains

Five strains of *Clostridium chauvoei* were used in this study, a reference vaccine strain CCM5735, (Harshey, Veterinary Research Institute, BRNO, Czechoslovakia), an Iranian strain C.IR and three local strains, ALG1, ALG2 and ALG3, isolated from calf carcasses in 1993 during a blackleg epidemic in the region of Setif (Algeria). The local strains were identified based on cultural and morphological characteristics, biochemical profiles using classic tests [3], API 20A and Rapid ID 32A strips (BioMérieux, Marcy l'Etoile, France), immunofluorescence [4] and PCR-restriction fragment length polymorphisms of 16S ribosomal RNA gene [5,6].

Strains have been stored in a lyophilized form in sealed vials and isolated pure cultures were maintained in liquid TGY medium in bottle of 50 mL at 4°C.

The strains were also propagated *in vivo* using guinea pigs (SPF, Hartley, Charles River, France) of 250 g to 300 g. A volume of 1 mL of a culture of 18 h on TGY medium was injected intramuscularly and the state of the animal was monitored for 48 hours. Blood samples from the heart, liver, leg muscle and sero-fibrinous exudate at the inoculation site were used to seed the TGY medium. After incubation, microscopic examination, isolation on Columbia Blood Agar, and pathogenicity and toxicity tests were performed.

Medium and Culture Conditions

Strains of *C. chauvoei* were cultivated under standard anaerobic conditions at 37°C on Columbia agar with 5% sheep blood (GBA), and in Trypticase Glucose Yeast (TGY) and CAM [7] liquid media.

Fermentation for vaccine production was carried out on CAM medium. First, a preculture of the strain was carried out with the same medium. A vial of lyophilized strain was inoculated into a fresh tube of TGY medium and incubated 18 h at 37°C. Then the TGY culture was inoculated into 750 mL of CAM medium in 1 liter capacity glass bottle and incubated at 37°C for 18 h. The anaerobic atmosphere was maintained by introducing nitrogen gas through a sterilizing filter. Controls were carried out at t=0 and t = 18 h.

Batch culture in laboratory fermenter was performed on 7 L of CAM medium inoculated with the preculture corresponding to 10% of total volume of the medium, at 37°C, pH 7.2, agitation of 100 rpm and with the introduction of nitrogen and antifoam agent (if necessary). Samples were taken at stationary phase and at 24 h, for the controls.

The checks carried out were as follows:

- *Morphology:* direct microscopic examination of cultures and after Gram staining.
- *Microbiological purity:* isolation on GBA and incubation in aerobic and anaerobic conditions.
- *Antigenic specificity:* slide agglutination reaction with *Clostridium chauvoei* anti-serum.
- *Microbial growth:* by measuring the OD at 650 nm and direct enumeration via culture on GBA and Thomas cell method.
- *Pathogenicity testing*
- *Toxicity testing in vitro and in vivo*

Pathogenicity and Toxicity Testing

The *in vitro* toxicity of strains was estimated by the hemolytic activity of the supernatant of a culture at concentration of 2 to $4x10^9$ organisms mL^{-1} [8]. The supernatant was recovered by centrifugation at 6,500 × g for 20 min at 4°C, filtered on 0.22 µm pore size Millipore and successively diluted by a factor of 2. A volume of 0.5 mL of each dilution was added to 0.5 mL of 2% sheep erythrocytes suspension in physiological saline, and then incubated 10 min at 70°C or 2 h at 37°C. Hemolysin titer was the highest dilution that produces 50% hemolysis. The 50% hemolysis was valued through a control consisting of the mixing of 0.25 mL of 2% sheep erythrocytes suspension (0% hemolysis), 0.25 mL of 2% sheep erythrocytes completely hemolyzed (100% hemolysis) and 0.5 mL of physiological saline.

The *in vivo* toxicity of the strains was evaluated by the lethal effect of the filtrate of the culture supernatant administered intravenously to five Balb/c mice and one guinea pig. The animals were observed for 3 days.

Pathogenicity of strains was assessed by intramuscular injection of a culture alone or added with $CaCl_2.2H_2O$ (final concentration of 0.225 M) to 2 guinea pigs (250 g to 300 g) at a dose of 1 mL and to 5 mice (18 g to 20 g) at a dose of 0.5 mL [9,10]. The controls were the culture medium alone and added with $CaCl_2.2H_2O$.

Anti-O and anti-H Immune Sera Preparation

O and H antigenic suspensions were prepared, respectively, by heating to 100°C for 1 h and by treatment with formalin (0.6%) [11] of an 18 h culture on TGY at concentration of $5x10^8$ bacteria mL^{-1}. After centrifugation and washing, the pellet was resuspended in physiological

saline. The immunization was performed on two a New Zealand albino rabbits weighing 2.5 to 3 kg (Pasteur Institute of Algeria) for each suspension according to the method of Micalizzi and de Guzman [12]. The anti-H serum was adsorbed with the antigen suspension O. The sera were stored at -20°C. Titrations of sera were performed by an agglutination test tube with dilutions from 1/10 to 1/1280.

Assessment of Passive Protection by Anti-H and Anti-O Immune Sera

The passive protection assay was performed according to the method of Micalizzi and de Guzman [12]. Mice in groups of 5 were inoculated intraperitoneally with 0.5 mL of anti-serum and of non-immunized rabbit serum. After 6 h, the mice were challenged by IM way with 0.5 mL of a culture of 18 h on TGY of a virulent strain. The animals were observed for 5 days, the survivors are considered fully protected.

A non-immunized control group of mice was directly inoculated with virulent strain, death must occur within 30 h.

Vaccine Formulation

Pure stationary-phase culture [13] was inactivated with formalin (0.6% final concentration) at 37°C for 14 days with stabilization of pH at 7 and frequent agitation. The adjuvant, aluminium hydroxide gel ($Al(OH)_3$), was added at the concentration of 6 mg mL^{-1} and leaving the adsorption for few hours at temperature of 12°C to 20°C with slow shaking. Then the vaccine was kept four days at 4°C to settle organisms. The vaccine composition was then as follows: *Clostridium chauvoei* anaculture, adjuvant and formaldehyde.

Sterility testing was performed by seeding GBA incubated for 48 h and TGY, Thioglycolate, nutrient broth, trypticase soja and Sabouraud media incubated for 14 days.

According to British Pharmacopoeia [14], abnormal toxicity was tested by inoculating 2 guinea pigs and 5 mice monitored for 7 days. No abnormal local or systemic reaction occurs during the test. The pH was also controlled as well as free formaldehyde and Al^{3+}.

Assessment of Vaccine Active Protection

Vaccine potency was tested according to the method of Mhoma [15]. The test was performed on 6 white guinea pigs weighing 250 to 300 g, of which 5 were inoculated subcutaneously with 2 mL of vaccine and the sixth control guinea pig was not vaccinated. A re-inoculation was made 21 days after. Ten days after the second dose, the animals were challenged with 1 mL of a virulent strain culture of 18 h. The animals were observed for 10 days.

All experiments were performed on triplicate, values given are averages.

Animal experiments were conducted in Animals Unit of Bacterial Vaccine Laboratory with the approval of authorities of Pasteur Institute of Algeria (reference: 02/ DLRD/IPA).

RESULTS

Strains cultured in batch on the CAM medium showed growth in rates of 0.85 h^{-1}, 0.84 h^{-1}, 0.5 h^{-1} and 0.4 h^{-1} for ALG2, CCM5735, ALG1 and ALG3, respectively. The stationary phase was reached at about 10 h for ALG2 and CCM5735 and at about 15 h for ALG1 and ALG3. Morphologically, the strain ALG2 had spindle, citron and rod shapes and CCM5735 strain showed spindle and rod shapes, with sporulation. The ALG1 strains and ALG3 had irregular shapes. The antigenic structure was uniformly maintained as indicated by the agglutination titer of 640 of a specific anti-serum anti-*C. chauvoei* observed for all strains. Mobility was very important for ALG2, medium for CCM5735 and absent in ALG1 and ALG3. Pathogenicity was present for all strains; however it was more pronounced for ALG2, followed by CCM5735 and ALG1, and then ALG3. *In vivo* toxicity was observed for ALG2 while it was absent for other strains. *In vitro* toxicity was present for all strains with a Minimal Hemolytic $Dose_{50}$ of >522 *(Table 1)*. The evaluation of titers of anti-H and anti-O immune sera prepared from strains ALG2 and CCM5735 gave a titer of 1280 for anti-H whatever the strain test, whereas anti-O had a titer of 1280 with homologous strain and 160 with heterologous strain *(Table 2)*. Passive protection of mice with immune sera anti-O and anti-H showed that anti-H prepared with ALG2 protected against ALG2, CCM5735 and ALG1 (5 survivors/5), while anti-H obtained from CCM 5735 partially protected against ALG2 and ALG1 (4 survivors/5). Anti-O from ALG2 fully protected against ALG1 and ALG2 and partially against CCM5735 (3 survivors/5). Anti-O from CCM5735 completely protected against CCM5735, very weakly against ALG1 (1 survivors/5) and not against ALG2 (0 survivors/5) *(Table 3)*. Full protection was observed in animals (5 survivors/5) against four strains (ALG2, CCM5735, ALG1 and C.IR) in guinea pigs vaccinated with the vaccine made from ALG2 strain *(Table 4)*.

DISCUSSION

The comparative study of characteristics of growth, micromorphology, mobility, antigenicity, pathogenicity and toxicity of strains distinguished the strain ALG2 from the other local strains ALG1 and ALG3. Indeed, ALG2 had a greater growth rate (0.85 h^{-1}), equivalent to that of reference strain CCM57335 and the stationary phase was reached after 10 h of growth for the two strains. The cell structural integrity, appraised by cell shape, antigenicity and mobility, was conserved for ALG2 and CCM5735 after 10 h and 24 h of growth; while a pleomorphism and lack of mobility were observed for ALG1 and ALG3. The occurrence

Table 1. *Characteristics of growth, morphology, antigenicity , mobility, pathogenicity and toxicity of C. chauvoei strains*
Tablo 1. *C. chauvoei suşlarının büyüme, morfoloji, antijenik, hareketlilik, patojenite ve toksisite özellikleri*

Strain	Growth Rate	Bacterial Structural Integrity			Pathogenicity	Toxicity	
		Morphology	Antigenicity (Slide Agglutination Titer)	Mobility		*In-vivo*	*In-vitro*
CCM 5735	0.84 h^{-1}	T=10 h : spindles, rods T=24 h : sporulated-spindles, rods	+ ++ (640)	+	++ (4 dead mice/5)	-	+ (Minimal Hemolytic Dose$_{50}$: >522)
ALG1	0. 5 h^{-1}	T=15 h and 24 h : irregular shapes	+++ (640)	-	++ (4/5)	-	+ (MHD$_{50}$ >522)
ALG2	0.85 h^{-1}	T=10 h spindles, citrons , rods T=24 h: sporulated-spindles and citrons, some rods	+++ (640)	++	+++ (5/5)	+	+ (MHD$_{50}$ >522)
ALG3	0.4h^{-1}	T=15 h and 24 h : irregular shapes	+++ (640)	-	+ (2/5)	-	+ (MHD$_{50}$ >522)

Table 2. *Titers of anti-H and anti-O immune-sera made from ALG2 and CCM5735 strains*
Tablo 2. *ALG2 ve CCM5735 suşlarından üretilen anti-H ve anti-O immune-sera titreleri*

Strain Test	Immune-Serum Titer			
	ALG2 Strain		CCM 5735 Strain	
	Anti-O	Anti-H	Anti-O	Anti-H
ALG2	1280	1280	160	1280
CCM5735	160	1280	1280	1280

Table 3. *Passive protection conferred by O and H antisera obtained with ALG2 and CCM5735 strains*
Tablo 3. *ALG2 ve CCM5735 suşları ile sağlanan O ve H antisera ile elde edilen pasif koruma*

Immune Serum	Number of Surviving Challenged Mice		
	ALG2	CCM 5735	ALG1
Anti-O from ALG2	5 /5	3/5	5/5
Anti-H from ALG2	5/5	5/5	5/5
Anti-O from CCM 5735	0/5	5/5	1/5
Anti-H from CCM 5735	4/5	5/5	4/5
Control serum from non immunized rabbit	0/5	0/5	1/5
Control: non-immunized mice challenged with virulent strain	0/5	0/5	0/5

Table 4. Active protection by vaccine from local strain ALG2
Tablo 4. Lokal suş ALG2'den üretilen aşı tarafından sağlanan aktif koruma

Challenge Virulent Strains	Responses of Guinea-Pigs to Challenge	
	Guinea-Pigs Vaccinated by ALG2 Strain	Guinea Pigs not Vaccinated
ALG_2	5 survivors/5	1 dead/1
ALG_1	5 survivors/5	1 dead/1
C.IR	5 survivors/5	1 dead/1
CCM 5735	5 survivors/5	1 dead/1

of non-motile variants is common in *C. chauvoei* [16].

Pathogenicity and toxicity were observed for ALG2, while pathogenicity was lower and *in vivo* toxicity was absent in other strains. The virulence character is variable in *C. chauvoei*. The ability to produce toxins varies greatly in *C. chauvoei* strains [17] and *in vivo* toxicity can be absent, despite the detection of the hemolytic activity in the filtrate of the culture supernatant [11,18]. Because of the importance of cell structural integrity, fitness and virulence in the immunogenicity and protective efficacy of a strain [11-13,19], strain ALG2 was selected for the formulation of a vaccine.

It is known that the protection is mainly provided by the structural antigens of bacteria, parietal somatic antigens O and mostly the flagellar antigens [20]. Indeed, flagella have been described as associated with full expression of virulence and immunoprotection against blackleg [16,21,22]. However, studies have reported the role of exotoxins and exo-enzymes in virulence and immunoprotection, hence the interest of introduce the culture supernatant in vaccine formulation [23].

In order to know the protective capacity of O and H antigens, anti-O and anti-H immune-sera were prepared from ALG2 strain and reference strain CCM5735.The results showed the same titers for anti-H and anti-O sera (1/1280) for the two strains. However, the titers of the anti-O varied according to the test strain, they were reduced to 1/160 when testing with heterologous strain; while the titers of anti H remained (1/1280) whatever the test strain. This result suggests a greater uniformity of H antigens among strains, unlike O antigens seems more variable, this finding is not consistent with results reported by Chandler and Gulasekharam [11]. Passive protection tests, performed on mice challenged with ALG2 or CCM5735, showed a protective capacity of both O and H antigens; however protection is more pronounced with H. Moreover, we noted the broader protective effect of immune sera from the strain ALG2 against both the strain ALG2 itself and the reference strain CCM5735.

Active protection testing with the vaccine prepared from the ALG2 strain showed that all vaccinated guinea pigs challenged with 4 wild strains ALG1, ALG2, CCM5735 and C.IR were survived; in contrast to unvaccinated animal controls that were died. These results highlighted the high and broader protective capacity of ALG2 strain. Because of the high variability of the protective capacity of vaccines according to strains used in vaccine formulation and the enzootic nature of blackleg, it is appropriate to use local strains in vaccine production; they have greater protector effect [12,13,20,24]. The observation is that the level of protection is high when the vaccine strain is homologous to local field strains [25].

We report in this study the immunogenic and protective capacity against blackleg disease of a strain of *C. chauvoei* ALG2 isolated in Algeria, it would be a good candidate for vaccine production. Assessment of vaccine potency should be performed in cattle.

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REFERENCES

1. Misra RP: Manual for the production of anthrax and blackleg vaccines. FAO animal production and health paper 87. Food and Agriculture Organization of the United Nations, Rome, Italy, 1991. http://www.fao.org/docrep/004/t0278e/t0278e00.htm

2. Araujo RF, Curci VCLM, Nobrega FLC, Ferreira RMM, Dutra IS: Vaccination protocol and bacterial strain affect the serological response of beef calves against blackleg. *Pesq Vet Bras,* 30, 554-558, 2010. DOI : 10.1590/S0100-736X2010000700008

3. Brefort G, Sebald M: Recherche et identification des anaérobies en bactériologie courante. *Rev Prat*, 27, 107-118, 1977 *(article in French)*.

4. Walker PD: The differentiation of *Clostridium septicum* and *Clostridium chauvoei* by the use of fluorescent labeled antibodies. *J Path Bact*, 85, 517-521, 1963.

5. Gacem F, Daube G, Bakour R: Molecular typing of *Clostridium chauvoei.* **In,** *ASM First International Conference on the Molecular Genetics and Pathogenesis of the Clostridia*. Arizona, USA, p.23, 1994.

6. Kuhnert P, Krampe M, Capaul SE, Frey J, Nicolet J: Identification of *Clostridium Chauvoei* in cultures and clinical material from blackleg using PCR. *Vet Microbiol*, 51, 291-298, 1997. DOI: 10.1016/S0378-1135(97)00129-6

7. Cameron CM, Botha WJS, Schoeman JH: Immunization of guinea pigs and cattle with a reduced dose *Clostridium chauvoei* vaccine produced in semi synthetic medium. *Onderstepoort J Vet Res*, 53, 51-53, 1986.

8. Tamura Y, Kijima M, Hamamoto K, Yoshimura H: Partial characterization of the hemolysin produced by *Clostridium chauvoei*. *J Vet Med Sci,* 54, 777-778, 1992.

9. Joubert L, Valette L: Les échecs de la vaccination anti-symptomatique et les méthodes de contrôle d'activité des vaccins. *Dev Biol Stand*, 32, 151-155, 1976 *(article in French)*.

10. Knight PA, Kent J: Some problems concerning the assay of *Clostridium chauvoei* vaccine in laboratory animals. *Dev Biol Stand*, 32, 143-150, 1976.

11. Chandler HM, Gulasekharam J: An evaluation of characteristics of *Clostridium chauvoei* which possibly indicate a highly protective strain. *Aust J Exp Biol Med Sci,* 48, 187-197, 1970. DOI: 10.1038/icb.1970.18

12. Micalizzi B, Stefanini de Guzman AM: Protective activity of different immunosera against *Clostridium chauvoei*. *Anaerobe*, 3, 127-129, 1997. DOI: 10.1006/anae.1997.0088

13. Mattar MA, Cortinas TA, Stefanini de Guzman AM: Immunogenic protein variations of *Clostridium chauvoei* cellular antigens associated with the culture growth phase. *FEMS Immunol Med Microbiol*, 33, 9-14, 2002. DOI: 10.1111/j.1574-695X.2002.tb00565.x

14. British Pharmacopoeia (Veterinary): V5 (appendix XIV E): 1; 2013.

15. Mhoma JRL: Observation on the influence of media composition on the efficacy of vaccines derived from some *Clostridium chauvoei* strain. *Bull Anim Hlth Prod Afr*, 33, 117-121, 1985.

16. Tamura Y, Kijima-Tanaka M, Aoki A, Ogikubo Y, Takahashi T: Reversible expression of motility and flagella in *Clostridium chauvoei* and their relationship to virulence. *Microbiology*, 141, 605-610, 1995. DOI: 10.1099/13500872-141-3-605

17. Cortinas TI, Mattar MA, de Guzman AMS: Alpha and beta toxin activities in local strains of *Clostridium chauvoei*. *Anaerobe*, 5, 297-299, 1999. DOI: 10.1006/anae.1999.0211

18. Frey J, Falquet L: Patho-genetics of *Clostridium chauvoei*. *Res Microbiol*, 2014. DOI: 10.1016/j.resmic.2014.10.013

19. Chandler HM, Gulasekharam J: The protective antigen of a highly immunogenic strain of *Clostridium chauvoei* including an evaluation of its flagella as a protective antigen. *J Gen Microbiol*, 84, 128-134, 1974. DOI: 10.1099/00221287-84-1-128

20. Tamura Y, Nimamoto N, Tanaka S: Demonstration of protective antigen carried by *Clostridium chauvoei*. *Microbiol Immunol*, 43, 612-616, 1984. DOI: 10.1111/j.1348-0421.1984.tb00790.x

21. Tanaka M, Harayaman N, Tamura Y: Production, characterization and protective effect of monoclonal antibodies to *Clostridium chauvoei* flagella. *Infect Immunol*, 5, 1779-1783, 1987.

22. Kojima A: Studies on gene encoding the flagellin of *Clostridium chauvoei* and diagnostic application for blackleg disease. Annual Report of the National Veterinary Assay Laboratory (NVAL), Tokyo, No 43, pp.9-17, 2006.

23. Mattar MA, Cortinas TI, Stefanini AM: Extracellular proteins of *Clostridium chauvoei* are protective in a mouse model. *Acta Vet Hung*, 55, 159-170, 2007. DOI: 10.1556/AVet.55.2007.2.1

24. Chandler HM, Hamilton RC: The preparation of immunogenic cell walls from a highly protective strain of *Clostridium chauvoei*. *J Gen Microbiol*, 88, 27-35, 1975. DOI: 10.1099/00221287-88-1-27

25. Schipper IA, Kelling CL, Mayer J, Pfeiffer NW: Effects of passive immunity on immune response in calves vaccinated against *Clostridium chauvoei* infection (blackleg). *Vet Med Small Anim Clin*, 73, 1564-1566, 1978.

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Research Article

The Effect of Xylazine HCl Used in Repeated Sedations for Sheep on Biochemical and Clinical Values [1]

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Abstract

The objective of the study is to determine the sedative effects of xylazine HCl administered to sheep in repeated fixed doses clinically and biochemically. Five Akkaraman breed rams identified to be clinically healthy were used in the study. Xylazine HCl was administered 4 times at a dose of 0.4 mg/kg intramuscular (fixed dose) with 4 day intervals to induce sedation. Rectal temperature, heart rate and respiratory rate along with rumen motility of all animals were evaluated before and after the administration. The depths of sedation and analgesia, control of reflexes along with various biochemical parameters were studied. The results showed that xylazine HCl led to change of less physiological parameters in the following administration in comparison with the first application. Whereas only moderate and deep sedation were observed in the first application on sheep; mild, moderate and deep sedation were observed in all remaining administration. The degree of analgesic effect was 0 during mild and moderate sedation periods whereas it was 1-2 during deep sedation period. It was concluded that repeated sedations of xylazine HCl administration reduce the degree of sedation in third and fourth administration in sheep.

Keywords: *Sheep, Xylazine HCl, Repeated sedation*

Koyunlarda Tekrarlanan Sedasyonlarda Kullanılan Xylazinin Biyokimyasal ve Klinik Değerlere Etkisi

Özet

Bu çalışmada ksilazinin tekrarlayan dozlarda klinik ve biyokimyasal açıdan sedatif etkilerini araştırmak amaçlanmıştır. Çalışmada klinik olarak sağlıklı olduğu belirlenen 5 adet yetişkin Akkaraman ırkı koç kullanıldı. Sedasyon oluşturmak amacıyla her uygulamada xylazin HCl 0.4 mg/kg dozunda kas içi olarak (sabit doz) 4 gün arayla 4 uygulama yapıldı. Uygulama öncesi ve uygulama sonrası rektal vücut sıcaklığı, nabız ve solunum sayısı, rumen hareketleri, takip edildi. Ayrıca sedasyon ve analjezinin derinliği, reflekslerin kontrolü ile biyokimyasal bazı parametreler araştırıldı. İlk uygulamaya göre tekrarlayan dozlarda ksilazin HCl fizyolojik parametrelerde daha az değişikliğe neden oldu. İlk ugulamada sadece orta ve ileri derecede sedasyon şekillenirken, sonraki uygulamalarda hafif, orta ve ileri derecede sedasyon şekillendi. Hafif ve orta derece sedasyonda analjezinin derecesi 0 iken, derin sedasyonda 1-2 olarak saptandı. Sonuç olarak koyunlarda tekrarlayan sedasyonlarda verilen ksilazin HCl'nin üçüncü ve dördüncü uygulamalarında sedasyonun derecesini azalttığı sonucuna varıldı.

Anahtar sözcükler: *Koyun, Ksilazin HCl, Tekrarlayan sedasyon*

INTRODUCTION

Sedation is required for the examination (endoscopy) of various surgical procedures (castration, cesarean section e.g.) in sheep and therefore α-2 adrenoreceptor agonists are used [1-4]. α-2 adrenoreceptor agonists cause respiratory distress, hypercapnia and hypoxemia. These effects may prolong the duration of sedation [5-9]. In addition they increase the amount of urine and cause hyperglycemia and hypoinsulinemia [7,10].

Xylazine HCl is the most preferred drug among α-2 adrenoreceptor agonists and can be administered intravenously, intramuscularly and subcutaneously [8,11,12]. Xylazine HCl has analgesic effects in addition to its sedative and myorelaxant effects. However, it has been stated that xylazine HCl has a different analgesic effect among sheep species [5,9,13]. The dose of the α-2 agonists and the temperament of the animal affect the degree of sedation [9]. It is also stated that increasing drug dose results in a sedation time increase whereas no change is observed in sedation depth [14].

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Pulse rate, respiratory rate, rumen motility and rectal temperature changes are observed in animals to which xylazine HCl is administered [10,11,15-17]. Various cases have been put forth especially for sheep in which clinical findings of pulmonary edema have been observed and which have ended in death [6,8,9,18].

A literature survey was carried out as a result of which no study was found in which the clinical, biochemical and sedative effects of repeated doses of xylazine HCl on sheep have been examined. The objective of our study is to determine the sedative effects of the repeated sedations of xylazine HCl from a clinical and biochemical perspective.

MATERIALS and METHODS

Animals

Five clinically healthy Akkaraman breed rams at the Yüzüncü Yıl University Research and Application Farm were used in the study. The live weights of the animals varied between 40-55 kg and their ages were between 20-24 months. The animals were subject to the same feeding program throughout the duration of the study. Consent of the Ethics Council of the University of Yüzüncü Yıl (2011/08) was taken prior to the start of the study.

Sedation Applications

The animals were not fed starting from 12 h prior to the study. Xylazine HCl (Rompun 2%, Bayer) was administered to induce sedation at a dose of 0.4 mg/kg intramuscularly (fixed dose) for 4 times with 4 day intervals.

Clinical Evaluation

Rectal temperature, pulse rate, respiration rate, pulmonary sounds and rumen activity were evaluated at times of 0 (before xylazine HCl administration -baseline) 5, 15, 30, 45, 60, 75, 90, 105 and 120 min following xylazine HCl administration. Rectal temperature was measured using a digital thermometer. Pulmonary sounds, rumen activities, pulse rate and respiration rate were measured using a stethescope. In addition, it was monitored whether oro-nasal discharge and urination started after administering xylazine HCl.

Evaluation of Reflexes

Eye movements, palpebral, corneal, pupillary, patellar and triceps brachii reflexes were controlled in all groups at times of 0 (before xylazine HCl administration-baseline) 5, 15, 30, 45, 60, 75, 90, 105 and 120 min following xylazine HCl administration.

Evaluation of Sedation

Sedation scoring was made by modifying the evaluations of Kastner et al.[19], as follows:

No sedation (before sedation-baseline): The movements, activity and standing posture of the animal are normal. The movements of the ears, neck positions and movements of the eyelids are at their physiological values.

Mild sedation: Decrease of the movements and interest of the animal towards its environment, sagging of the ear, bending of the head, uncoordinared movements of the animal while walking but not attaining a laying posture were accepted as symptoms of mild sedation.

Moderate sedation: Lying down of the animal in the sterno-abdominal position following mild sedation findings was accepted as moderate sedation.

Deep sedation: Direct lateral laying down of the animal following moderate or mild sedation conditions along with the head and extremities not moving were accepted as deep sedation.

Ending sedation: The passing of a deeply sedated animal to a sterno-abdominal or standing posture are accepted as the end of this period. Whereas this period was accepted to have ended when a moderately sedated animal attained a standing posture. Mild sedation period was accepted to have ended when the physiological behavior of a mildly sedated animal returned back to normal. Therefore, behavior of the animals was evaluted at 5 min intervals.

Evaluation of the Analgesic Effect

Analgesia was evaluated before drug administration (baseline) and at 5 min interval after drug administration until complete recovery. Onset and duration of analgesia was evaluated by applying painful stimuli with 23-gauge needle inserted through the skin and the underlying tissues in different parts of the body (perineal, left front and hind limbs interdigital and corona regions). All animal procedures were performed in the same points. Analgesic effect were measured by a single investigator throughout the experiment.

Analgesic assessment score was modified according to the evaluation of Khan et al.[11]. The grading made for no analgesic effect (normal strong reaction to painful stimuli) was 0 score, for mild analgesic effect (depressed reaction to painful stimulus) it was 1-2 score, for moderate analgesic effect (no response to needle-prick stimulation of the skin but there are response to needle-prick stimulation of the skin underlying tissue) it was 3-4 score and deep analgesic effect (no response to insertion of the needle deep into skin underlying tissue- no leg movement or contraction) was graded as 5-6 score.

Biochemical Evaluation

Blood samples were taken from jugular vein of all animals in all administrations prior to sedation, during

sedation (on the 45th min after xylazine HCl administration) and after sedation (on the 120th min after xylazine HCl administration). The obtained blood samples were centrifuged at 5.000 rpm to remove the serums which were then frozen at -18°C and were stored until the day of the analyses. The serums were then thawed after which their glucose, total bilirubin, BUN, AST, ALT, ALP, Ca, Na, K and Cl values were measured using an analyzer (Roche - Hitachi, Germany).

Statistical Evaluation

The comparison of the numerical values obtained during the applications was carried out via SPSS (Statistical Package for the Social Sciences Program) for Windows, ver. 21.0 statistical package software using Student-t test. A value of P<0.05 was considered as statistically significant.

RESULTS

Clinical Evalation Results

Oro-nasal discharge and urination started earliest on the 5th min after injection in all administrations and continued until at most the 90th min with various intervals. At most 2 urinations occured in all animals during the time period of 120 min. Penis prolapse was observed in only one animal during all the administrations. No pathological pulmonary sound was determined in any animal during sedation.

Rectal temperature increased in all administrations following xylazine HCl administration and even though it decreased below the control values after a certain period of time (15-60 min) it remained within physiological limits.

Respiratory rate increased in all administrations until the 45th min in comparison with the control values, after which it started to decrease below the pre-sedation values and remained at a low level until the 120th min.

Rumen activity decreased in all groups until the 30th min in comparison with the control values after which it increased until the 120th min however it could not reach the control values.

The changes in rectal temperature, respiration rate and rumen activity were statistically significant for the 1st aplication (P<0.05) and statistically insignificant for the other three applications (P>0.05) *(Table 1)*.

Pulse rate remained at a low level in all applications for 120 min. This decrease was statistically significant for the 2st, 3st and 4st applications (P>0.05) and statistically insignificant for the first application (P<0.05) *(Table 2)*.

Reflex Evaluation Results

Reflexes (eye movements, palpebral, corneal, pupillary, patellar and triceps brachii reflexes) were not lost during the sedation periods.

Table 1. *Average rectal temperature, respiration rate and rumen activity values according to 1st application periods.*

Tablo 1. *1. Uygulamada ortalama rektal ısı, solunum sayısı ve rumen sayısı değerleri*

Time (min)	Rectal Temperature (°C)	Respiration Rate (breaths/min)	Ruminal Contraction (Contractions/5 min)
0	39.68±0.39^{x}	51.80±16.31^{xyz}	5.20±2.59^{x}
5	39.72±0.40^{x}	73.00±28.23^{xy}	1.40±1.67^{yz}
15	39.60±0.35^{x}	70.20±26.25^{xy}	1.00±1.00^{z}
30	39.12±0.38^{xy}	79.80±32.15^{x}	1.00±1.00^{z}
45	38.62±0.26^{y}	54.80±18.75^{xyz}	1.20±1.09^{yx}
60	38.56±0.18^{y}	35.60±20.20^{yz}	2.20±0.84^{xyz}
75	38.52±0.26^{y}	27.00±10.22^{z}	3.20±1.30^{xyz}
90	38.52±0.26^{y}	26.00±7.58^{z}	3.20±0.84^{xyz}
120	38.78±0.41^{y}	2.500±4.00^{z}	4.20±1.79^{xy}
p	*	*	*

^{x,y} the difference between the averages on the same column is statistically significant (P<0.05)

Sedation Evaluation Results

The first animal: Mild sedation started on the 5th min after injection in the first application. The animal attained a sterno-abdominal laying posture on the 10th min of mild sedation. The animal attained a standing posture 30 min later and the animal was out of sedation completely after the 35th min.

Mild sedation started on the 10th min after injection in the 2nd application. On the 50th min of this period the animal attained a sterno-abdominal laying posture and attained a standing posture 17 min later. The animal was out of mild sedation after 20 min.

A mild sedation started on the 8th and 12th min during the 3rd and 4th applications. The mild sedation occurred after 75 and 85 min.

The second animal: Mild sedation started on the 4th min after injection in the first application. The animal attained a sterno-abdominal laying posture 8 min later and attained a standing posture 7 min later. The animal attained a sterno-abdominal laying posture again 10 min later and stood up completely after 11 min. The animal was out of mild sedation after 35th min.

It was identified in the 2nd application that mild sedation started at the 5th min after injection. On the 5th min of this period, the animal attained a sterno-abdominal laying position and stood up 14 min later. 6 min later the animal attained a lateral laying position and stood up 25 min later. The animal was out of mild sedation after 30 min.

It was determined in the 3rd application that mild sedation started 3 min after injection and 4 min later the

Table 2. *Average pulse rate according to application periods*
Tablo 2. *Uygulama peryodu dönemlerinde ortalama nabız sayısı*

Time (min)	Pulse Rate (beats/min)			
	1st application	2nd application	3rd application	4th application
0	80.20±25.26	74.80±9.36^{x}	76.40 ± 7.09^{x}	75.80 ± 13.31^{x}
5	75.20±15.00	55.40±7.67^{y}	69.00 ± 8.89^{yz}	60.00 ± 4.89^{xy}
15	69.80±8.95	62.20±4.66^{xy}	70.20±6.94^{yz}	61.00±7.81^{xy}
30	72.00±16.75	58.60±2.88^{y}	66.40±5.41^{yz}	62.20±10.47^{xy}
45	60.60±5.78	57.20±68.0^{y}	62.60±6.46^{z}	59.00±10.07^{y}
60	65.60±9.21	58.40±5.55^{y}	67.00±3.39^{yz}	57.00±1.87^{y}
75	62.60±84.4	60.60±9.32^{xy}	74.80±7.36^{yz}	58.20±4.02^{y}
90	67.00±13.00	62.80±11.52^{xy}	69.80±11.05^{yz}	58.20±2.28^{y}
120	69.80±8.81	63.40±6.56^{xy}	71.60±16.83^{xy}	58.60±8.96^{y}
p	-	*	*	*

^{x,y,z} *the difference between the averages on the same column is statistically significant (P<0.05)*

animal attained a sterno-abdominal laying position and 2 min after that attained a lateral position and stood up after staying in the same position for 20 min. It was decided that mild sedation symptoms disappeared 40 min after that.

In the last application, mild sedation started in the 3rd min after injection and 5 min later the animal attained a sterno-abdominal laying position and after staying in the same position for 55 min, it stood up. The mild sedation ended 15 min later.

The third animal: Mild sedation started on the 6th min after injection in the first application. The animal attained a lateral laying posture after 5 min and stood up after staying in the same position for 25 min. It was decided that mild sedation symptoms disappeared after 45 min.

Mild sedation started at the 8 min after injection in the second administration. The animal attained a sterno-abdominal laying posture after 6 minutes and attained a lateral laying posture after 20 min. It again attained a sterno-abdominal position after staying in the same position for 7 min and stood up 13 min later. It was decided that mild sedation symptoms disappeared 45 min after that.

Mild sedation started 5 min after injection in the third application. The animal attained a sterno-abdominal laying posture after 4 min and attained a lateral laying posture after 24 min. It stood up after staying in the same position for 11 min. The animal was out of mild sedation after 35th min.

Sedation started 6 min after injection in the fourth application. The animal attained a sterno-abdominal laying posture after 10 min and 25 min later it attained a lateral laying posture. It attained a sterno-abdominal position after staying in the same position for 30 min and stood up 20 min later. It was decided that mild sedation ended 10 min after that.

The fourth animal: Sedation started 5 min after injection in the first application. The animal attained a sterno-abdominal laying posture after 10 min and stood up 15 min later. It was decided that the animal was out of sedation 40 min after that.

Only mild sedation started in the 2nd, 3rd and 4th applications. The sedation times were determined as 60, 75 and 70 min respectively.

The fifth animal: Sedation started 10 min after injection in the first administration. The animal attained a sterno-abdominal laying posture after 10 min and attained a lateral laying posture15 min later. It again attained a sterno-abdominal position after staying in the same position for 20 min and stood up 10 min later. The mild sedation ended 35 min after that.

Sedation started 8 min after injection in the second administration. The animal attained a sterno-abdominal laying posture after 15 min and attained a lateral position 10 min later. It again attained a sterno-abdominal position after staying in the same position for 8 min. It stood up after staying in the sterno-abdominal position for 12 min. The mild sedation symptoms disappeared 40 min after that.

Sedation started 6 min after injection in the third application. The animal attained a sterno-abdominal laying posture after 18 min and stood up 14 min after that. After 35 min the mild sedation ended.

Sedation started 12 min after injection in the final application. The animal attained a sterno-abdominal laying posture after 28 min and stood up 11 min after that. After 25 min the mild sedation ended *(Table 3)*.

Analgesic Effect Evaluation Results

As a result of the applied pain tests, the analgesic effect score was 0 (normal strong reaction to painful stimuli)

Table 3. *Sedation level of sheep in the application periods, number of sheep and rations*
Tablo 3. *Uygulama peryodu dönemlerinde koyunların sedasyon dereceleri, koyun sayısı ve oranları*

Applications	Mild Sedation (No recumbency)		Moderate Sedation (Sterno-abdominal recumbency)		Deep Sedation (Lateral recumbency)	
1	-	(0%)	3[n]	(60%)	2[n]	(40%)
2	1[n]	(20%)	1[n]	(20%)	3[n]	(60%)
3	2[n]	(40%)	1[n]	(20%)	2[n]	(40%)
4	2[n]	(40%)	2[n]	(40%)	1[n]	(20%)

during mild and moderate sedation periods and 1-2 score (depressed reaction to painful stimulus) during deep sedation period.

The onset of analgesia occured betwen 10 and 40 min after xylazine HCl administration in sheep during deep sedation period. The duration of analgesia was determined between 10 and 30 min.

Biochemical Evluation Results

It was observed as a result of the examination of biochemical parameters that xylazine HCl caused hyperglycemia ($P<0.01$). Even though some of the other parameters (total bilirubin, BUN, AST, ALT, ALP, Ca, Na, K and Cl) were statistically insignificant, they were within the physiological values ($P>0.01$).

DISCUSSION

It is stated in literature that xylazine HCl can cause oro-nasal discharge and urination [7,11,16,20,21]. In our study, it was observed that oro-nasal discharge started as early as 5 min after injection and continued at various intervals until at most the 90th min. Urination was observed at most twice in the animals during the 5th and 30th min after injection. In addition, penis prolapse was observed only in one animal in all applications.

Rams which are administered with xylazine HCl at a dose of 0.3 mg/kg intramuscularly [22] and 0.2 mg/kg intramuscularly [11] laid down 15 min after injection. In our study, sheep attained a laying posture between the 3rd and 12th min after the first drug was administered in all applications.

Sheep in sedation have remained in a laying posture for 55 min [22], whereas Khan et al.[11] have stated that it was between 90 and 105 min. In our study, animals in moderate sedation laid down for 11-55 min whereas animals in deep sedation laid down for 7-30 min. The shortest sedation time in all groups was 60 min whereas the longest sedation time was 95.

Some animals are mildly sedated until the 15th min after injection and the sedation deepens on the 45th min after which 60% of the animals continue to do so on the 60th min and they are completely out of sedation 120 min later [11].

In our study, sedation time for animals which were only mildly sedated varied between 20 and 85 min. Whereas the sedation times of animals in moderate sedation varied between 11 and 55 min. Sedation times of animals in deep sedation were observed to be between 7 and 30 min.

It has been observed in more recent studies [11,16,17] that rectal temperature decreases for a short period of time for sheep to which xylazine HCl is administered. The initial increase of rectal temperature in all applications after xylazine HCl is injected and its decrease afterwards is in accordance with the statement of Dart [23], saying that hypothermia or hyperthermia can occur in animals due to the depression of the thermoregulation center by α-2 agonists.

Some investigators [11,15-17] stated that pulse rate has decreased. Pulse rates decreased in all applications in this study for a period of 120 min after xylazine HCl is administered.

Respiratory rate decreased in animals to which xylazine HCl has been administered [11,15,16]. In contrast, Ismaila et al.[17] have reported an increase in respiration rate. Whereas some researchers put forth that α-2 agonists cause tachypnea in sheep and bradypnea in other ruminants. In addition, they also state that α-2 receptor agonists have different effects on different sheep species and that there are even individual differences among the species [10]. The increase of respiration rate in the first 45 minutes and the decrease that started after the 45th minute to values below the pre-sedation values as well as the low values for 120 min is in accordance with the aforementioned references.

Studies carried out [16,17,20,24-26] indicate that xylazine HCl decreases ruminal activity in sheep and causes tympani. In this study, rumen activity decreased until the 30th min in all applications after which it increased until the 120th min; however failing to reach the control values.

The animals did not give any reaction to various stimuli on the 45th min during the evaluation of reflexes; such responses started after the 60th min and all responses were observed on the 90th min [11]. It was observed in our study that all reflexes were observed during all sedation periods.

The analgesia level with xylazine HCl in sheep was determined by Khan et al.[11] with the score of between

4-6 was determined. Whereas Grant and Upton [12] Grant et al.[27] states that a complete analgesic effect does not occur in sheep when xylazine HCl is administered in an intramuscular way. The analgesic effect of xylazine HCl differs among sheep species [28]. In this study the degree of analgesia was determined as 1-2 score during deep sedation period. We think that xylazine HCl has a mild analgesic effect on sheep during deep sedation period.

It has been put forth in various studies in literature [7,10] that xylazine HCl causes hyperglycemia. In this study, hyperglycemia was observed in all animals during and after sedation.

It was concluded that even though biochemical parameters started changing after the first application, repeating sedations did not change enzyme and mineral levels since normal limits were not exceeded.

It was decided that repeating sedations can be carried out since xylazine HCl application changed the respiration rate, rectal temperature and rumen activity number less in the following applications in comparison with the first application.

The results of this study indicate that repeated fixed doses of xylazine HCl in sheep as first application caused moderate and deep sedation while mild sedation did not occur. However, the sedation depth decreased significantly in third and fourth applications. We suggest that this should be taken into account during repeated sedations in sheep.

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REFERENCES

1. Brounts SH, Hawkins JF, Baird AN, Glickman LT: Outcome and subsequent fertility of sheep and goats undergoing cesarean section because of dystocia: 110 cases (1981-2001). *J Am Vet Med Assoc,* 224, 275-281, 2004. DOI: 10.2460/javma.2004.224.275

2. Stierschneider M, Franz S, Baumgartner W: Endoscopic examination of the upper respiratory tract and oesophagus in small ruminants: Technique and normal appearance. *Vet J,* 173, 101-108, 2007. DOI: 10.1016/j.tvjl.2005.09.002

3. Melches S, Mellema SC, Doherr MG, Wechsler B, Steiner A: A castration of lambs: A welfare comparison of different castration techniques in lambs over 10 weeks of age. *Vet J*, 173, 554-563, 2007. DOI: 10.1016/j.tvjl.2006.01.006

4. Stafford K: Castrating older lambs: What are the issues? *Vet J*, 173, 477, 2007.

5. Taylor PM: Anesthesia in sheep and goats. *In Pract,* 13, 31-36, 1991.

6. Celly CS, Mc Donell WN, Young SS, Black WD: The comparative hypoxaemic effect of four alpha-2 adrenoceptor agonists (xylazine, romifidine, detomidine and medetomidine) in sheep. *J Vet Pharmacol Ther,* 20, 464-471, 1997. DOI: 10.1046/j.1365-2885.1997.00097.x

7. Carroll GL, Hartsfield SM, Champney TH, Geller SC, Martinez EA, Haley EL: Effect of medetomidine and its antagonism with atipamezole on stress-related hormones, metabolites, physiologic responses, sedation, and mechanical threshold in goats. *Vet Anaesth Analg,* 32, 147-157, 2005. DOI: 10.1111/j.1467-2995.2005.00187.x

8. Kästner SBR: A_2-agonists in sheep: A review. *Vet Anaesth Analg,* 33, 79-96, 2006. DOI: 10.1111/j.1467-2995.2005.00243.x

9. Riebold TW: Ruminants. **In,** Tranquilli WJ, Thurmon JC, Grimm KA (Eds): Lumb & Jones' Veterinary Anesthesia and Analgesia. 731-746, Wiley-Blackwell, Ames, IA, 2007.

10. Valverde A, Doherty TJ: Anesthesia and analgesia of ruminants. **In,** Fish RE, Brown MJ, Danneman PJ, Karas AZ (Eds): Anesthesia and Analgesia in Laboratory Animals. 285-411, Academic Press, London, UK, 2008.

11. Khan MA, Ashraf M, Pervez K, Rashid HB, Mahmood AK, Chaudhry M: Comparative effects of detomidineand xylazine as sedative and analgesic agents in small ruminants. *Pak Vet J,* 24, 62-69, 2004.

12. Grant C, Upton RN: Comparison of the analgesic effects of xylazine in sheep via three different administration routes. *Aust Vet J,* 82, 304-307, 2004. DOI: 10.1111/j.1751-0813.2004.tb12712.x

13. Carroll GL, Hartsfield SM: General anaesthetic techniques in ruminants. *Vet Clin North Am: Food Anim Pract,* 12, 627-661, 1996.

14. Dugdale A: Veterinary Anaesthesia Principles to Practice. 255, Wiley-Blackwell Malaysia, 2010.

15. Doherty TJ, Pascoe PJ, Mc Donell WN, Monteith G: Cardiopulmonary effects of xylazine and yohimbine in laterally recumbent sheep. *Can J Vet Res,* 50, 517-521, 1986.

16. Habib S, Das BC, Islam MN, Hossain MK, Ahmed MF: A Comparison of xylazine, diazepam, chlorpromazine and promethazine in relation to certain clinical and hematological parameters of indigenous sheep *(Ovis aries). Pak J Biol Sci,* 5, 484-488, 2002.

17. Ismaila MS, Onifade KI, Agaie BM, Elsa AT, Chafe UM: The influence of environmental temperature on physiological parameters in xylazine sedated sheep. *Int J Anim Veter Adv,* 2, 37-42, 2010.

18. Hall LW, Clarke KW, Trim CM: Anaesthesia of sheep, goats and other herbivores **In,** Hall LW, Clarke KW, Trim CM (Eds): Veterinary Anaesthesia. 341-366, WB Saunders, London, UK, 2001.

19. Kästner SBR, Wapf P, Feige K, Demuth D, Bettschart-Wolfensberger R, Akens MK, Huntinen M: Pharmacokinetics and sedative effects of intramuscular medetomidine in domestic sheep. *J Vet Pharmacol Ther,* 26, 271-276, 2003. DOI: 10.1046/j.1365-2885.2003.00492.x

20. Powell JD, Denhart JW, Lloyd WE: Effectiveness of tolazoline in reversing xylazine-induced sedation in calves. *J Am Vet Med Assoc,* 212, 90-92, 1998.

21. Galatos AD: Anesthesia and analgesia in sheep and goats. *Vet Clin North Am: Food Anim Pract,* 27, 47-59, 2011. DOI: 10.1016/j.cvfa.2010.10.007

22. Shokry M, Morad HM, Khalil IA: Studies on the effect of rompun in sheep. *Vet Med Rev,* 2, 237-243, 1976.

23. Dart CM: Advantages and disadvantages of using alpha-2 agonists in veterinary practice. *Aust Vet J,* 77, 720-721, 1999. DOI: 10.1111/j.1751-0813.1999.tb12907.x

24. Toutain PL, Zingoni MR, Ruckbush Y: Assessment of alpha-2 adrenergic antagonists on the central nervous system using reticular contraction in sheep as a model. *J Pharmacol Exp Ther,* 223, 215-218, 1992.

25. Deghani S, Behbodikhah A, Khorsand N: Clinical, haematological and biochemical effects of xylazine, ketamine and their combination in cattle and sheep. *Proceedings of the 4th International Congress of Veterinary Anaesthesia,* 123-128, Utrecht, The Netherlands, 1991.

26. Mohammad FK, Wahed RA, Dabbagh BK: Stimulation of food intake by xylazine in sheep. *Zentralbl Veterinarmed A,* 43, 387-391, 1996. DOI: 10.1111/j.1439-0442.1996.tb00466.x

27. Grant C, Summersides GE, Kuchel TR: A xylazine infusion regimen to provide analgesia in sheep. *Lab Anim,* 35, 277-281, 2001. DOI: 10.1258/0023677011911606

28. Ley S, Waterman A, Livingston A: Variation in the analgesic effects of xylazine in different breeds of sheep. *Vet Rec,* 26, 508, 1990. DOI:10.1136/vr.126.20.508

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Research Article

Th1/Th2 Cytokine Balance and SOCS3 Levels of Female Offspring Born from Rats with Gestational Diabetes Mellitus [1]

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Abstract

Diabetes mellitus during pregnancy is a metabolic disorder that is very important in regard to both the health of the mother and the baby. We determine the effect of diabetes mellitus generated by streptozotocin with different dosages in pregnant rats on serum Th1/Th2 cytokine balance and SOCS3 levels. Twenty-one pregnant rats in their late pregnancy were used for this purpose. The rats were divided into three groups randomly and the rats in the first group were used as the control group. Streptozotocin was administered intraperitoneally at a dosage of 40 mg/kg to the rats in the second group and at a dosage of 60 mg/kg to the rats in the third group. One female offspring of each rat was decapitated and the blood of the decapitated rats was collected and Th1 [Interferon gamma (IFNγ), Interleukin 2 (IL-2), Tumor necrosis factor alpha (TNFα)], Th2 [Interleukin 4 (IL-4), Interleukin 5 (IL-5), Interleukin 10 (IL-10)] cytokine levels were measured using a multiplex immunoassay based on xMAP® detection technology and the suppressor of cytokine signaling 3 (SOCS3) levels were measured using a commercial ELISA kit. The IFN-γ (1.59±0.28 pg/mL) levels in group 1 were lower than those in the other groups. The TNF-α (2.24±0.20, 2.21±0.19 pg/mL) levels in groups 2 and 3 were higher than in group 1, and the IL-4, IL-6, IL-10 and SOCS3 concentrations were not significantly different among groups. The SOCS3 levels of the offspring were not different among the groups, and the IFN-γ and TNF-α blood concentrations were increased; the Th1/Th2 cytokine balance was shifted toward Th1. We have observed that the SOCS3 levels of offspring born from mothers generated to be diabetic by administration of streptozotocin in late pregnancy were not different among various groups, and the IFN-γ and TNF-α blood concentration were increased. As a result, the Th1/Th2 cytokine balance was shifted toward Th1. This suggests that more prominent cellular immunity.

Keywords: *Cytokine, Diabetes Mellitus, Offspring, Pregnancy, Rat, SOCS3*

Gebelikte Diabetes Mellitus Şekillenmiş Ratlardan Doğan Dişi Yavrularda Th1/Th2 Sitokin Dengesi Ve SOCS3 Düzeyleri

Özet

Gebelikte şekillenen diabetes mellitus, hem anne sağlığı hem de yavru sağlığı için son derece önemli ve giderek görülme sıklığı artan bir metabolik bozukluktur. Bu hastalığın gelişimi ve tedavisine yönelik yeni araştırmalara ihtiyaç vardır. Bu çalışmada da ileri gebe ratlarda farklı dozlarda streptozotosinle oluşturulan diabetes mellitusun yavruların, kan serumlarındaki Th1/Th2 sitokin dengesi ve SOCS3 düzeyleri üzerine etkisinin belirlenmesi amaçlanmıştır. Bu amaçla, 21 adet ileri gebe rat kullanıldı. Ratlar rastgele üç gruba ayrılarak 1. gruptaki hayvanlar kontrol grubu olarak ayrıldı. Streptozotosin 2. gruptaki hayvanlara 40 mg/kg, 3. gruptaki hayvanlara 60 mg/kg dozda intraperitonal olarak uygulandı. Daha sonra hayvanların doğumları takip edildi. Yavrular 1 aylık olduktan sonra her hayvanın 1 dişi yavrusu dekapite edildi. Dekapite edilen hayvanların kanları alındı ve kan serumlarında Th1 [Interferon gamma (IFNγ), Interleukin 2 (IL-2), Tumor necrosis factor alpha (TNFα)], Th2 [Interleukin 4 (IL-4), Interleukin 5 (IL-5), Interleukin 10 (IL-10)] sitokin düzeyleri xMAP® tespit teknolojisini esas alan multiplex immünoassay ve Suppressor of cytokine signalling 3 (SOCS3) düzeyleri ise ticari ELİSA kiti ölçüldü. Sonuç olarak, grup 1'deki IFN-γ (1,59±0,28 pg/ml) değerinin diğer gruplara nazaran daha düşük olduğu, grup 2 ve 3'teki TNF-α (2,24±0,20, 2,21±0,19 pg/ml) değerlerinin de grup 1'den fazla olduğu, tüm gruplardaki IL-4, IL-6, IL-10 ve SOCS3 konsantrasyonlarının karşılaştırılması sonucunda ise gruplar arasında istatistiki anlamda bir fark olmadığı belirlendi. Sonuç olarak; Th1/Th2 sitokin dengesinin Th1'e doğru kaydığı gözlendi. Bu da hücresel immünitenin daha ön planda olduğunu göstermektedir.

Anahtar sözcükler: *Sitokin, Diabetes mellitus, Yavru, Gebelik, Rat, SOCS3*

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INTRODUCTION

Gestational diabetes mellitus (GDM) appearing as a glucose tolerance disorder during pregnancy negatively affects the health of both the mother and the baby. Maternal diabetes creates an unfavorable environment for embryonic and placental development. Both type 1 and type 2 GDM patients are at high risk of developing complications such as abortion, stillbirth, congenital malformations, placental anomalies and intrauterine development impairment [1].

It has been reported that the immune system of the babies born from diabetic mothers may be affected at different levels and even long-term effects such as immunodeficiency have been observed in babies [2].

In particular, the cytokines released from CD4+ (cluster of differentiation 4) T lymphocytes play an important role in the regulation of immunological reactions. Th0 lymphocytes differentiate into two subgroups: Th1 and Th2. IL–2, IL–12, IL–15, IL–18, IFNγ and TNFβ are released from the Th1 group CD4+ T lymphocytes, and IL–4, IL–5, IL–6, IL–10, IL–13 and Granulocyte-macrophage colony-stimulating factor (GM-CSF) are released from the Th2 group CD4+ T lymphocytes. Th1 cells are responsible for cellular immunity whereas Th2 cells are responsible for humoral immunity [3,4].

Etiology, genetics and pathogenesis of type 1 and 2 DM are different. However, there is strong evidence that inflammatory mediators play an important role in the pathogenesis of both diseases [5,6]. Loss of beta cells in both type 1 and type 2 DM may be caused by apoptosis and necrosis triggered by inflammatory mediators. In type 1 diabetes is destruction of beta cells due to immune causes. Pro-inflammatory cytokines emerging during the inflammation of pancreatic islets cause apoptosis and necrosis of beta cells [5,6]. Both the lack and apoptosis of beta cells and the high levels of pro-inflammatory cytokines during the early periods of the disease are causes of type 2 DM [7-10]. Furthermore, it is known that various pro-inflammatory cytokines stimulate insulin resistance [11-13].

Natural inhibitors of some cytokines have recently been defined [14]. Suppressor of cytokine signaling (SOCS) proteins are also part of this group of natural inhibitors that regulate IFN-γ signals via negative feedback [14]. These proteins are inhibitors of the cytokine signal network and are important physiological regulators playing a role in the regulation of innate and adaptive immunity. Signal Transducers and Activators of Transcription (STAT) signal networks play a role in many cellular events, such as the release of cytokines and growth factors. Cytokines lead to cell proliferation or death over gene expression by triggering the JAK/STAT pathway. SOCS3 proteins down-regulate IL-1 signals and restrict the severity and duration of the cytokine response in a negative direction through STAT3 activation [15,16]. These molecules play an essential role in T cell development and differentiation [17].

In this study, we investigated the effect of streptozotocin induced gestational diabetes mellitus on serum Th1/Th2 cytokine balance and SOCS3 levels in pregnant rats.

MATERIAL and METHODS

Animals

A total of 21 Wistar rats, aged 3-4 months and weighing 200-250 g, were used in this study. The rats were obtained from Firat University Experimental Research Center. The rats were kept in separate cages in groups of seven, and a 12-h light and 12-h dark regime was applied. The rats were fed as much as they could eat and drink. The Ethical Committee Report was obtained from the Firat University Experiment Animals Ethical Committee (FUHADEK 2014/8).

Selection of Animals

Twenty-one rats that were detected to be in estrous were selected by vaginal irrigation. The rats were placed in cages in groups of three. One male rat was placed in each case and coitus was followed.

Vaginal Irrigation

Vaginal irrigations were performed as described by Rişvanlı et al.[18]. Irrigations were performed using elastic pipette bulb and pipette tip with distilled water. Fluid obtained by irrigation was placed on the slide and examined under the microscope with 40× magnifications. The intensity of the cell types included in the samples were evaluated as +, ++ and +++. Rats with a superficial cell intensity of +++ were accepted as being in estrous. Rats that had spermatozoids in their samples prepared with vaginal irrigation were accepted as being fertilized. The date of examination was accepted as the first day of pregnancy.

Experimental Groups

The rats were randomly divided into three groups and group 2 and 3 rats were exposed to the following treatment on day 13 of the study:

Group 1: Control group, intraperitoneal normal saline (n=7)

Group 2: Intraperitoneal streptozotocin (SIGMA, USA) 40 mg/kg (n=7) [19],

Group 3: intraperitoneal streptozotocin (SIGMA, USA) 60 mg/kg (n=7) [19].

Streptozotocin was prepared in doses indicated above in solution of 0.1 M citrate phosphate tampon.

Glucose concentrations were measured with a glucometer using blood collected from the tails of the rats 3 days after the injections, on day 16 of pregnancy. Normal blood glucose levels were accepted as 90-110 mg/dl; rats that had a blood glucose level over 250 mg/dl were accepted as diabetic [20].

Analyses

Afterwards, the rats were placed in separate individual cages and the delivery of each rat was followed. One female offspring of each rat was decapitated after the offspring were 1 month of age. The blood of the decapitated rats was collected and kept at -20°C until the assay after separating the serum. Th1 (IFN-γ, IL-2, TNF-α) and Th2 (IL-4, IL-5, IL-10) cytokine levels were measured with a multiplex immunoassay (Procarta® CytokineAssay Service, Diax, Italy) based on xMAP® detection technology [21]. SOCS3 levels were measured using a commercial ELISA kit (Catalog No: MBS2019020, MyBiosource Inc., USA) and read by an ELISA reader (BioTek Instruments, USA).

Statistical Analysis

The Kruskal-Wallis test was used to compare the blood serum levels of the offspring rats among the groups. The significance level was determined by the Mann-Whitney U-test in cases in which significance was present as a result of the Kruskal-Wallis test. Statistical analysis was performed using the SPSS 11.5 program.

RESULTS

The IFN-γ (1.59±0.28 pg/mL) levels in group 1 animals - were lower than those of other group rats (P<0.001). In addition, the TNF-α (2.24±0.20 pg/mL and 2.21±0.19 pg/mL) levels in groups 2 and 3 were higher than that of group 1 (P<0.001). The IL-2 concentrations in all groups were determined to be below measurable levels *(Table 1)*.

No significant difference was determined among groups in terms of IL-4, IL-6, IL-10 and SOCS3 concentrations *(Table 1)*.

DISCUSSION

The cytokine environment around the fetus is important for the continuation of the pregnancy. According to the immunotropic hypothesis described by Wegmann et al.[22], the Th1/Th2 cytokine balance is an important mechanism to maintain the vitality of the fetus in the uterus. Local production of Th2-type cytokines in the feto-maternal relation is required for the constitution of the pregnancy. The Th1/Th2 cell balance is in favor of Th2 in diabetic pregnancies of both animals and humans. However, the Th1/Th2 balance of macrosomic and obese babies born from diabetic mothers is directed toward pro-inflammatory Th1. The direction toward Th1 in obese babies plays a role in the development of diabetogenic status that exhibits hyperglycemia and hyperinsulinemia seen in later years [23]. In this study the IFN-γ (Group 2: 2.16±0.24, Group 3: 2.27±0.24 pg/mL) and TNF-α (Group 2: 2.24±0.20, Group 3: 2.21±0.19 pg/mL) levels of female offsprings born from mothers with DM were determined to be higher as well.

TNF-α stimulates insulin resistance by indirectly stimulating the stress hormone or continuous induction of SOCS3 proteins [11,24]. Furthermore, SOCS3 is claimed to inhibit leptin insulin signaling. Growth hormone also causes the development of insulin resistance by causing the release of SOCS3 [25]. Therefore; SOCS3 plays an important role as a candidate gene in the pathogenesis of type 1 diabetes and insulin resistance. In a study by Gylvin et al.[5] no mutation in the SOCS3 gene identification was found in people with type 1 DM. No data were found about the SOCS3 levels of babies born from mothers that had diabetes during pregnancy. In the present study, the serum SOCS3 levels of female offsprings born from mothers with GDM were determined to be different from that of the control group.

The immune status of the babies born from diabetic mothers is important for the postpartum period and also for the rest of the life of the offspring, since any damage to the immune system may cause life-threatening problems also in the future. In conclusion, we demonstrated that

Table 1. *Comparison of the cytokine and SOCS3 levels among groups*
Tablo 1. *Gruplar arasında sitokin ve SOCS3 düzeylerinin karşılaştırılması*

Parameters	Group 1 (n=7)	Group 2 (n=7)	Group 3 (n=7)	P
IFN-γ (pg/mL)	1.59±0.28[a]	2.16±0.24[b]	2.27±0.24[b]	*
IL-2 (pg/mL)	BD	BD	BD	-
TNF-α (pg/mL)	BD	2.24±0.20[b]	2.21±0.19[b]	*
IL-4 (pg/mL)	0.22±0.11	0.22±0.10	0.22±0.08	-
IL-6 (pg/mL)	25.51±1.86	25.43±0.75	24.69±2.05	-
IL-10 (pg/mL)	2.41±0.27	2.44±0.22	2.34±0.24	-
SOCS3 (ng/mL)	0.38±0.08	0.36±0.06	0.33±0.15	-

*- The difference between the groups was insignificant (P>0.05); * The difference between groups was significant (P<0.05); [ab] The difference between the values indicated with different letters in the same line was significant (P<0.001);* ***BD:*** *below detection limits*

the IFN-γ and TNF-α blood concentrations of offsprings born from experimentally induced GDM female rats were increased where as the SOCS3 levels were not different among groups. As a result, the Th1/Th2 cytokine balance was shifted towards Th1. This suggests that more prominent cellular immunity.

REFERENCES

1. Eriksson UJ: Congenital anomalies in diabetic pregnancy. *Semin Fetal Neonatal Med,* 14, 85-93, 2009. DOI: 10.1016/j.siny.2008.11.001

2. Yessoufou A, Moutairou K: Maternal diabetes in pregnancy: Early and long-term outcomes on the offspring and the concept of "Metabolic Memory". *Experimen Diab Res*, Article ID 218598, 2011. DOI: 10.1155/2011/218598

3. Belardelli F: Role of Interferons and other cytokines in the regulation of the immune response. *APMIS,* 103, 161-179, 1995. DOI: 10.1111/j.1699-0463.1995.tb01092.x

4. Raghupathy R: Th1-type immunity is incompatible with successful pregnancy. *Immunol Today,* 18, 478-482, 1997.

5. Gylvin T, Nolsøe R, Hansen T, Nielsen EM, Bergholdt R, Karlsen AE, Billestrup N, Borch-Johnsen K, Pedersen O, Mandrup-Poulsen T, Nerup J, Pociot F: Mutation analysis of suppressor of cytokine signalling 3, a candidate gene in Type 1 diabetes and insulin sensitivity. *Diabetologia,* 47, 1273-1277, 2004.

6. Nerup J, Mandrup-Poulsen T, Helqvist S, Andersen HU, Pociot F, Reimers JI, Cuartero BG, Karlsen AE, Bjerre U, Lorenzen T: On the pathogenesis of IDDM. *Diabetologia,* 37, S82-S89, 1994.

7. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC: Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes,* 52, 102-110, 2003. DOI: 10.2337/diabetes.52.1.102

8. Esposito K, Nappo F, Giugliano F: Cytokine milieu tends toward inflammation in type 2 diabetes. *Diabetes Care,* 26, 1647, 2003. DOI: 10.2337/diacare.26.5.1647

9. Kubaszek A, Pihlajamäki J, Komarovski V: Promoter polymorphisms of the TNF-alpha (G-308A) and IL-6 (C-174G) genes predict the conversion from impaired glucose tolerance to type 2 diabetes: The Finnish diabetes prevention study. *Diabetes* 52, 1872-1876, 2003. DOI: 10.2337/diabetes.52.7.1872

10. Spranger J, Kroke A, Möhlig M: Inflammatory cytokines and the risk to develop type 2 diabetes: Results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. *Diabetes,* 52, 812-817, 2003. DOI: 10.2337/diabetes.52.3.812

11. Grimble RF: Inflammatory status and insulin resistance. *Curr Opin Clin Nutr Metab Care,* 5, 551-559, 2002.

12. Kim F, Gallis B, Corson MA: TNF-alpha inhibits flow and insulin signaling leading to NO production in aortic endothelial cells. *Am J Physiol Cell Physiol,* 280, 1057-1065, 2001.

13. Starr R, Willson TA, Viney EM: A family of cytokine-inducible inhibitors of signalling. *Nature,* 387, 917-921, 1997. DOI: 10.1038/43206

14. Krebs DL, Hilton DJ: SOCS proteins: Negative regulators of cytokine signaling. *Stem Cells,* 19, 378-387, 2001.

15. Karlsen AE, Rønn SG, Lindberg K: Suppressor of cytokine signaling 3 (SOCS-3) protects beta cells against interleukin-1beta- and interferon-gamma-mediated toxicity. *Proc Natl Acad Sci USA,* 98, 12191-12196, 2001. DOI: 10.1073/pnas.211445998

16. Carow B, Reuschl AK, Gavier-Widén D: Critical and Independent Role for SOCS3 in either myeloid or t cells in resistance to *Mycobacterium tuberculosis*. *PLoS Pathog,* 9, e1003442, 2013. DOI: 10.1371/journal.ppat.1003442

17. Yoshimura A, Naka T, Kubo M: SOCS proteins, cytokine signalling and immune regulation. *Nat Rev Immunol,* 7, 454-465, 2007. DOI: 10.1038/nri2093

18. Risvanli A, Aydin M, Kaygusuzoglu E, Timurkan H: The effect of thyroidectomy on sexual cycle and pregnancy rates in rats. *Turk J Vet Anim Sci,* 27, 873-877, 2003.

19. Thamotharan M, McKnight RA, Thamotharan S, Kao DJ, Devaskar SU: Aberrant insulin-induced GLUT4 translocation predicts glucose intolerance in the offspring of a diabetic mother. *Am J Physiol Endocrinol Metab,* 284, 901-914, 2003. DOI: 10.1152/ajpendo.00516.2002

20. Buyukleblebici O, Karagul H: The biochemical effects of dietary chromium in experimental diabetic rats with streptozotocin. *Kafkas Univ Vet Fak Derg,* 18, 21-26, 2012. DOI: 10.9775/kvfd.2011.4501

21. de Jager W, Prakken B, Rijkers GT: Cytokine multiplex immunoassay: Methodology and (clinical) applications. *Meth Mol Biol,* 514, 119-133, 2009. DOI: 10.1007/978-1-60327-527-9_9

22. Wegmann TG, Lin H, Guilbert L, Mosmann TR: Bidirectional cytokine interactions in the maternal-fetal relationship: Is successful pregnancy a Th2 phenomenon? *Immunol Today,* 14, 353-356, 1993. DOI: 10.1016/0167-5699(93)90235-D

23. Soulimane-Mokhtari NA, Guermouche B, Yessoufou A: Modulation of lipid metabolism by n-3 polyunsaturated fatty acids in gestational diabetic rats and their macrosomic offspring. *Clin Sci,* 109, 287-295, 2005.

24. Rui L, Yuan M, Frantz D, Shoelson S, White MF: SOCS-1 and SOCS-3 block insulin signaling by ubiquitinmediated degradation of IRS1 and IRS2. *J Biol Chem,* 277, 42394-42398, 2002.

25. Luger A, Prager R,Gaube S, Graf H, Klauser R, Schernthaner G: Decreased peripheral insulin sensitivity in acromegalic patients. *Exp Clin Endocrinol,* 95, 339-343, 1990.

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Research Article

Antihelmintik Triklabendazolun Yapay Besin ile Beslenen *Galleria mellonella* (Lepidoptera: Pyralidae) Larvalarının Yaşama ve Gelişimine Etkisi [1]

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Özet

Benzimidazol grubu bir antihelmintik olan triklabendazolun yapay besin kullanılarak Büyük bal mumu güvesi *Galleria mellonella* (L.) larvalarının ergin evreye kadar farklı gelişme evrelerinde yaşama oranına ve gelişme süresine etkisi incelendi. Triklabendazol yapay besine %0.001, 0.01 ve 0.1 oranlarında ilave edilerek birinci evre larvaları besinlerde ergin oluncaya kadar yetiştirildi. Antihelmintik maddenin düşük konsantrasyonlarında (0.001 ve 0.01 g/100 g besin), son evre larva oranı (7. evre) ve ergin olma oranı kontrole göre önemli derecede düşük bulunurken, pupa olma oranı bakımından kontrol ile arasında istatistiksel farklılık oluşmadı. Bunun tersine, denenen antihelmintik maddenin en yüksek besinsel konsantrasyonunda, pupa evresine ve ergin evreye ulaşma oranı kontrol grubundan önemli derecede düşük tespit edilirken 7. evreye ulaşan larva oranı bakımından kontrol ile arasında farklılık görülmedi. Triklabendazolun en yüksek konsantrasyonu (0.1 g/100 g besin) pupa olma oranını %81.6±4.32'den %43.3±7.45'e, ergin olma oranını %73.3±2.36'dan %13.3±2.36'ya düşürdü. En yüksek antihelmintik konsantrasyonu 7. evreye ulaşma süresini yaklaşık 3.8 gün uzatarak 22.8±1.54 güne ulaştırdı. Triklabendazolun %0.1 oranını içeren besin kontrol besinindeki 24.6±1.24 gün olan pupa olma süresini 28.4±1.24 güne, 35.3±1.27 gün olan ergin olma süresini 42.0±1.62 güne önemli derecede uzattı. Bu çalışma farklı kimyasal yapı ve etki mekanizmasına sahip antihelmintiklerin zararlı böceklerin mücadelesinde hedef olmayan canlılara ve çevreye karşı en az zararla kullanılabilirliğinin araştırılması açısından önemlidir.

Anahtar sözcükler: *Galleria mellonella, Triklabendazol, Yaşama oranı, Beslenme*

The Effect of Anthelmintic Triclabendazole on Survival and Development of *Galleria mellonella* (Lepidoptera: Pyralidae) L. Reared on Artificial Diet

Abstract

The effect of triclabendazole, which is a benzimidazole anthelmintic, on the survival rate and developmental time in different stages of greater wax moth *Galleria mellonella* (L.) was investigated by rearing the larvae on the artificial diets. Triclabendazole was incorporated into diets at concentrations of 0.001, 0.01 or 0.1%. *G. mellonella* larvae was reared from first instar larvae to adult emergence on the artificial diets with different concentrations of triclabendazole. The survival rate in seventh instar (7^{th}-instar) and adult stage were significantly lower at low concentrations of this anthelmintic agent (0.001 and 0.01 g per 100 g of diet) than the control, while there were no differences on pupation in comparison to control diet. However, the pupation and adult emergence were significantly lower at the highest dietary concentration of triclabendazole than control group but there were no differences on survival of 7^{th}-instar larvae. The highest concentration of the anthelmintic agent (0.1 g/100 g diet) decreased pupation from 81.6±4.32% to 43.3±7.45%, adult emergence rate from 73.3±2.36 to 13.3±2.36. Triclabendazole at the highest concentration prolonged developmental time to 7^{th} larval stage by 3.8 days reaching total 22.8±1.54 days. This diet containing 0.1% of triclabendazole significantly prolonged pupal developmental time from 24.6±1.24 days to 28.4±1.24 days, adult developmental time from 35.3±1.27 days to 42.0±1.62 days. This study is of importance in appreciation for usage of antihelmintic with different structure and mode of actions in the management of pest insects to reduce damage to environment and nontarget organisms.

Keywords: *Galleria mellonella, Triclabendazole, Survival rate, Nutrition*

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GİRİŞ

Böceklerin laboratuar şartlarında yapay besinler ile yetiştirilmesi biyolojik, biyokimyasal, fizyolojik ve moleküler araştırmaların yürütülebilmesi için önemli araçtır. Kimyasal yapısı belirli yapay besinlerin içeriğindeki besinsel değeri yüksek bileşenler mikrobiyal gelişim için oldukça uygun bir ortam oluşturmaktadır [1-3]. Laboratuvar şartlarında böcekleri yetiştirmek için kullanılan yapay besinlere bakteriyel, fungal ve diğer kaynaklı kontaminasyonlar ile mücadele amacıyla ilave edilen antimikrobiyal maddelerin kontaminasyonu önleme özelliği yanında böceklerin yaşamını, gelişimini ve bazı fizyolojik özelliklerini olumsuz yönde etkilediği tespit edilmiştir [1,4-14]. Böcek üzerinde bu maddelerin düşük miktarlarda olumsuz etkiye sahip olması zararlı böcekler ile mücadelede etkin ve çevreye duyarlı yeni kimyasal mücadele yöntemlerinin geliştirilmesi açısından önemlidir [15,16]. Bu amaçla son zamanlarda laboratuvar şartlarında yaptığımız bir beslenme çalışmasında yeni kuşak bazı antihelmintik maddelerin Büyük bal mumu güvesi *Galleria mellonella* (L.) üzerinde olumsuz etkiye sahip olduğu gözlenmiştir [17].

Galleria mellonella larvaları arı kovanlarındaki ürünler (bal peteği, mum ve bal) ile beslendiğinden arıcılık sektöründe önemli ekonomik kayıplara sebep olmaktadır [18]. Mum güvesi larvalarının laboratuar ortamında yapay besinler ile yetiştirilmesi sırasında mikrobiyal kontaminasyonu önlemek ve fizyolojik araştırmalar için mikroorganizmadan arındırılmış steril böcekler yetiştirmek amacıyla bazı antibiyotikler besinlere ilave edilmiştir [19,20-23]. Bu böcek laboratuar şartlarında ucuz yapay besinlerde bol miktarda üretilebildiği için fizyoloji, biyokimya ve moleküler biyoloji çalışmalarında yaygın olarak model böcek olarak kullanılmaktadır. Biyolojik mücadelede kullanılan parazitoid böceklerin laboratuvarda yetiştirilmesinde doğal konak olarak ve insektisit etkinlik denemelerinde, hatta insan ve diğer memelilerde hastalık yapan mikroorganizmaların patojenitesinin belirlenmesinde yaygın kullanılmasından [24] dolayı önemi gittikçe artmaktadır.

Antibakteriyel, antifungal, antiviral maddelerin yüksek organizasyonlu ökaryotik organizmalardan, insanın da dahil olduğu memelilerin DNA replikasyonunu, transkripsiyonu, protein sentezini ve hücre ara metabolizmasını olumsuz yönde etkilediği bilinmektedir [25]. Memeli hücrelerinde hücre iskeletinin oluşumunu veya sterol biyosentezini engelleyen geleneksel bazı antifungal maddeler ile RNA sentezi inhibitörü antibakteriyel maddelerin çeşitli zararlı böcek türlerinin biyolojik özelliklerini olumsuz yönde etkilediğine dair bilgiler mevcuttur [26,27].

Triklabendazol [5-kloro-6-(2,3-diklorofenoksi)-2-(metiltiyo)-1*H*-benzimidazol] benzimidazol grubu yeni kuşak bir antihelmintik olup insan ve hayvanlarda enfeksiyona neden olan parazitlerin ergin evre ve ergin öncesi evreleri üzerinde oldukça etkilidir [28-31]. Triklabendazolün de içinde bulunduğu bu bileşiklerinin etki mekanizması tam olarak anlaşılmamasına rağmen, benzimidazoller mikrotübüllerin yapısal bir proteini olan β-tubulin molekülüne bağlanarak [32-36], hücre bölünmesi ve hücre içi taşıma gibi hücre işlevlerini bozarlar. Triklabendazolun metabolizma ürünleri (sülfoksit türevleri ve sülfon) aracılığıyla parazitlerde mikrotübül oluşumunu bozması sonucu glukoz alımı yapılamadığından hücredeki enerji mekanizmaları dolayısıyla homeostazi bozulmaktadır [37,38-40].

Antihelmintiklerin insan ve hayvanlarda enfeksiyona neden olan parazitlerde olduğu gibi zararlı böceklerde de benzer olumsuz etkilere sahip oldukları hakkında detaylı bilgi bulunmamaktadır. Ancak *G. mellonella*'nın mücadelesinde karbon dioksit, metil bromid, fosfin, sülfür, naftalin ve paradiklorobenzen gibi toksik kimyasalların uygulandığı yöntemlere kıyasla [18] triklabendazolun parazitlerdeki seçici etki mekanizmasından dolayı hedef olmayan memeli hayvanlara ve çevreye toksisitesinin düşük olduğu [35] bilinmektedir. Bu çalışmada insan ve hayvan sağlığı açısından klinik öneme sahip antihelmintiklerin zararlı böceklere etkilerinin model böcek *G. mellonella* üzerinde araştırılması amaçlanmıştır. Böylece antihelmintiklerin ekonomik öneme sahip zararlı böceklerin biyolojisi üzerindeki etkilerinin daha detaylı bilinmesine yönelik diğer çalışmalara öncülük edilmiş olacaktır. Benzimidazol grubu bir antihelmintik olan triklabendazol *G. mellonella*'nın tüm gelişme evrelerindeki yaşama oranını ve gelişme süresini olumsuz yönde etkilemiştir.

MATERYAL ve METOT

Böceklerin Laboratuvarda Yetiştirilmesi

Dişilerin bıraktığı yumurtaların açılması ile serbest kalan *G. mellonella* birinci evre larvaları laboratuvarda yapay besin ortamlarında beslenerek erginleşen bireylerden sürekli böcek kültürü oluşturuldu. Böcekler inkübatörde (Nüve, ES 500) 28±2°C sıcaklık ve %65±5 bağıl nemde yetiştirildi. Yaşama ve gelişme ile ilgili deneylerde yumurtadan yeni çıkmış birinci evre larvaları kullanıldı.

Laboratuvar ortamında *G. mellonella* larvalarını yetiştirmek ve çoğaltmak için yapay besin kullanıldı [41]. Besinin bileşiminde, 420 g buğday kepeği, 150 ml süzme bal, 150 ml gliserin, 20 g öğütülmüş koyu renkli eski petek ve 30 ml saf su bulunmaktadır. Bileşenler hazırlanacak besinin belirli bir miktarı için gerekli miktarlarda tartılarak karıştırıldı ve özel bir karıştırıcı ile homojen bir ortam sağlandı. Besinler cam kavanozlara taksim edildi. Kavanozun içindeki besinin üstüne bırakılacak dişilerin yumurta bırakmasını kolaylaştırmak ve bırakılan yumurtalardan yeni açılan larvaların ilk anda beslenmesi için besinin üzerine küçük bir parça kuru boş bal peteği konuldu [42]. Kavanozlardaki besinlerin üzerine 5-10 adet dişi bırakılarak kavanozların ağızları solunumu sağlamak için tel kafes içeren vida kapak ile kapatıldı. Gelişimlerini normal tamamlayan 7. evre larvaları

alınarak pupa olmalarını sağlamak için başka bir kavanoza bırakıldı. Larvalara kuru bir ortam sağlamak üzere pelur kağıt parçaları katlanarak kavanozun içine bırakıldı [43]. Pupa olan bireyler gözlendi ve pupalardan erginleşen bireyler alındı. Stok böcek kültürünün devamı bu erginlerin bir bölümü ile sağlandı. Diğer erginler ise triklabendazol ile ilgili deneylerde kullanılacak larvaları elde etmek için ayrıldı.

Böceğin Yaşama ve Gelişmesi Üzerine Triklabendazolun Etkisi

Stok kültürden erginleşen sağlıklı ve iyi görünümlü dişiler 30 ml'lik geniş ağızlı, vida kapaklı bir plastik kabın (ORLAB, L190030, 35x55 mm) içine bırakıldı. Dişilerin yumurta bırakması için bir kaç gün beklendi. Bu süre içerisinde bırakılan yumurtaların açılması için kaplar 28±2°C ve %65±5 bağıl nemde bekletildi. Laboratuvarda gerçekleştirilen beslenme deneylerinde bu yumurtaların açılması ile serbest kalan larvalar kullanıldı. Açılan larvalar tel kafes kapaklı cam kavanozların (60x120 mm) içindeki yaklaşık 200 g besinin üzerine bırakıldı. Larvaların kavanozlardaki besinlere aktarılmasında zarar görmemeleri için yumuşak uçlu bir fırça (No: 0, Goya Toray) kullanıldı.

Triklabendazol benzimidazol grubu bir antihelmintiktir ($C_{14}H_9Cl_3N_2OS$) (5-kloro-6-(2,3-diklorofenoksi)-2-(metiltiyo)-1*H*-benzimidazol). Antihelmintik ilacın beslenme deneylerinde denenen miktarları 100 g besine katılacak gram miktar (% a/a) olarak belirlendi ve besin hazırlanırken doğrudan ilave edildi. Triklabendazol içermeyen kontrol besini ve triklabendazolün %0.001, 0.01 ve 0.1'ini içeren besinler olmak üzere dört besin denendi. Çalışmada denenen triklabendazol konsantrasyonları *G. mellonella* [21-23] ve bazı parazitoid böcek türleri üzerinde antibiyotiklerin etkisinin araştırıldığı önceki çalışmalara göre tespit edildi [11-13]. Ayrıca daha önce antihelmintik niklozamid ile ilgili yapılan çalışma da dikkate alındı [17]. Bu çalışmalardan yola çıkarak öncelikle denenecek konsantrasyonların aralığını belirlemek amacıyla ön beslenme deneyleri yürütüldü. Bunun için en düşük %0.001 ve en yüksek %3.5 oranlarındaki aralıklarda triklabendazol besinlere katılarak biyolojik parametreler üzerindeki etkilerine bakıldı. Yaşama ve gelişme üzerine etkilerinin belirlenebilmesi için larvaların ergin evreye kadar gelişebildiği konsantrasyon aralıkları tespit edildi. Daha sonra bu konsantrasyonların böceğin gelişim evrelerindeki yaşama oranı ve gelişme süresine etkisi tespit edildi.

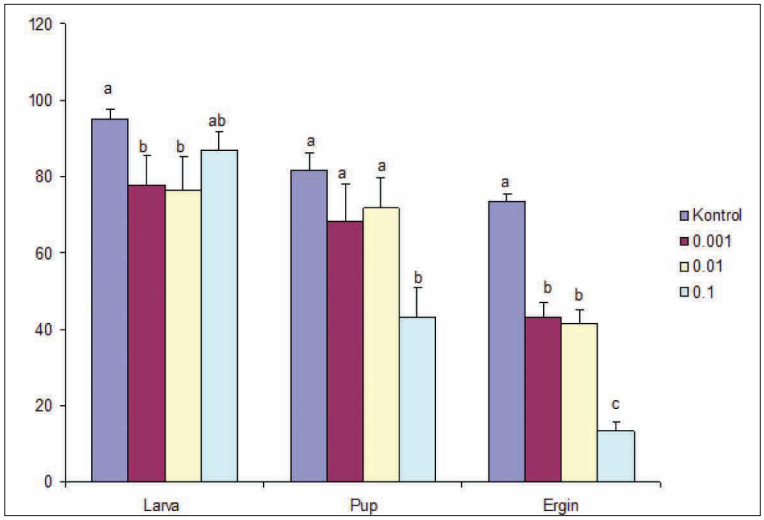
Beslenme deneylerinde antihelmintik maddenin miktarlarını içeren her bir besin ve kontrol besini için 20 adet birinci evre larvası seçilerek besinlere bırakıldı. Her deney dört defa tekrar edildi. Yedinci (7. evre) evreye ulaşan larvalar besinden alınarak pupa olmaları için içlerinde katlanmış, ince pelur kağıt bulunan 30 ml'lik plastik örnek kaplarına (ORLAB, L190030, 35x55 mm) teker teker bırakıldı. Kavanozlara pelur kağıtlar larvaların pupa olmaları için kuru ortam sağlamak amacıyla konuldu. Pupa evresine ulaşan ve pupalardan erginleşen bireylerin sayısı belirlendi, yüzde oranları hesaplandı ve bu evrelere ulaşan bireylerin ortalama gelişme süresi ayrıca hesaplandı.

Triklabendazolun farklı konsantrasyonlarını içeren besinlerdeki *G. mellonella'nın* yaşama oranı ve gelişimi üzerindeki etkileri 7. larval evre, pupa ve ergin evreye ulaşan bireylerin yüzdesi ve bu evreye ulaşmak için geçen ortalama süre (gün) belirlendi. Gelişme süresi ile ilgili verilerin değerlendirilmesinde, tek yönlü "Varyans Analizi" [44] testi yapıldı. Ortalamalar arasındaki farkın önemini saptamak için "LSD Testi" [44] uygulandı. Yaşama ile ilgili verilerin değerlendirilmesinde ise "$\chi 2$ (Chi square) Testi" [45] kullanıldı. 0.05 olasılık düzeyinde ortalamaların önemi incelendi.

BULGULAR

Triklabendazolün denenen en yüksek konsantrasyonu hariç besine ilave edilen miktarlarının yedinci evreye (7. evre) kadar gelişen larvaların oranını önemli derecede düşürdüğü tespit edildi. Kontrol besini ile karşılaştırıldığında bu antihelmintik ilacın belirtilen düşük konsantrasyonları (0.001 ve 0.01 g/100 g besin) besine katılan en düşük konsantrasyondan itibaren son evre larva oranını önemli derecede düşürdü ($P<0.05$). Triklabendazol larval yaşama oranında olduğu gibi en yüksek besinsel konsantrasyonda ergin evreye ulaşma oranını da önemli derecede düşürdü. Triklabendazol içermeyen kontrol besininde larvaların %94.8±2.77'si 7. evreye ulaşırken, besine %0.01 oranında triklabendazol ilave edildiğinde larvaların %77.9±7.45'inin 7. evreye ulaşması sağlamış olup kontrole göre yaşama oranı önemli derecede azaldı ($P<0.05$). Kontrol besini ile yetiştirilen larvaların %81.6±4.32'si pupa olurken %73.3±2.36'sı ise ergin olabildi. Triklabendazolün en düşük besinsel miktarı ise pupa ve ergin evredeki yaşama oranını sırasıyla %68.3±9.83 ve 43.3±3.72'ye kadar düşürdüğü halde pupa olma oranında kontrole göre istatistiksel olarak önemli bir fark oluşmadı. Besine ilave edilen triklabendazol miktarı 10 katı artırıldığında (0.01 g/100 g besin) besinle beslenen larvaların %76.6±8.34'ü 7 evreye ulaşarak kontrol besinine göre yaklaşık %30'luk bir azalma kaydedildi. Triklabendazolun %0.01 oranı erginleşen bireylerin yüzdesini önemli derecede azaltarak, yaşama oranını %41.6±3.63'e düşürdü ($P<0.05$) *(Şekil 1)*. Antihelmintik maddenin besindeki %0.1 oranı böceğin larval evredeki yaşama oranı dışında diğer gelişme evrelerindeki yaşama oranını önemli derecede düşürdü ($P<0.05$). Antihelmintik maddenin bu konsantrasyonu pupa olma oranını %81.6±4.32'den 43.3±7.45'e, ergin olma oranını %73.3±2.36'dan 13.3±2.36'ya azalttı.

Antihelmintik maddenin besine ilave edilen düşük miktarlarının larvaların yedinci evreye (7. evre) kadar gelişmeleri için gerekli olan süre üzerinde önemli derecede etkili olmadığı tespit edildi. Triklabendazol, %0.001 ve 0.01 oranında besine ilave edildiğinde, larvalar kontrol besinine göre sırasıyla yaklaşık 1.8 ve 1.0 gün daha geç 7. evreye ulaşmalarına rağmen gelişme süresindeki uzama

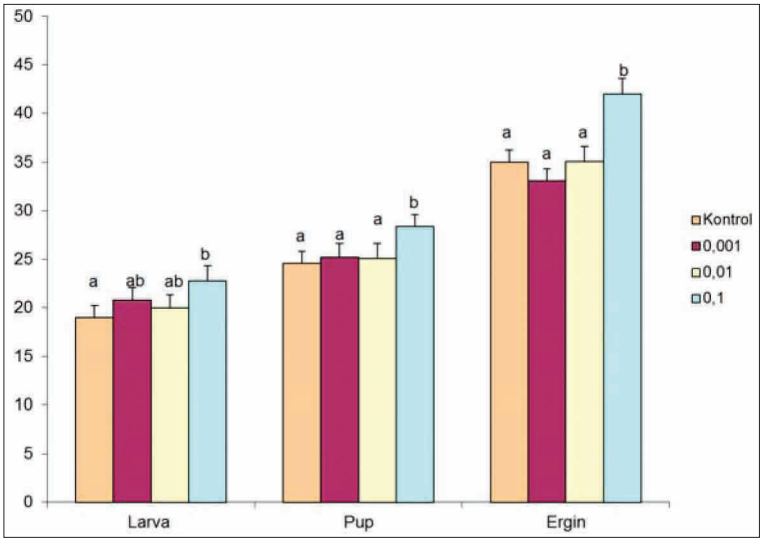


Şekil 1. *G. mellonella* larvalarının yaşama oranına triklabendazolün etkisi. Veriler dört tekrarın ortalaması olarak verilmiştir, her tekrarda yirmi larva kullanılmıştır. Aynı evrede farklı harfi içeren ortalamalar birbirinden farklıdır ($P<0.05$) (c^2 testi). (a-b) 7. larval evre ve ergin evredeki yaşama oranında kontrol ile triklabendazolün %0.001 ve 0.01'lik konsantrasyonlarının karşılaştırılması ($P<0.05$) ve pup evresindeki yaşama oranında kontrol ile triklabendazolün %0.1'lik konsantrasyonun karşılaştırılması ($P<0.05$). (a-c) Ergin olma oranında kontrol ile triklabendazolün % 0.1'lik konsantrasyonun karşılaştırılması ($P<0.05$).

Fig 1. The effect of triclabendazole on survival rate of *G. mellonella* larvae. Data are the average of four replicates, with 20 larvae per replicate. Values followed by the same letter in the same stage are significantly different from each other (P<0.05) (c^2 test). (a-b) Comparison of 0.001 and 0.01% of triclabendazole with control in survival rate of seventh instar and adult stage (P<0.05), and comparison of 0.1% of triclabendazole with control in pupation rate (P<0.05). (a-c) comparison of 0.1% of triclabendazole with control in survival rate of adult stage (P<0.05)

Şekil 2. *G. mellonella* larvalarının gelişme süresi üzerine triklabendazolün etkisi. Veriler dört tekrarın ortalaması olarak verilmiştir, her tekrarda yirmi larva kullanılmıştır. Aynı evrede farklı harfi içeren ortalamalar birbirinden farklıdır ($P<0.05$) (LSD Testi). (a-b) 7. larval evre, pupa ve ergin evreye gelişme süresinde kontrol ile triklabendazolün %0.1'lik konsantrasyonun karşılaştırılması ($P<0.05$)

Fig 2. The effect of triclabendazole on developmental time of *G. mellonella* larvae. Data are the average of four replicates, with 20 larvae per replicate. Values followed by the same letter in the same stage are significantly different from each other (P<0.05) (LSD Testi). (a-b) Comparison of 0.1% of triclabendazole with control in developmental time to seventh instar, pupal and adult stage (P<0.05)



istatistiksel olarak önemli bir fark oluşturmadı ($P>0.05$). Buna göre kontrol besini ile yetiştirilen larvalar ortalama 19.0±1.22 günde son larval evreye ulaşırken %0.001 ve 0.01 oranında antihelmintik içeren besinlerde larvalar ortalama 20 ve 21 günde son larval evreye ulaştı. Triklabendazolün besindeki en yüksek konsantrasyonu 7. evreye ulaşma süresini yaklaşık 3.8 gün geciktirerek 22.8±1.54 güne uzattı. Kontrol besini ile karşılaştırıldığında antihelmintik maddenin besine katılan en düşük konsantrasyonu (%0.001) pupa evresine ulaşma süresini önemli derecede etkilemezken ergin evreye ulaşma süresini önemli olmayan derecede kısalttı. Antihelmintik maddenin bu konsantrasyonunu içeren besinde larvalar 25.2±1.43 günde pupa evresine, 33.1±1.27 günde ergin evreye ulaştı.

Triklabendazol içermeyen kontrol besininde larvalar 24.6±1.24 günde pupa, 35.0±1.27 günde ergin olurken besine %0.01 oranında triklabendazol ilave edildiğinde larvalar 25.1±1.54 günde pupa, 35.1±1.54 günde ise ergin oldu. Kontrol besininine %0.1 oranında triklabendazol ilave edildiğinde ise 7. larva evresine, pupa ve ergin evreye ulaşma süresinin kontrol besinine göre önemli derecede uzadığı tespit edildi ($P<0.05$). Bu besin kontrol besinindeki 24.6±1.24 olan pupa olma süresini 28.4±1.24 güne, 35.3±1.27 olan ergin olma süresini 42.0±1.62 güne uzattı. Gelişme süresindeki uzama pupa evresine ulaşma süresinde yaklaşık 4 gün, ergin evreye ulaşma süresinde ise yaklaşık 7 gün olarak belirlendi *(Şekil 2)*.

TARTIŞMA ve SONUÇ

Bu çalışmada triklabendazolün *G. mellonella*'nın biyolojisi üzerindeki etkisinin böceğin gelişme evrelerine ve denenen antihelmintik maddenin konsantrasyonlarına göre değiştiği tespit edildi. Triklabendazolun yüksek konsantrasyonlarının *G. mellonella* larvalarının ergin evreye doğru yaşama oranını düşürdüğü, gelişme süresini uzattığı belirlendi. Triklabendazolun *G. mellonella* üzerindeki etkilerine benzer etkiler diğer takımlara ait böcekler üzerinde bazı antibiyotikler ile yapılan çalışmalar ile de elde edilmiştir. Örneğin, yumurta endoparazitoidi olan *Trichogramma* türleri ile yapılan önceki araştırmalar yapay besin ortamlarına küf ve mantar kontaminasyonunu

önlemek için ilave edilen nistatin, sodyum benzoat ve metil p-hidroksibenzoat gibi bazı geleneksel antifungallerin bırakılan yumurtaların açılma oranını azalttığı, farklı gelişme evrelerindeki ölüm oranını artırdığını göstermiştir [9,46,47]. Bu sonuçlara benzer şekilde, denenen triklabendazolun yüksek konsantrasyonları *G. mellonella*'nın hem larval gelişme hem de larva sonrası gelişme evrelerinde etkili olduğu, böceğin tüm gelişme evrelerindeki yaşama oranını ve gelişme süresini olumsuz yönde etkilediği tespit edildi.

Besine ilave edilen triklabendazol konsantrasyonlarının besinin kimyasal ve fiziksel bileşimini etkilemesi ve bunun sonucunda besinsel kaliteyi değiştirmesi beklenebilir. Besinsel içeriklerin oranlarının değişmesi ile larvaların beslenme davranışı değişmiş ve buna bağlı olarak böceğin biyolojik özellikleri olumsuz yönde etkilenmiş olabilir. Slansky ve Scriber'ın [48] yapay besin ortamlarının yapısının bozulması sonucu besin kalitesinin azalmasının besinle beslenen larvalardan oluşan erginlerin biyolojik özelliklerini olumsuz etkilediğini belirtmesi bizim görüşümüzü desteklemektedir. Elde edilen bulgularımızı toksikolojik açıdan destekleyen diğer bir çalışma ise kimyasal yapısı bozulmuş doğal besin ile yürütülmüştür. Belirtilen doğal besin ile beslenen bir örümcek türü *Pardosa prativaga* (L. Koch)'da, besin bileşenlerinin oksidatif hasarına bağlı olarak artan oksidatif stres sonucu detoksifikasyon enzimi glutatyon *S*-transferaz (GST)'ın aktivitesinin azaldığı ileri sürülmüştür [49].

Ekonomik öneme sahip böceklerin laboratuvar şartlarında yapay besinler ile yetiştirilmeleri sırasında olası mikrobiyal kontaminasyonların önlenmesi amacıyla besinlere ilave edilen antimikrobiyal maddelerin böcekler üzerinde bazı olumsuz etkilerine rastlanmıştır [50,51]. Bu konuda en önemli bir çalışma Singh ve House [4] tarafından yapılmıştır. Bir dipter tür olan *Agria affinis* (Fall.) (Diptera: Sarcophagidae)'in farklı antibakteriyel ve antifungalleri içeren besinler ile yetiştirilmesi sonucunda böceklerin son larva evresine gelişmesinin geciktiği, pupa ve erginlerin oranının azaldığı gözlenmiştir. Benzer etkiler Diptera takımına ait diğer bir böcek *Phryxe caudata* (Rondani) (Diptera: Tachinidae) ile de elde edilmiştir [7]. Bir parazitik hymenopter olan *Micropilis croceipes* Cresson larvaları ile yapılan beslenme çalışmalarında antifungal bir madde olan manganez etilenbisditiyokarbamat'ın 2.600 ppm'lik konsantrasyonu konak böceklerde yetiştirilen parazitik böceğin larvalarının gelişimini önlemiştir [52]. Ülkemizde antibiyotikler ile ilgili başlangıç niteliğinde bir çalışma Büyükgüzel ve Yazgan tarafından yapılmıştır. Çalışmada kimyasal yapısı bilinen sentetik besine ilave edilen penisilin, streptomisin ve rifampisinin endoparazitoid hymenopter türü olan *P. turionellae*'nın yaşama oranını düşürdüğü ve gelişimini geciktirdiği belirlenmiştir [11]. Belirtilen çalışmalarda çeşitli takımlara ait böcekler üzerinde denenen farklı etki mekanizmalarına sahip antibakteriyel ve antifungallerin böceklerin biyolojik parametreleri üzerindeki olumsuz etkileri benzer olmuştur. Bu çalışmada antimikrobiyal maddelerden farklı yapı ve etki mekanizmasına sahip antihelmintik triklabendazol *G. mellonella*'nın yaşama oranı ve gelişmesi üzerine benzer olumsuz etki yapmıştır. Olumsuz etkilerin farklı böcek takımlarına ve antibiyotiklerin asıl etki mekanizmalarına göre değişmediği açıkça görülmekte olup triklabendazol de dahil tüm klinik öneme sahip maddelerin böceklerde etki mekanizmaları dışında farklı bir mekanizma ile etkili olduğu düşünülmektedir. Bu düşüncenin doğrulanması için detaylı çalışmalara ihtiyaç bulunmaktadır.

Timmermann ve ark.[53] besin bileşimlerinin birbirleri ile veya bunların besin katkı maddeleri ile etkileşiminin besin kalitesini bozduğunu buna bağlı olarak oluşan serbest radikallerin oksidatif etkisinden böceklerin biyolojik ve fizyolojik aktivitelerinin etkilendiğini açıklamışlardır. Cohen ve Crittenden [54] böceklerin biyolojik yaşam parametrelerinin olumsuz etkilenmesinin çoğunlukla serbest radikallerin toksisitesine bağlı olduğunu açıklamışlardır. Bu durumun böceklerin besin tüketim oranını da değiştirdiğini belirtilmiştir. Besinsel olmayan bir katkı maddesi olarak triklabendazol çalışmada kullanılan besinin kalitesini herhangi bir şekilde olumsuz yönde değiştirmiş olabilir. Antihelmintik bir madde olan niklozamid ile *G. mellonellla* üzerinde yapılan önceki beslenme çalışması, maddenin böceğin yaşama ve gelişim evrelerindeki olumsuz etkilerinin oksidatif stres ile ilişkili olabileceğini göstermiştir [17]. Triklabendazol ile yürütülen bu çalışmada da olumsuz etkilerin oksidatif stres ile ilişkilendirilebilmesi için ilave deneylere ihtiyaç bulunmaktadır. Diğer taraftan bazı antibiyotikler ile *G. mellonella* üzerinde yapılan çalışmalarda görüşümüzü destekleyen sonuçlar elde edilmiştir. Büyükgüzel ve Kalender [21-23] tarafından yürütülen böcek beslenmesi çalışmalarında insan ve hayvan hastalıklarının tedavisinde kullanılan antibiyotiklerden penisilin ve streptomisinin *G. mellonella*'nın yaşama, gelişme, vücut ağırlığı ve total protein miktarını etkilediği ortaya çıkarılmıştır. Etkiler böceğin gelişme evreleri ile denenen antibiyotiklerin türü, yapısı ve besinsel konsantrasyonuna göre değişmiştir. Çalışmalarda ayrıca antibiyotiklerin böceğin biyolojisi üzerine olan olumsuz etkileri ile ilişkili olarak *G. mellonella* larvalarının orta bağırsağında lipid peroksidasyonu ürünü malondialdehit (MDA) miktarı ve antioksidan enzimlerden süperoksit dismutaz (SOD), katalaz (CAT), GST, glutatyon peroksidaz (GPx) aktivitelerinin böceğin larval evrelerine (3-7 evreler) göre değiştiği belirlenmiştir [21,22].

Triklabendazol geniş spektrumlu, sentetik bir antihelmintik ilaçtır. İnsan ve hayvanlardaki paraziter enfeksiyonların tedavisinde kullanılmaktadır. Triklabendazolun çeşitli helmint türlerinde yumurta ölümüne neden olduğu ve açılmasını engellediği bilinmektedir. Bu antihelmintik madde duyarlı helmintlerin hücrelerinde tübülin alt birimine bağlanarak mikrotübüller halinde polimerizasyonunu inhibe eder. Mikrotübüllerin kaybı glukoz alımını ve ATP yapımını azaltır ve glikojen rezervlerini tüketir. Parazitler ATP üretimi için yeterli enerji kaynağına

sahip olamaz ve çoğalamazlar veya canlılıklarını kaybederler [55]. Tübülin memeli konakların hücrelerinde de bulunmasına rağmen benzimidazoller parazit tübülinlerine yüz kat daha fazla afinite ile bağlanırlar. Bu nedenle memeli hücrelerine toksisiteleri minimal düzeydedir. Triklabendazolun *G. mellonella*'nın yaşama ve gelişmesi üzerinde olumsuz etki göstermesi tarımsal zararlı böceklerin mücadelesi açısından değerlendirildiğinde ümit verici bir sonuçtur. Ayrıca triklabendazolun parazit tübülinlerine memeli konak organizmalarınkine göre yüz kat daha fazla ilgi ile bağlanması sebebiyle insan dahil hedef olmayan oganizmalara etkilerinin düşük olabileceğini göstermektedir. Buna karşılık model olarak üzerinde çalıştığımız zararlı böcek ile mücadelede kullanılan kimyasallar ve zararlı böceklerin kontrolünde yaygın kullanılan organofosfatlı insektisitler omurgasızlardan omurgalılara kadar tüm hayvanlar için ortak olan sinir sistemini bozarak etki göstermektedir [18]. Triklabendazolun böceklerdeki metabolizması bilinmemekte olup muhtemelen memelilerdeki metabolitleri olan triklabendazol sülfoksit ve triklabendazol sülfon veya sindirim kanalındaki oksidasyon ürünleri ile [39,40] böcekte etkisini gösterebilir. Ancak konunun aydınlatılabilmesi için ilave çalışmalara ihtiyaç bulunmaktadır.

Triklabendazolun *G. mellonella*'nın biyolojisine etkisinin araştırıldığı bu çalışmada, böceğin yaşama oranı ve gelişme süresinin olumsuz etkilendiği tespit edildi. Yaşamsal parametrelerin triklabendazolun insektisit olarak kullanılabilirliğinin incelenmesinde önemli kriterler olduğu anlaşılmıştır. Larval evrede alınan besinin larva sonrası evrelerde ve özellikle erginlerde biyolojik ve fizyolojik özellikleri etkilediği göz önüne alındığında besinle alınan triklabendazolun yüksek konsantrasyonlarda böceğin biyolojik özelliklerini olumsuz etkilemesi normaldir. Ancak besinsel etkileşim dışında hangi mekanizmayla olumsuz etki gösterdiğini saptamak amacıyla ilave çalışmalar yapılmalıdır. Diğer yandan triklabendazol asıl etkisini parazit hücrelerinlerde mikrotübül polimerizasyonunu inhibe ederek gösterdiği için hedef olmayan insan dahil yüksek organizasyonlu ökaryotik canlılarda benzer etki mekanizmasını göstermesi ihtimali düşüktür. Böyle bir durumda antihelmintiklerin asıl etki mekanizmaları dışında başka bir etki mekanizması ile böcekler üzerinde etkili olduğu düşünülmektedir. Besinlere antimikrobiyal maddeler ilave edilerek yapılan daha önceki çalışmalar [16,22,56] bu maddelerin böceğin yaşama ve gelişmesi üzerindeki asıl etkilerinin belirlenmesinde böceklerin farklı evrelerindeki bireylerin dokularında biyokimyasal analizlerinin yapılması ve fizyolojik değişimlerin ortaya konulmasının gerektiğini ortaya çıkarmıştır.

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KAYNAKLAR

1. Yazgan Ş: A chemically-defined synthetic diet and larval nutritional requirements of the endoparasitoid *Itoplectis conquisitor (Hymenoptera)*. *J Insect Physiol*, 18, 2123-2141, 1972. DOI: 10.1016/0022-1910(72)90244-2

2. Yazgan Ş: A meridic diet and quantitative effects of tween 80, fatty acid mixtures and inorganic salts on development and survival of the endoparasitoid *Pimpla turionellae*. *Z Angew Ent*omol, 91, 433-441, 1981. DOI: 10.1111/j.1439-0418.1981.tb04501.x

3. Kulkarni N, Kushwaha DK, Mishara VK, Paunikar S: Effect of economical modification in artificial diet of Greater wax moth *Galleria mellonella* (Lepidoptera: Pyralidae). *Indian J Entomol*, 74 (4): 369-374, 2012.

4. Singh P, House HL: Antimicrobials safe levels in a synthetic diet of an insect, *Agria affinis*. *J Insect Physiol*, 16, 1769-1782,1970. DOI: 10.1016/0022-1910(70)90275-1

5. Singh P, House HL: Effects of streptomycin and potassium sorbate levels in relation to nutrient levels on the larvae of *Agria affinis*. *J Econ Entomol*, 63, 449-454, 1970. DOI: 10.1093/jee/63.2.449

6. Singh P, House HL: Antimicrobial agents: Their detrimental effects on size of an insect, *Agria affinis*. *Can Entomol*, 102, 1340-1344, 1970. DOI: 10.4039/Ent1021340-10

7. Grenier S: Effects nocif de la nipagine M.sur le parasitoide *Phryxe caudata* [Dipt.: Tachinidae]. *Entomophaga*, 22, 223-236, 1977. DOI: 10.1007/BF02377847

8. Thompson SN: Nutrition and *In vitro* culture of insect parasitoids. *Ann Rev Entomol*, 31, 197-220, 1986. DOI: 10.1146/annurev.en.31.010186.001213

9. Xie ZN, Nettles Jr CW, Morrison RK, Irie K, Vinson SB: Three methods for the *In vitro* culture of *Trichogramma pretiosum* Riley. *J Entomol Sci*, 21 (2): 133-138, 1986.

10. Rotundo G, Cavalloro R, Tremblay E: *In vitro* rearing of *Lysiphlebus fabarum* (Hym.: Braconidae). *Entomophaga*, 33, 261-267, 1988. DOI: 10.1007/BF02372615

11. Büyükgüzel K, Yazgan Ş: Bazı antibiyotiklerin endoparazitoid *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae)'nın yaşama ve gelişimine etkileri. *Turk J Zool*, 20, 1-7,1996.

12. Büyükgüzel K, Yazgan Ş: Combinational effects of some antimicrobial agents on the survival and development of the endoparasitoid *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae). *Commun Fac Sci Univ Ank (Séries C.)*, 48, 1-14, 1999. DOI: 10.1501/Commuc_0000000090

13. Büyükgüzel K: Positive effects of some gyrase inhibitors on survival and development of *Pimpla turionellae* (Hymenoptera: Ichneumonidae) larvae reared on an artificial diet. *J Econ Entomol*, 94 (1): 21-26, 2001. DOI: 10.1603/0022-0493-94.1.21

14. Büyükgüzel K: DNA gyrase inhibitors: Novobiocin enhances the survival of *Pimpla turionellae* larvae reared on an artificial diet but other antibiotics do not. *J Appl Entomol*, 125, 583-587, 2001. DOI: 10.1046/j.1439-0418.2001.00585.x

15. Büyükgüzel K: Antimicrobial agents: Their combined effects on total protein content of the endoparasitoid *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae). *Turk J Zool*, 26, 229-237, 2002.

16. Büyükgüzel K, İçen E: Effects of gyrase inhibitors on the total protein content of *Pimpla turionellae* (Hymenoptera: Ichneumonidae) larvae reared on an artificial diet. *J Entomol Sci*, 39, 108-116, 2004.

17. Büyükgüzel E, Kayaoğlu S: The effect of niclosamide on some biological and physiological aspects of *Galleria mellonella* L. (Lepidoptera: Pyralidae). *Türk Entomol Derg*, 38 (1): 83-99, 2014.

18. Charriere JD, Imdorf A: Protection of honey combs from moth damage. Swiss Bee Research Center Federal Dairy Research Station. *Comm*, 24, 1-14, 1997.

19. Jarosz J: Use of oxytetracycline-nystatin combination in obtaining germ-free larvae of *Galleria mellonella* for gnotobiotic studies. *Cytobios*, 32, 107-120, 1981.

20. Jarosz J: Simplified technique for preparing germ-free specimens of greater wax moth (Lepidoptera: Pyralidae) larvae. *J Econ Entomol*, 82,

1478-1481, 1989. DOI: 10.1093/jee/82.5.1478

21. Büyükgüzel E, Kalender Y: Penicillin-induced oxidative stress: effects on antioxidative response of midgut tissues in larval instars of *G. mellonella*. *J Econ Entomol*, 100, 1533-1541, 2007. DOI: 10.1093/jee/100.5.1533

22. Büyükgüzel E, Kalender Y: *Galleria mellonella* survivorship, development and protein content in response to dietary antibiotics. *J Entomol Sci*, 43, 27-40, 2008.

23. Büyükgüzel E, Kalender Y: Exposure to streptomycin alters oxidative and antioxidative response in larval midgut tissues of *Galleria mellonella*. *Pest Biochem Physiol,* 94, 112-118, 2009. DOI: 10.1016/j.pestbp.2009.04.008

24. Mukherjee K, Raju R, Fischer R, Vilcinskas A: *Galleria mellonella* as a model host to study gut microbe homeostasis and brain ınfection by the human pathogen *Listeria monocytogenes*. *Adv Biochem Eng Biotechnol,* 135, 27-39, 2013. DOI: 10.1007/10_2013_203

25. Kuhlmann I: The prophylactic use of antibiotics in cell culture. *Cytotechnology*, 19, 95-105, 1996. DOI: 10.1007/BF00749764

26. Alverson J, Cohen AC: Effect of antifungal agents on biological fitness of *Lygus hesperus* (Heteroptera: Miridae). *J Econ Entomol,* 95, 256-260, 2002. DOI: 10.1603/0022-0493-95.2.256

27. Ruan YM, Xu J, Liu SS: Effects of antibiotics on fitness of the B. biotype and a non-B. biotype of the whitefly *Bemisia tabaci*. *Entomol Exp Appl,* 121, 159-166, 2006. DOI: 10.1111/j.1570-8703.2006.00466.x

28. Boray JC, Crowfoot PD, Strong MB, Allison JR, Schellenbaum M, Von Orelli M, Sarasin G: Treatment of immature and mature *Fasciola hepatica* infections in sheep with triclabendazole. *Vet Rec,* 113, 315-317, 1983. DOI: 10.1136/vr.113.14.315

29. Fisher MA, Jacobs DE, Hutchinson MJ, Abbott EM: Efficacy of fenbendazole and piperazine against developing stages of *Toxocara* and *Toxascaris* in dogs. *Vet Rec,* 132, 473-475, 1993. DOI: 10.1136/vr.132.19.473

30. Calvopina M, Guderian RH, Paredes W, Cooper PJ: Comparison of two single day regimens of triclabendazole for the treatment of human pulmonary paragonimiasis. *Trans R Soc Trop Med Hyg,* 97, 451-454, 2003. DOI: 10.1016/S0035-9203(03)90088-5

31. Keiser J, Engels D, Büscher G, Utzinger J: Triclabendazole for the treatment of fascioliasis and paragonimiasis. *Expert Opin Investig Drugs*, 14, 1513-1526, 2005. DOI: 10.1517/13543784.14.12.1513

32. McKellar QA, Scott EW: The benzimidazole anthelmintic agents - A review. *J Vet Pharmacol Ther,* 13, 223-247, 1990. DOI: 10.1111/j.1365-2885.1990.tb00773.x

33. Lubega GW, Prichard RK: Specific interaction of benzimidazole anthelmintics with tubulin from developing stages of thiabendazole-susceptible and -resistant *Haemonchus contortus*. *Biochem Pharmacol* 41, 93-101, 1991.

34. Martin RJ: Modes of action of anthelmintic drugs. *Vet J,* 154, 11-34, 1997. DOI: 10.1016/S1090-0233(05)80005-X

35. Lacey E, Brady RL, Prichard RK, Watson TR: Comparison of inhibition of polymerisation of mammalian tubulin and helminth ovicidal activity by benzimidazole carbamates. *Vet Parasitol,* 23, 105-119, 1987. DOI: 10.1016/0304-4017(87)90029-X

36. Shompole S, Yao C, Cheng X, Knox D, Johnson S, Jasmer DP: Distinct characteristics of two intestinal protein compartments discriminated by using fenbendazole and a benzimidazole resistant isolate of *Haemonchus contortus*. *Exp Parasitol,* 101, 200-209, 2002. DOI: 10.1016/S0014-4894(02)00135-2

37. Schmidt J: Effects of benzimidazole anthelmintics as microtubule-active drugs on the synthesis and transport of surface glycoconjugates in *Hymenolepis microstoma, Echinostoma caproni*, and *Schistosoma mansoni*. *Parasitol Res,* 84, 362-368, 1998. DOI: 10.1007/s004360050411

38. Cumino AC, Elissondo MC, Denegri GM: Flubendazole interferes with a wide spectrum of cell homeostatic mechanisms in *Echinococcus granulosus* protoscoleces. *Parasitol Int*, 58, 270-277, 2009. DOI: 10.1016/j.parint.2009.03.005

39. Apt W, Aguilera X, Vega F, Miranda C, Zulantay I, Perez C, Gabor M, Apt P: Treatment of human chronic fascioliasis with triclabendazole: Drug efficacy and serologic response. *Am J Trop Med Hyg,* 19, 532-535, 1995.

40. Sanyal PK: Pharmacokinetic study of triclabendazole in sheep and goat using a high performance liquid chromatography method. *Indian J Pharmacol,* 22, 200-203, 1994.

41. Bronskill J: A cage to simplify the rearing of the greater wax moth, *Galleria mellonella* (Pralidae). *J Lep Soc,* 15 (2): 102-104, 1961.

42. Ortel J: Accumulation of cd and pb in successive stages of *Galleria mellonella* and metal transfer to the pupal parasitoid *Pimpla turionellae*. *Entomol Exp Appl*, 77, 89-97, 1995. DOI: 10.1111/j.1570-7458.1995.tb01989.x

43. Campos F, Donskov N, Arnason JT, Philogene BJR, Atkinson PM, Werstiuk NH: Biological effects and toxicokinetics of DIMBOA in *Diadegma terebrans* (Hymenoptera: Ichneumonidae), an endoparasitoid of *Ostrina nubilalis* (Lepidoptera: Pyralide). *J Econ Entomol*, 83, 356-360, 1990. DOI: 10.1093/jee/83.2.356

44. SPSS: User's Manual. Version 10. SPSS, Chicago, IL., 1997.

45. Snedecor GS, Cochran WG: Statistical Methods. Iowa State University Press, 8th ed., 158-160, Ames, IA. 1989.

46. Grenier S, Liu WH : Antifungals: Mold control and safe levels in artificial media for *Trichogramma* [Hymenoptera: Trichogrammatidae]. *Entomophaga*, 35, 283-291, 1990. DOI: 10.1007/BF02374804

47. Grenier S, Liu WH: Mold control and safe levels of antifungals in artificial media for egg parasitoids *(Hymenoptera)*. *Les Colloques de l'INRA*, 56, 141-144, 1991.

48. Slansky JrF, Scriber JM: Food consumption and utilization. **In,** Kerkut GA, Gilbert LI (Eds): Comprehensive Insect Physiology, Biochemistry and Pharmacology. 87-163, Pergamon Press, Oxford, 1985.

49. Nielsen SA, Toft S: Responses of a detoxification ezyme to diet quality in the wolf spider. **In,** Toft S, Scharff N (Eds): Pardosa Prativaga. European Arachnolog. 65-70, Aarhus University Press, Aarhus, 2002.

50. Clark AG, Dick GL, Martindale SM, Smith JN: Glutathione s-transferases from the New Zealand grass grub. *Costelytra zealandica*. *Insect Biochem*, 15, 35-44, 1985. DOI: 10.1016/0020-1790(85)90041-1

51. Costa HS, Thomas JH, Nick CT: Effect of antibacterial materials on *Bemisia argentifolii* (Homoptera: Aleyrodidae) oviposition, growth, survival and sex ratio. *J Econ Entomol,* 90, 333-339, 1997. DOI: 10.1093/jee/90.2.333

52. Felton GW, Dahlman DL: Nontarget effect of a fungicide: Toxicity of Maneb to the parasitoid *Microplitis croceipes* (Hymenoptera: Braconidae). *J Econ Entomol*, 77, 847-850, 1984. DOI: 10.1093/jee/77.4.847

53. Timmermann SE, Zangerl AR, Berenbaum MR: Ascorbic and uric acid responses to xanthotoxin ingestion in a generalis and specialist caterpillar. *Arch Insect Biochem Physiol*, 42, 26-36, 1999. DOI: 10.1002/(SICI)1520-6327(199909)42:1<26::AID-ARCH4>3.0.CO;2-G

54. Cohen AC, Crittenden P: Deliberately added and cryptic antioxidants in three artificial diets for insects. *J Econ Entomol*, 97, 265-272, 2004. DOI: 10.1093/jee/97.2.265

55. Diniyanti L, Syahril P, Panusunan L: Current status of the efficacy and effectiveness of albendazole and mebendazole for the treatment of *Ascaris lumbricoides* in North-Western Indonesia. *Asian Pac J Trop Med,* 5, 605-609, 2012. DOI: 10.1016/S1995-7645(12)60125-4

56. Eid MAA, El-Nakkadı AN, Saleh MA: Functional adaptation of silk glands after administration of antibiotic to larvae of *Philosamaia ricini* (Boisd). *Insect Sci Appl*, 10, 139-143, 1989.

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Research Article

Availability, Cyst Characteristics and Hook Morphology of *Echinococcus granulosus* Isolates from Livestock (Cattle, Sheep and Goats) in Central Punjab, Pakistan

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Abstract

Cystic Echinococcosis (CE) is a zoonotic infection caused by larval (metacestode) stages of cestodes belonging to the genus *Echinococcus* and the family *Taeniidae*. The aim of current study was to determine the availability and organ placement of hydatid cysts in some ruminants of Pakistan and to study of rostellar hooks morphology of protoscoleces of *E. granulosus*. A total of 2803 animals comprising 925 sheep, 939 goats and 939 cattle (n=) from both sexes were examined to find out the prevalence of hydatid cysts in different regions of Central Punjab from January to December 2013. The overall prevalence of hydatidosis was determined as 3.24%, 2.44% and 2.44% in examined sheep, goats and cattle, respectively. The localization of hydatid cysts in the livers of infected sheep, goats and cattle was found as 1.4%, 1.17% and 1.17%, respectively while lung localizations were determined as 1.83%, 1.27% and 1.27% in the same order. Among the 33, 25 and 30 examined hydatid cysts in sheep, goats and cattle, 8 (24.2%), 11 (33.3%), 8 (24.2%), 6 (18.1%); 9 (36%), 9 (36%), 4 (16%), 3 (12%) and 10 (33.3%), 11 (36.6%), 4 (13.3%), 5 (16.6%) were characterized as fertile, sterile, calcified and under-developed, respectively. The total number of hooks on protoscoleces was 28.68±3.80 (sheep origin), 26.0±2.59 (goat origin) and 27.70±1.11 (cattle origin). In conclusion our investigation revealed that availability of hydatid cysts is still significantly higher among all examined livestocks.

Keywords: *Hydatid cyst, Protoscoleces, Availability, Cattle, Sheep, Goat, Pakistan*

Pakistan'ın Pencap Eyaletindeki Çiftlik Hayvanlarında (Sığır, Koyun ve Keçi) *Echinococcus granulosus* Izolatlarının Mevcudiyeti, Kist Karakteristiği ve Çengel Morfolojisi

Özet

Kistik Ekinokokkozis (KE) *Taeniidae* ailesinde *Echinococcus* soyuna bağlı sestodların larval (metasestod) dönemlerinin sebep olduğu zoonotic bir enfeksiyondur. Bu çalışmanın amacı, Pakistan'daki bazı ruminantlarda hidatik kistlerin mevcudiyeti ve organ yerleşimini tespit etmek ve *E. granulosus* protoskolekslerinin çengel morfolojisini belirlemekti. Ocak - Aralık 2013 tarihleri arasında Pencap eyaletinin farklı bölgelerindeki 2803 ruminant (925 koyun, 939 keçi ve 939 sığır) hidatik kist yaygınlığını belirlemek için muayene edilmiştir. Hidatidozisin ortalama yaygınlığı koyunlarda %3.24, keçilerde %2.44 ve sığırlarda %2.44 olarak belirlenmiştir. Organlara göre yaygınlık koyun karaciğerinde %1.4, akciğerlerinde %1.83; keçi karaciğerinde %1.17, akciğerlerinde %1.27 iken sığır karaciğerlerinde %1.17 akciğerlerinde %1.27 olarak belirlenmiştir. Koyunlarda muayene edilen 33 hidatik kistin keçilerde ise muayene edilen 25 kistin fertile, steril, kalsifiye ve yeni gelişmekte olma durumları koyunlarda sırasıyla 8 (%24.2), 11 (%33.3), 8 (%24.2), 6 (%18.1) ve keçilerde sırasıyla 9 (%36), 9 (%36), 4 (%16) ve 3 (%12) olarak belirlenmiştir. Sığırlarda muayene edilen 30 hidatik kistin 10'u (%33.3) fertil, 11'i (%36.6) steril, 4'ü (%13.3) kalsifiye ve 5'i (%16.6) de yeni gelişmekte olan kist olarak belirlenmiştir. Protoskolekslerdeki toplam çengel sayısı koyun kistlerinde 28.40±1.72, keçi kistlerinde 21.0±1.06 ve sığır kistlerinde 27.70±1.11 adet olarak belirlenmiştir. Bu çalışma ile çiftlik hayvanlarındaki hidatik kist mevcudiyetinin halen yüksek olduğu belirlenmiştir.

Anahtar sözcükler: *Hidatid kist, Protoskoleks, Yaygınlık, Sığır, Koyun, Keçi, Pakistan*

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INTRODUCTION

Livestock plays a vital role in economic development and play a major role in the life of farmers in developing Asian countries. In the Asian region, livestock contributes a major role to agriculture through draft power, manure, fuel, and as a fertilizer. Parasitism is a significant problem affecting livestock in many parts of the world. The low productivity in livestock sectors is due to the parasitism. The parasitic infestation is very common in Pakistan and responsible for about 26.5 million (Pakistani Rupees) costs annually to livestock sector [1]. Hydatidosis is an important zoonosis and the disease is widespread worldwide [2,3]. In the developed countries such as in North American continent this disease is about 1/100.000 but in developing countries the prevalence was 10% [4]. In the central Asia, echinococcosis results serious health problems such as about 466 million people mostly inhabitants and farmers in Kyrgyzstan, Turkmenistan, Kazakhstan, Tajikistan, Uzbekistan, Afghanistan, Mangolia, Iran, western China and Pakistan are under high risk of *E. granulosus* and *E. multilocularis* as well [5-9]. The strains of *E. granulosus* larvae can also be identified by studying an appropriate principle of protoscolex hook morphology and have been considered by [10-12] the rostellar hooks of protoscolex are positioned in two rows of larger and smaller [13,14].

The objectives of the current study was to determine the availability and organ localizations of hydatid cysts in ruminants of Central Punjab (Sargodha district), Pakistan and to identify the types of cysts on the basis of fertile, sterile, calcified and under-developed configurations as well. Besides, we aimed to study rostellar hooks morphology of protoscoleces from some livestock *E. granulosus* isolates.

MATERIALS and METHODS

Location

Sargodha district has a latitude and longitude of 32° 10′ north and 72° 40′ east respectively, with an average temperature in summer is ranging from 25-49°C and in winter 5-23°C and yearly rainfall of 526 millimeter.

Study Design

The present study was conducted to find out the prevalence of hydatidosis in goats, sheep and cattle. The clinical data on sampled animals were collected from different abattoirs and butcher shops of Central Punjab (Sargodha), Pakistan. A cross-sectional study was conducted to find out the occurrence of hydatidosis. The cysts were collected from infected organs in different abattoirs and butcher shops. These abattoirs were visited twice a week in 2013 (January to December) for the collection of hydatid cysts from lungs and liver of slaughtered sheep, cattle and goats.

Sample Collection and Laboratory Investigations

Following parameters was carried out in order to investigation of hydatid cysts: Prevalence of hydatidosis, organ specificity (lungs and liver) and types of cysts (fertile, sterile, calcified, under-developed).

Postmortem Examination

During postmortem examinations, detected hydatid cysts from liver and lungs were collected and distribution of cysts in different organs was recorded. Hydatid cysts were carefully detached and individually collected (in organ basis) into the clean containers for further cyst description.

Cyst Characteristics and Viability

Cysts were incised with a sterile scalpel blade and the substance (fluid) was poured into the glass Petri dishes and examined. The existence of protoscoleces either closes to the germinal layer in the form of brood capsule or presence in the cyst fluid was considered as indicative of fertility. Fertile cysts were more subjected to viability test. A drop of fluid from cyst containing the protoscoleces were placed on the microscope glass slide and covered with cover slip and observed for amoeboid like peristaltic movements with ×40 objective. For clear vision, a drop of 0.1% aqueous eosin solution was added to equal volume of protoscoleces. Sterile hydatid cysts are characterized by their smooth inner lining, generally with a slight turbidity of the enclosed fluid and usually calcified cyst that produced a rough sound feeling upon opening [15]. Calcified cysts were hard and nodular with at least one internal chamber or had calcified, chalky deposits in the cyst wall. Under-developed cysts were firm in texture and quite small in calibrate (1-2 mm), had a little fluid in the cyst wall and defined germinal layer but no protoscoleces [16].

Morphology of Protoscoleces

Protoscoleces were mounted in polyvinyl-lactophenol and sufficient pressure was applied to the cover slip to flatten but not to damage the hooks. All measurements were made by single person using a calibrated eye-piece micrometer under oil immersion. Three large and small hooks were individually measured and all the hooks were counted from each of 6 protoscoleces from each isolate. Morphometric analysis was done as described by Hobbs *et al.*[13].

Statistical Analysis

The statistical analyses were carried out by using SPSS (Version 18).

RESULTS

Prevalence of Hydatid Cysts

Among the examined 2803 livestock, the overall prevalence of hydatid cysts was determined as 3.24%,

2.44% and 2.44% in sheep, goats and cattle, respectively *(Table 1)*. The statistical analysis (chi-square) showed that there were no differences between the prevalence of hydatid cysts in sheep and the others.

Organ Specificity of Hydatid Cysts

The prevalence of hydatid cysts in sheep liver and lungs was 1.40% and 1.83% while 1.17% and 1.27% of the liver and lungs from goats were found to be infected with hydatid cysts. The distribution of hydatid cysts in the liver and lungs of the examined cattle and goats were designated as 1.17% and 1.27% *(Table 1)*. The analysis indicated that there were no statistical differences in the prevalence of different infected organs from goats, sheep and cattle.

Types of Cysts

Prevalence of cysts was recorded on the basis of fertile, sterile, calcified and under-developed type. In sheep 8 (24.2%), 11 (33.3%), 8 (24.2%) and 6 (18.1%) of the examined hydatid cysts (n=33) were classified as fertile, sterile, calcified and under-developed, respectively. While in goats 9 (36%), 9 (36%), 4 (16%) and 3 (12%) out of 25 and in cattle 10 (33.3%), 11 (36.6%), 4 (13.3%) and 5 (16.6%) out of 30 hydatid cysts were characterized in the same order as in sheep *(Table 2)*. Among all animal species various numbers of cysts selected for comparisons. Cattle showed comparatively maximum number of fertile cysts (n=10) than in sheep and goats. Maximum number of sterile cysts (n=11) were found in sheep and cattle and maximum numbers of calcified cysts (n=8) were observed in sheep. Sheep showed relatively maximum number of under-developed cysts (n=6) while goats showed minimum under-developed cyst. The statistical analysis (chi-square) showed that there were no statistical differences between fertile and sterile cysts also between calcified and under developed cysts ($P<0.05$). While there were statistical differences among the groups ($P>0.05$) *(Table 2)*.

Rostellar Hooks Morphology

In present study the measuring factors were consist of the total number of hooks (NH), their total length (TL) (µm) and blade length (BL) (µm), (in case of small hook blade length was measured in L/m) *(Table 3)*.

Average Number of Hooks (NH)

The average number of hooks on protoscoleces from hydatid cysts in sheep (28.68±3.80), goat (26.0±2.59)

Table 1. *Showing the infection rate of hydatid cysts in examined livestock*
Tablo 1. *İncelenen çiftlik hayvanlarında hidatik kist enfeksiyon oranları*

Animals	Examined (n)	Infected Liver		Infected Lung		Total	
		Inf (n)	Prev (%)	Inf (n)	Prev (%)	Inf (n)	Prev (%)
Sheep	925	13	1.40	17	1.83	30	3.24
		X^2=1.067 P=0.302					
Goat	939	11	1.17	12	1.27	23	2.44
		X^2= 0.087 P=0.768					
Cattle	939	11	1.17	12	1.27	23	2.44
		X^2=0.087 P=0.768					
Total	2803					76	2.71

Table 2. *Cyst types of hydatid cyst in sheep, goats and cattle*
Tablo 2. *Koyun, keçi ve sığırlardaki hidatik kistlerin kist tipleri*

Animals	Total No. of Cysts	Fertile		Sterile		Calcified		Under-developed	
		Cysts (n)	Infected (%)	Cysts (n)	Infected (%)	Cysts (n)	Infected (%)	Cysts (n)	Infected (%)
Sheep	33	8	24.2	11	33.3	8	24.2	6	18.1
		X^2=2.06 P=0.560							
Goat	25	9	36	9	36	4	16	3	12
		X^2=6.56 P=0.087							
Cattle	30	10	33.3	11	36.6	4	13.3	5	16.6
		X^2=6.57 P=0.087							
Total	89	27	30.3[a]	31	34.8[a]	16	17.9[b]	14	15.7[b]
		X^2=12.43 P=0.006							

[a,b] *Different letters represent statistically significant (P>0.05) difference between groups*

Table 3. *Rostellar hooks morphology of protoscoleces of Echinococcus granulosus from various hydatid cyst isolates. Values are given for (Mean±S.E) 10 samples of three reciprocal groups*

Tablo 3. *Çeşitli hidatik kist izolatlarından elde edilen Echinococcus granulosus protoskolekslerinin çengel morfolojisi. Değerler üç karşılıklı gruptan 10 örnek için verilmiştir (Ortalama±S.H)*

Parameters	Mean + S.E			P
	Sheep	Goat	Cattle	
Total Number of Hooks (NH)	28.68±3.80 [a]	26±2.59[b]	27.70±1.11[ab]	**
Large Hook Length (LHL) (µm)	28.15±1.77[a]	27.14± 1.84[a]	25.87±1.92[b]	***
Large Hook Blade Length (LBL) (µm)	9.55±0.89[b]	9.39±0.9[b]	15.22±0.96[a]	***
Small Hook Length (STL) (L/m)	18.62±2.28	17.22±2.08	18.59±2.79	NS
Small Hook Blade Length (SBL) (L/m)	7.31±0.45[b]	7.20±0.84[b]	8.53±0.88[a]	***

NS: *Not significant; ** P<0.01; *** P<0.001*

and cattle origin (27.70±1.11) are shown in *Table 3*. The results showed that the maximum number of hooks were present on protoscoleces from sheep origin and minimum on those from goat origin. The values of NH significantly varied among all examined species. Average number of hooks in goat isolates found significantly varied from other species but showed least variance from NH of sheep isolates. The one way ANOVA applied to the data of total number of hooks on protoscoleces indicated a significant difference among the isolates from all animal species.

Total Large Hook Length (LHL) (µm)

The mean length of large hooks was measured as 28.15±1.77 µm, 27.14±1.84 µm and 25.87±1.92 µm for the protoscoleces from sheep, goats and cattle origin, respectively *(Table 3)*. It is evident from these values that the maximum length of large hooks was recorded in protoscoleces of sheep origin and minimum in those of cattle origin. Total length of large hook (LHL) was significant among all these species (P<0.001).

Large Hook Blade Length (LBL) (µm)

The blade lengths of large hooks on protoscoleces from sheep (9.55±0.89), goats (9.39±0.9) and cattle origin (15.22±0.96) were shown in *Table 3*. It is evident from these values that the LBL was found maximum in cattle origin and minimum in goat origin. Blade length showed significant variation in large hooks (LBL) (P<0.001).

Small Hook Length (SHL) (L/m)

The total length of small hooks measured on protoscoleces from sheep (18.62±2.28), goats (17.22±2.08) and cattle origin (18.59±2.79) was presented in *Table 3*. It is evident from these values that the maximum and minimum values of SHL were observed in sheep and goats origin, respectively. Small hook length (SHL) was not-significant among all species (P>0.05).

Small Hook Blade Length (SBL) (L/m)

The blade length of small hooks on protoscoleces was measured as 7.31±0.45 L/m, 7.20±0.84 L/m and 8.53±0.88 L/m from sheep, goat and cattle origin, respectively *(Table 3)*. It is evident from these values that the SBL was measured maximum in cattle, while minimum in goat origin. There was significant difference among small hook blade lengths (SBL) from all these species (P<0.001).

DISCUSSION

The results of the present study showed the actual prevalence of hydatidosis in sheep (3.24%), goats (2.44%) and cattle (2.44%) in Pakistan. The prevalence of hydatidosis was also reported by various investigators as 49% in buffaloes, 33% in cattle, 14.8% in sheep, and 5.9% in goats in Pakistan. Pal and Jamil [17] reported the hydatidosis prevalence of 31.5%, 1.79% and 5.3% in cattle, goats and sheep, respectively at Rawalpindi abattoir. Our results are different from previously reported those results because they studied before 20 to 25 years and they collected information only from one abattoir from each selected city. Only the prevalence in goats determined in this study was similar with the findings of Iqbal *et al.*[18]. Our results also showed that the location of hydatid cysts in various organs (lungs and liver) differed significantly among sheep, goats and cattle as well as between different organs of the same species of animal. Ahmed *et al.*[19] also reported the prevalence of hydatidosis in liver of sheep (46.74%) and goats (23.28%). The liver was the predominant site of infection in sheep and goats [20]. Tavakoli *et al.*[21] also reported the prevalence of hydatidosis in liver and lung in cows as 4.84% and 4.41%, in sheep as 5.05% and 6.84% respectively. In the present study lung was found to be more infected organ as compared to livers among all animals. The present study revealed that significantly higher occurrence of hydatid cysts (P<0.05) in lungs and liver of sheep than goats and cattle. Similar observations were reported by Getachew *et al.*[22]. They reported that the prevalence of hydatid cyst in sheep was 60% in lung and 36% in liver. In goats, hydatid cysts were recovered from 70% of the lung, 18.5% of the liver. In both sheep and goats the infection was more in lungs, followed by liver.

The results showed that total number of hooks (NH), large hook length (LHL), large hook blade length (LBL) and small hook blade length (SBL) of protoscoleces were varied significantly among all species. Blade length showed non-significant variation in small hook length (STL). The results showed that large hook length (28.15±1.77) and blade length (9.55±0.89) of sheep isolate was similar to sheep strain reported by some other researchers [23,24]. Small hook length (18.62±2.28) and blade length (7.31±0.45) of protoscoleces from sheep isolates in the present study was similar to sheep isolates identified by Gordo and Bandera [10]. Our investigations presented the morphometric analysis of protoscoleces, total number of hooks, large hook length, large hook blade length and small hook and blade length in sheep, goat and cattle origin. Morphological study and statistical analyses showed that hooks morphology is not sufficient for strains identification of *E. granulosus* in Central Punjab, Pakistan. Molecular study is required for describing better criteria for strain identification among different species.

There is a trend of declining prevalence of cystic echinococcosis among livestock likely due to the presence of over 180 local council or municipal owned abattoirs in peri-urban regions. However, in stating this, it is likely that in the present study, the government-run abattoirs that were sampled are more likely to attract livestock from large-scale livestock production facilities that are intensively managed rather than the poorly resourced rural farmer. This study is therefore unlikely to represent the prevalence of hydatid disease of food producing animals in poorly resourced rural communities, which is expected to be significantly higher.

In conclusion, our investigation revealed a mild prevalence of fertile, sterile and under-developed cysts among all animal species. These findings showed variations from previous reports which might be due to geographical distributions. Keeping in view, it is concluded that hydatidosis is still out of control due to stray dogs and their easy approach to the abattoirs, improper disposal of hydatid organs and unhygienic conditions of abattoirs in Pakistan. Thus it is suggested that more effort should be done for the prevention of hydatidosis.

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REFERENCES

1. Anwar AH, Buriro SN, Phulan A: A hydatidosis veterinary perspective in Pakistan. *The Veterinarian*, 4, 11-14, 1995.

2. Pednekar RP, Gatne ML: Molecular and morphological characterization of *Echinococcus* from food producing animals in India. *Vet Parasitol*, 165, 58-65, 2009. DOI: 10.1016/J.VETPAR.2009.06.021

3. Ibrahim MM: Study of cystic echinococcosis in slaughtered animals in Al Baha region, Saudi Arabia: Interaction between some biotic and abiotic factors. *Acta Tropica*, 113, 26-33, 2010. DOI: 10.1016/J.ACTATROPICA.2009.08.029

4. Hussain A, Maqbool A, Hussain S, Athar M, Shakoor A, Amin MK: Studies on prevalence and organ specificity of hydatidosis in ruminants slaughtered at Karachi and Faisalabad abattoir, Pakistan. *Ind J Dairy Sci*, 45, 454-456, 1992.

5. Bai Y, Cheng N, Wang Q, Cao D: An epidemiological survey of cystic echinococcosis among Tibetan school pupils in western China. *Ann Trop Paed*, 21, 235-238, 2001. DOI: 10.1080/027249301200777817

6. Craig PS: Epidemiology of human alveolar echinococcosis in China. *Parasitol Int*, 55, 221-225, 2006. DOI: 10.1016/j.parint.2005.11.034

7. Wang Q, Qiu J, Yang W, Schantz PM, Raoul F, Craig PS, Giraudoux P, Vuitton DA: Socioeconomic and behavior risk factors of human alveolar echinococcosis in Tibetan communities in Sichuan, People's Republic of China. *Am J Trop Med Hyg*, 74, 856-862. 2006.

8. Yang YR, Sun T, Li Z, Zhang J, Teng J, Liu X, Liu R, Zhao R, Jones MK, Wang Y, Wen H, Feng X, Zhao Q, Zhao Y, Shi D, Bartholomot B, Vuitton DA, Pleydell D, Giraudoux P, Ito A, Danson MF, Boufana B, Craig PS, Williams GM, McManus DP: Community surveys and risk factor analysis of human alveolar and cystic echinococcosis in Ningxia Hui autonomous region, China. *Bull World Health Organ*, 84, 714-721, 2006.

9. Yu SH, Wang H, Wu XH, Ma X, Liu PY, Liu YF, Zhao YM, Morishima Y, Kawanaka M: Cystic and alveolar echinococcosis: An epidemiological survey in a tibetan population in southeast Qinghai, China. *Jpn J Inf Dis*, 61, 242-246, 2008.

10. Gordo FP, Bandera CC: Differentiation of Spanish strains of *Echinococcus granulosus* using larval rostellar hook morphometry. *Int J Parasitol*, 27, 41-49, 1997. DOI: 10.1016/S0020-7519(96)00173-7

11. Thompson RCA, Boxell AC, Ralston BJ, Constantine CC, Hobbs R, Shury T, Olson ME: Molecular and morphological characterization of *Echinococcus* in cervids from North America. *Parasitology*, 132, 439-447, 2006. DOI: 10.1017/S0031182005009170

12. Latif AA, Tanveer A, Riaz-Ud-Din S, Maqbool A, Qureshi AW: Morphometry of protoscoleces rostellar hooks of *Echinococcus granulosus* isolates from Punjab, Pakistan. *Pak J Sci*, 61, 223-228, 2009.

13. Hobbs RP, Lymbery AJ, Thompson RCA: Rostellar host morphology of *Echinococcus granulosus* (Batsch, 1786) from natural and experimental Australian hosts, and its implication for strain recognition. *Parasitology*, 101, 273-281, 1990.

14. Almeida FB, Rodrigues Silva R, Neves RH, Romani ELS, Machado-Silva JR: Intraspecific variation of *Echinococcus granulosus* in livestock from Peru. *Vet Parasitol*, 143, 50-58, 2007. DOI: 10.1016/J.VETPAR.2009.06.021

15. Parija SC: Medical Parasitology, Protozoology and Helminthology Text and Atlas. 2nd ed., 221-229, Chennai Medical Book Publisher, India, 2004.

16. Simsek S, Koroglu E: Evaluation of enzyme-linked immunosorbent assay (ELISA) and enzyme-linked immunoelectrotransfer blot (EITB) for immunodiagnosis of hydatid diseases in sheep. *Acta Tropica*, 92, 17-24, 2004. DOI: 10.1016/J.ACTATROPICA.2009.08.029

17. Pal RA, Jamil K: Incidence of hydatidosis in goats, sheep and cattle. *Pak Vet J*, 6, 65-69. 1986.

18. Iqbal Z, Hayat CS, Hayat B, Khan MN: Prevalence of organ distribution and economics of hydatidosis in meat animals at Faisalabad abattoir. *Pak Vet J*, 9, 70-74, 1989.

19. Ahmed S, Nawaz M, Gul R, Zakir M, Razzaq A: Some epidemiological aspects of hydatidosis of lungs and livers of sheep and goats in Quetta, Pakistan. *Pak J Zool*, 38, 1-6. 2006.

20. Baswaid SH: Prevalence of hydatid cysts in slaughtered sheep and goats in hadramout (Yemen). *Ass Univ Bul Environ Res*, 10, 67-71, 2007.

21. Tavakoli H, Junaedi JN, Izadi M, Parsa M: Epidemiological investigation of hydatidosis infection in Iran. *18th European Congress of Clinical Microbiology and Infectious Diseases*, 19-22 April, Barcelona, 2008.

22. Getachew D, Almaw G, Terefe G: Occurrence and fertility rates of

hydatid cysts in sheep and goats slaughtered at Modjo Luna Export Slaughter House, Ethiopia. *Eth Vet J*, 16, 83-91, 2012. DOI: 10.4314/evj.v16i1.7

23. Ahmadi N, Dalimi A: Characterization of *Echinococcus granulosus* isolates from human, sheep and camel in Iran. *Inf Gen Evol*, 6, 85-90, 2006. DOI: 10.1016/J.ACTATROPICA.2009.08.029

24. Karimi A, Dianatpour R: Genotypic and phenotypic characterization of *Echinococcus granulosus* of Iran. *Biotechnology*, 7, 757-762, 2008.

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Research Article

Türkiye'de Veteriner Hekimlerin Girişimcilik Düzeyi ve Niyetine Etkili Faktörler [1]

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Özet

Bu çalışmanın amacı Türkiye'de veteriner hekimlerinin girişimcilik düzeyinin belirlenmesi ve girişimcilik niyeti (iş kurma) üzerine etkili faktörlerin tahmin edilmesidir. Girişimcilik düzeyi girişimcilik bileşenleriyle belirlenirken, girişimcilik niyetine (GN) etkili faktörler lojistik regresyon modeliyle (LRM) tahmin edilmiştir. Çalışma, Türkiye'nin 7 farklı bölgesinden Haziran–Aralık 2014 tarihleri arasında tesadüfi örnekleme yöntemiyle seçilen 455 veteriner hekimle bir anket aracılığıyla yürütülmüştür. Araştırma bulgularına göre ortalama girişimcilik skoru (GS) 24.53'dür. Klinisyenlerin kamuda çalışanlardan (P<0.01), babası işveren olanların olmayanlardan (P<0.05), kendi işini kurmak isteyenlerin istemeyenlerden (P<0.01) daha yüksek bir girişimcilik skoruna sahip olduğu bulunmuştur. Türkiye'nin farklı coğrafi bölgeleri arasında GS açısından anlamlı bir farklılık yoktur. LRM P<0.001 düzeyinde anlamlıdır ve modele dâhil edilen değişkenler GN'deki varyasyonun %35'ini açıklamaktadır. GN üzerinde yaş (P<0.001) ve GS (P<0.05) pozitif; eğitim düzeyi ise (P<0.05) negatif bir etkiye sahiptir. GS ve yaştaki bir birimlik artış, girişimcilik niyetinde sırasıyla %6.1 (Odds ratio-OR=1.061) ve %5.5 (OR=1.055) oranında artışa neden olmaktadır. Özel sektörde çalışan veteriner hekimlerde GN kamuda çalışanlara göre 6.1 kat (OR=6.104) daha fazladır. Özellikle genç veteriner hekimlerde GN'ni artırmak için meslek örgütleri, üniversiteler ve özel sektör işbirliğiyle eğitim programları ve mesleki etkinlikler düzenlenebilir.

Anahtar sözcükler: *Girişimci, Lojistik regresyon, Girişimcilik niyeti, Türkiye, Veteriner hekim*

Factors Influencing Entrepreneurship Level and Intention of Veterinarians in Turkey

Abstract

The aims of the study were to determine entrepreneurship level of veterinarians and to estimate factors affecting entrepreneurial intention (to set up business) of them, in Turkey. Entrepreneurship level was determined by entrepreneurial components and the factors influencing entrepreneurial intention (EI) was estimated using the logistic regression model (LRM). The study was conducted in 7 different regions of the Turkey between June 2014 and December 2014 through a questionnaire completed by a total of 455 randomly selected veterinarians. According to the study result, the mean entrepreneurship score (ES) was 24.53. It was found that clinicians have higher ES than public employees (P<0.01), those veterinarians whose fathers had set up their own business have higher ES than those who did not (P<0.05), and those veterinarians who want to set up their own business have higher ES than those who don't want it (P<0.01). There were no significant differences among the different geographical regions of the Turkey in respect to ES. The overall LGM was significant at the P<0.001 level and variables included in the model explained 35% of the variation regarding the EI. Age (P<0.001) and ES (P<0.05) have a positive, whereas education level (P<0.05) has a negative impact on EI. One unit increase in age and ES will lead to 6.1% (Odds ratio-OR=1.061) and 5.5% (OR=1.055) increase in EI, respectively. EI of the veterinarians working at the private sector is higher than 6.1 times (OR=6.104) compared to public employees. In order to increase the EI especially in young veterinarians, training programs and occupational activities could be arranged by the collaboration among professional organizations, universities and the private sector.

Keywords: *Entrepreneur, Logistic regression, Entrepreneurship intention, Turkey, Veterinarian*

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GİRİŞ

Geleneksel olarak "girişimcilik", ekonomik ve sosyal riskler üstlenerek kar amacıyla üretim faktörlerinin bir araya getirilmesi sonucunda mal/hizmet üretilmesi ve pazarlanması olarak tanımlanabilir [1-4]. Cantillon, Baptiste Say ve Schumpeter gibi girişimcilik literatürüne önemli katkılar sağlayan yazarların ise günümüz ihtiyaçlarına da yanıt veren biçimde "yenilik yapma, fırsatları görme ve risk alabilme" kavramlarında birleştikleri dikkati çekmektedir [2,5,6]. Ekonomik büyüme ve gelişmenin sağlanması, işsizliğin önlenmesi ve yenilikçi süreçlerin başlatılmasında çok ciddi roller üstlenen girişimciler; vergi gelirlerine, istihdama ve Gayri Safi Milli Hâsılaya önemli katkılar sağlamaktadır [5,7-9]. Girişimciliğin yalnızca müteşebbisler için değil, kamu ve özel sektörde ücretli çalışanlar için de geçerli olduğunu gösteren "iç girişimcilik" kavramı ise, çalışanların risk alarak örgütlerinin/kurumlarının geleneksel kalıplarına karşılık ürettikleri yeni fikirler, davranışlar, yenilikçi süreçler ve uygulamalar [3,10,11] olarak açıklanmaktadır.

Girişimcilik düzeyi ve niyetine etki eden başlıca faktörler sosyoekonomik ve demografik yapı, gelenekler, aile yapısı ve genetik özellikler, genel ekonomik koşullar ve devletin girişimcilere yönelik uyguladığı politikalardır [2,5,12,13]. Gelir sağlama, iş garantisi, bağımsızlık ve kişisel tatmin girişimciliği motive ederken; girişimcileri harekete geçiren düşüncenin gelişmiş ülkelerde "kendini kanıtlama"; gelişmekte olanlarda ise "kazanç sağlama" olduğu belirtilmektedir. Bu durum kendini Türkiye'de özellikle kendi ve ailesi için bir iş garantisi ve gelir sağlayabilme olarak göstermektedir [7].

Kendisine duyulan ihtiyaç evcilleştirmeyle birlikte başlayan [14] ve eğitim-öğretim alanındaki kurumsallaşması 18. yy'ın ikinci yarısına kadar uzanan [2] veteriner hekimliği mesleği, Dünya Veteriner Hekimler Birliğine üye ülkelerde 500.000 veteriner hekim tarafından icra edilmektedir [15]. Türkiye'de 3.000'i GTHB (Gıda Tarım ve Hayvancılık Bakanlığı) ve 8.500'ü kliniklerde çalışan hekimlere [16] özel sektörün %9 ile %20 arasında değişen payı da [2,17] ilave edildiğinde, bugün için yaklaşık 12.700-14.300 arasında veteriner hekimin mesleki alanda faal olarak çalıştığı anlaşılmaktadır. Ülkemizde 5996 ve 6343 sayılı yasalar [18,19] veteriner hekimlere kamu ve özel sektörde geniş bir yetki ve istihdam alanı sunmaktadır. Türkiye'de veteriner hekimlerin mesleki hak ve sorumlulukları konsey, birlik, oda ve dernekler aracılığıyla korunmakla birlikte; dünyada ekonomik meselelerinin Amerika Birleşik Devletlerindeki (ABD) gibi özel olarak ele alındığı komisyonlar da (The National Commission on Veterinary Economic Issues) mevcuttur [20]. Girişimciliğin sermaye dışındaki değişkenlerden de etkilenmesi [2,5,12,13], Avrupa Birliğindeki (AB) işletmelerin %99'unun küçük ve orta ölçekli olması [7], hayvancılık sektöründeki geniş ürün/hizmet yelpazesi ve kamuda istihdamın belirli bir doygunluğa ulaşması [2,16] günümüzde mesleki girişimciliği daha da önemli kılmaktadır.

Konuya ilişkin çalışmalar incelendiğinde, veteriner hekimlere yönelik bölgesel veya ulusal düzeyde bugüne kadar herhangi bir araştırmanın yapılmadığı; Can [2] tarafından yürütülen çalışmanın ise yerel düzeyde ve veteriner fakültesi öğrencilerine yönelik gerçekleştirildiği görülmektedir. Bu çalışmanın amacı, Türkiye genelinde 7 farklı bölgede kamu ve özel sektörde görev yapan veteriner hekimlerin farklı alt gruplara göre girişimcilik skorlarının (GS) belirlenmesi ve veteriner hekimlerin girişimcilik niyetleri (GN) yani kendi işini kurma kararları üzerinde etkili faktörlerin lojistik regresyon modeliyle tahmin edilmesidir. Elde edilecek bulguların, mesleki istihdam ve eğitim politikalarının yanı sıra, mesleki eğilim ve girişimcilik özellikleri hakkında da ilgili kurum ve araştırmacılara yararlı bilgiler sunacağı düşünülmektedir.

MATERYAL ve METOT

Örneklemin Belirlenmesi

Veriler bir anket vasıtasıyla Haziran-Aralık 2014 tarihleri arasında basit tesadüfi örnekleme yöntemiyle Türkiye'nin 7 coğrafi bölgesindeki 24 farklı ilden (Antalya, Isparta, Hatay, Kahramanmaraş, Gaziantep, Adıyaman, Elazığ, Malatya, Iğdır, Ankara, Çankırı, Kırklareli, Edirne, İstanbul, Çanakkale, Bursa, Bolu, İzmir, Aydın, Denizli, Amasya, Samsun, Sinop ve Kastamonu) alınmıştır. Veteriner hekimlerin sosyoekonomik/demografik özellikleri, mesleki görüş ve tercihleri ile 9 farklı girişimcilik bileşenine yönelik tutumlarının sorgulandığı çalışmada, minimum örneklem hacmi aşağıdaki formül yardımıyla belirlenmiştir;

$$n = \frac{N}{1 + N(e^2)}$$

Bu formüldeki; "n" örnek büyüklüğünü, ortalama 20.000 olarak alınan "*N*" ülkedeki tahmini veteriner hekim sayısını, %5 (0.05) olarak alınan "*e*" ise kabul edilen maksimum hata payını, göstermektedir. Formül ile 392 olarak hesaplanan örneğin bölgelere göre dağılımı ülke nüfusu içindeki ağırlıklı ortalamalarına göre yapılmıştır [21-23]. Tutarsız ve/veya eksik yanıtların olabileceği ihtimalinin yanında temsil gücünü de artırabilmek amacıyla çalışma 455 veteriner hekimle tamamlanmıştır.

Girişimcilik Bileşenlerinin Güvenilirlik ve Geçerliliği

Bu çalışmadaki girişimcilik bileşenleri, daha önce veteriner fakültesi lisans öğrencilerine yönelik yapılan ve güvenilirlik ve geçerliliği sınanmış bir çalışmadan alınmıştır [2]. Mesleki alanda (I) "yenilikçi düşünebilirim", (II) "fırsatları değerlendiririm", (III) "rekabet edebilirim", (IV) "risk alabilirim", (V) "liderlik vasfına sahibim", (VI) "problemlere karşı kolay motive olabilirim", (VII) "problemlere karşı sabırlı/kararlı davranabilirim", (VIII) "eleştirilere karşı olumlu tavır geliştirebilirim", (IX) "kişi/kurumlarla güçlü sosyal ilişkiler kurabilirim" dikkate alınan bileşenler olmuştur. Çalışmada soruların güvenilirlik ve geçerliliğinin bir kez daha

kontrol edildiği pilot çalışmada, soruların katılımcılarca aynı biçimde algılanabilme düzeyinin test edildiği Cronbach Alpha katsayısı 0.82, faktör analizi için verilerin uygunluğunun belirlendiği Kaiser-Meyer-Olkin katsayısı 0.768 ve Barlett's Test of Sphericity sonucu anlamlı (P<0.001) olarak bulunmuştur.

Verilerin Değerlendirilmesi

Araştırmada 5'li likert ölçeğiyle sorgulanan girişimcilik bileşenleri "hiçbir zaman" için 0 puan; "nadiren" için 1 puan; "bazen" için 2 puan; "sık sık" için 3 puan ve "her zaman" için 4 puan olacak biçimde puanlandırılmıştır. En düşük ve en yüksek GS sırasıyla 0 ve 36 puan olmuştur. Sürekli değişkenlerin normal dağılıma uygunluğunda alt grup sayılarına göre Kolmogorov-Smirnov veya Shapiro-Wilks testleri kullanılmıştır. İkiden fazla sayıda sosyoekonomik ve coğrafik alt grupların karşılaştırılmasında, normal dağılım şartının sağlanamaması nedeniyle Kruskal Wallis H testinden yararlanılmış ve P<0.05 düzeyindeki anlamlı farklılıklar için ikili karşılaştırmalar Mann Whitney U testi ile gerçekleştirilmiştir. GS ile sosyoekonomik değişkenler arasındaki ilişki Spearman's rho ile belirlenmiştir [8,24,25].

Lojistik Regresyon Modeli

Bu çalışmada bağımsız değişkenlerin, kategorik (sınıflı) bağımlı değişken üzerindeki etkisini tahmin etmek amacıyla İkili Lojistik Regresyon (Binary Logistic Regression) analizinden yararlanılmıştır. LR (lojistik regresyon) analizinde kesikli değer alan bağımlı değişkenin alabileceği değerlerden birinin gerçekleşme olasılığı tahmin edilmektedir. Esnek, kolay kullanılabilir ve yorumlanabilir bir özelik taşıyan LR, doğrusal regresyon analizine alternatif bir tekniktir [26-28]. Bu çalışmada "girişimcilik niyeti" bağımlı değişken; GS, yaş, cinsiyet, eğitim düzeyi, gelir düzeyi, çalışma alanı ve babanın ücretli/işveren olma durumu ise bağımsız değişken olarak dikkate alınmıştır. Model sonuçları değişkenlerin katsayıları (B), Wald istatistikleri ve olasılık oranları (Odds ratios-OR) ile beraber yorumlanmıştır.

BULGULAR

Tablo 1 incelendiğinde yaklaşık 13 yıllık deneyime sahip ve çoğunluğunu erkeklerin ve kamu çalışanlarının oluşturduğu veteriner hekimlerin yalnızca %13.7'sinin

Tablo 1. *Veteriner hekimlerin sosyoekonomik özellikleri, mesleki eğilimleri ve görüşleri*
Table 1. *Socioeconomic characteristics, professional trends and opinions of veterinarians*

Katılımcıların Sosyoekonomik Özellikleri ve Mesleki Profilleri	Tanımlayıcı İstatistikler	
Katılımcıların Cinsiyeti	Frekans N (%)	
–Bayan		101 (22)
–Erkek		350 (78)
Katılımcıların Girişimcilik Niyeti/Kendi İşini Kurma Niyeti		
–Evet		189 (42)
–Hayır		265 (58)
Katılımcıların Görev Yaptığı Alan/Çalıştığı Kurum		
–Kamuda Çalışanlar		238 (52)
–Klinisyenler		162 (36)
–Özel Sektörde Çalışanlar		55 (12)
Katılımcıların Geçmişte İş Değiştirme Durumları		
–Evet		62 (14)
–Hayır		388 (86)
Katılımcıların Bugüne Kadarki İş Değiştirme Sayısı (adet)	X ± SD	0.77±1.17
Katılımcıların Müteşebbis/İşveren Yakın Akraba Sayısı (adet)		1.18±1.73
Katılımcıların Mesleki Deneyim Süresi (yıl)		12.56±8.63
Katılımcıların Aile Birey Sayısı (adet)		3.47±1.26
Katılımcıların Yaşı (yıl)		37.26±8.89
Katılımcıların Fakültelerinin Girişimcilik Eğitimine İlişkin Düşünceleri	Medyan (min–mak)	2 (1–5)[a]
Katılımcıların Meslektaşlarının Girişimcilik Düzeyine İlişkin Düşünceleri		3 (1–5)[a]
Katılımcıların Aylık Gelir Düzeyi (ordinal/sıralı)		2 (1–3)[b]
Katılımcıların Eğitim Düzeyi (ordinal/sıralı)		1 (1–4)[c]
Katılımcıların Anne Eğitim Düzeyi (ordinal/sıralı)		2 (1–5)[d]
Katılımcıların Baba Eğitim Düzeyi (ordinal/sıralı)		3 (1–5)[d]

[a] 1: çok yetersiz, 2: yetersiz, 3: orta düzeyde, 4: yeterli, 5: çok yeterli. [b] 1: 1999 TL ve altı, 2: 2000 4999 TL 3: 5000 TL ve üstü. [c] 1: lisans, 2: yüksek lisans, 3: doktora, 4: öğretim üyesi. [d] 1: ilköğretim altı, 2: ilköğretim, 3: lise, 4: üniversite, 5: yüksek lisans/doktora

Tablo 2. *Farklı alt gruplara göre veteriner hekimlerinin girişimcilik skorları (GS)*
Table 2. *Entrepreneurship scores (ES) of veterinarians according to different subgroups*

Veteriner Hekimlerin Kategorize Edildiği Alt Gruplar	N	Girişimcilik Skorları ve Anlamlılıkları	
		Ortalama ± Standart Sapma	Sıralama Ortalaması
1. Sektörlere/çalışma alanlarına göre		([a] Kruskal-Wallis H testi = 14.259; P=0.000)	
1.a. Kamuda çalışanlar	238	23.55±5.75	191.60
1.b. Klinisyenler	162	25.80±6.21	239.37
1.c. Özel sektörde çalışanlar	55	25.12±5.47	224.86
2. Yaşanılan coğrafik bölgeleye göre		(Kruskal-Wallis H testi = 8.239; P=0.221)	
2.a. Marmara Bölgesi	123	24.84±5.77	220.28
2.b. Ege Bölgesi	70	25.36±6.24	222.16
2.c. Akdeniz Bölgesi	60	23.44±7.01	196.95
2.d. Karadeniz Bölgesi	74	25.45±5.65	230.24
2.e. İç Anadolu Bölgesi	57	24.64±5.37	210.15
2.f. Doğu Anadolu Bölgesi	45	23.21±5.78	183.37
2.g. Güneydoğu Anadolu Bölgesi	26	22.54±5.42	173.63
3. Cinsiyet durumuna göre		(Mann–Whitney U testi = 12.788; P=0.061)	
3.a. Bayanlar	101	23.48±5.63	188,69
3.b. Erkekler	350	24.73±6.00	215.75
4. Babanın mesleki durumuna göre		(Mann–Whitney U testi = 10.107; P=0.014)	
4.a. Ücretli	339	24.20±5.97	199.81
4.b. İşveren	73	26.06±5.98	237.55
5. Annenin çalışma durumuna göre		(Mann–Whitney U testi = 14.376; P=0.260)	
5.a. Ev hanımı	324	24.71±5.91	214.13
5.b. Çalışan	96	23.96±6.16	198.26
6. Kendi işini kurma niyetine göre		(Mann–Whitney U testi = 16.961; P=0.000)	
6.a. Hayır	265	23.70±5.76	194.28
6.b. Evet	189	25.77±6.09	236.44
Ortalama Girişimcilik Skoru		24.53±5.96	Medyan: 24 (9–36)

[a] Kruskal-Wallis analizi ile gruplar arasında P<0.05 düzeyinde anlamlı bir farklılık belirlenmiştir. Mann–Whitney U testi ile gerçekleştirilen ikili karşılaştırmalar, gruplar arasındaki farklılığın P<0.001 düzeyinde "klinisyen" ile "kamuda çalışan" gruplarından kaynaklandığını göstermiştir

Tablo 3. *Sosyoekonomik/demografik özellikler ile GS arasındaki ilişkiler*
Table 3. *Relationships between socioeconomic/demographic characteristics and ES*

Bazı Değişkenlerin İlişkileri	Yaş	Aile Nüfusu	Mesleki Deneyim Süresi	Gelir Düzeyi	Eğitim Düzeyi	Anne Eğitim Düzeyi	Baba Eğitim Düzeyi	Müteşebbis Akraba Sayısı
Girişimcilik Skoru (GS)	-0.058	-0.080	-0.034	0.166[a]	0.024	-0.005	-0.009	0.199[a]

[a] P<0.01

meslek hayatları boyunca "iş değiştirdiği"; yarısına yakınının (%41.7) ise kendi işini kurduğu/kurma niyetinde olduğu anlaşılmaktadır. Katılımcılar, veteriner fakültelerinin girişimcilik konusunda kendilerini iş hayatına "2" yani "yetersiz" düzeyde hazırlayabildiğini, kendi mesletaşlarının ise girişimcilik konusunda "3" yani "orta düzeyde yeterli" olduğunu düşünmektedir.

Çalışma neticesinde, Türkiye geneli için veteriner hekimlerin ortalama girişimcilik skoru 24.53 bulunmuştur. İkinci girişimcilik bileşeni olan "mesleki alanda fırsatları değerlendiririm" için ortanca değer 2 yani "bazen", geriye kalan diğer sekiz bileşen içinse ortanca değer 3 yani "sık sık" olarak belirlenmiştir. Kendi işini kurma tercihinde %33.1 ile "bağımsızlık", %25.8 ile "mesleki tatmin", %24.2 ile "yüksek kazanç sağlama", %12.4 ile "seçeneksizlik" ve %4,5 ile "saygınlık kazanma" rol oynamaktadır. Müteşebbis olmama tercihinde ise %32.0 ile "mali yetersizlikler", %20.3 ile "potansiyel riskler", %19.9 ile "isteksizlik/ilgisizlik", %16.9 ile "deneyimsizlik" ve %10.8 ile "aile ve/veya çevre etkisi" gerekçe olarak gösterilmiştir.

Tablo 4. *Girişimcilik niyetine etkili faktörlerle ilgili lojistik regresyon sonuçları*
Table 4. *Results of logistic regression regarding the factors influencing entrepreneurial intention*

Modele Alınan Bağımsız Değişkenler	Katsayı (B)	Standart Hata	Wald İstatistiği	P	Olasılık Oranı (Odds Ratio)
Sabit	-4.334	0.986	19.393	<0.001	0.013
Yaş	0.059	0.015	16.278	<0.001	1.061
Girişimcilik Skoru	0.054	0.021	6.329	<0.05	1.055
Cinsiyet (1) = Erkek (referans = kadın)	-0.162	0.302	0.287	>0.05	0.851
Eğitim (1) = Y. Lisans / Doktora (referans = Lisans düzeyi)	-0.565	0.252	5.046	<0.05	0.568
Gelir			3.988	>0.05	
Gelir (1) = 2000-4999 TL (referans = 1999 TL ve altı)	0.262	0.516	0.257	>0.05	1.299
Gelir (2) = 5000 TL ve üstü (referans = 1999 TL ve altı)	-0.491	0.333	2.179	>0.05	0.612
Çalışma Alanı			47.843	<0.001	
Çalışma Alanı (1) = Klinisyen (referans = kamu çalışanı)	-0.100	0.372	0.073	>0.05	0.905
Çalışma Alanı (2) = Özel Sektör (referans = kamu çalışanı)	1.825	0.376	23.502	<0.001	6.104
Baba (1) = İşveren (referans = ücretli çalışan)	0.511	0.336	2.311	>0.05	1.666
Model X^2	117.973 (P<0.001)				
-2log likelihood	407.421				
Pseudo R^2	0.352				

Tablo 2'de çeşitli alt gruplara göre veteriner hekimlerin aldığı girişimcilik skorları verilmiştir. Klinisyenler kamuda çalışanlara göre; babası işveren olanlar ve kendi işini kurma niyetindekiler olmayanlara göre istatistiksel olarak anlamlı daha yüksek bir skora sahiptir. *Tablo 3*'te katılımcıların girişimcilik skorunun 9 farklı sosyoekonomik/demografik faktörden yalnızca "gelir düzeyi" ve "sahip olunan girişimci yakın akraba sayısı" ile anlamlı bir ilişki içinde olduğu anlaşılmaktadır.

Tablo 4'te, girişimcilik niyeti üzerinde etkili değişkenlerin ve bunlara ait alt kategoriler arasındaki farklılıkların oransal olarak tahmin edilebildiği lojistik regresyon model sonuçları özetlenmiştir. Modelin X^2 değerine göre anlamı olduğu (P<0.001) belirlenmiştir. Ayrıca, Hosmer-Lemesov test sonucundan (χ2=11.443 ve p değeri 0.178) modelin bir bütün olarak uyumlu olduğu, yani gözlenen ve model tarafından kestirilen değerler arasında anlamlı fark olmadığı anlaşılmıştır. Modelin belirleme katsayısı (Pseudo R^2) bağımsız değişkenlerin girişimcilik niyetindeki değişimin %35.2'sini açıkladığını göstermektedir. Cinsiyet ve gelir dışındaki tüm bağımsız değişkenlerin model üzerinde anlamlı etkileri bulunmaktadır. Girişimcilik niyeti üzerinde yaş ve GS pozitif; eğitim düzeyi ise negatif bir etkiye sahiptir. Yaş ve girişimcilik skorundaki bir birimlik artış girişimcilik niyetini sırasıyla %6.1 (Odds Ratio-OR=1.061) ve %5.5 (OR=1.055) oranında artırmaktadır. Tablodaki olasılık oranlarından, yüksek lisans ve doktora yapanların lisans mezunlarından %58 (OR=0.584) daha düşük; özel sektörde çalışanların ise kamudakilerden 6.1 kat (OR=6.104) daha yüksek bir girişimcilik niyetine sahip olduğu anlaşılmaktadır.

TARTIŞMA ve SONUÇ

Ülke geneli için bulunan 24.53'lük GS, veteriner hekimlerin meslektaşlarını girişimcilik hakkında "orta düzeyde yeterli görmeleri" bulgusuyla örtüşmektedir. Kendi işini kurmayı düşünen ve düşünmeyenlerdeki GS arasında bulunan anlamlı farklılık ise, çalışmada kullanılan girişimcilik bileşenlerinin güvenilirlik ve geçerliliğini desteklemektedir. Kendi işini kurma niyetindekilerin ortalama GS (25.77) güven aralıklarının alt sınırı (%99 CI: 24.54-27.00) bir veteriner hekimin girişimci potansiyeli taşıyabilmesi açısından sahip olması gereken minimum düzey hakkında bir fikir vermektedir. Veteriner hekimler için belirlenen düzeyin, daha önce veteriner fakültesi öğrencileri için bulunan 25.93'lük skordan [2] daha düşük çıkması ise, gençlerin girişimciliğe daha yatkın olmaları, geleceğe daha iyimser bakmaları veya çalışmaların farklı düzeylerde (yerel ve ulusal) yapılmış olmasıyla açıklanabilir.

Veteriner hekimlerin büyük çoğunluğunun özel sektörü kamuya göre daha avantajlı gördükleri bildirilse de [29], 1990-2011 yılları arasında Kafkas Üniversitesi Veteriner Fakültesi mezunlarının yalnızca %9'unun özel sektörü tercih ettikleri; %64'ünün kamu, %27'sinin ise kliniklerde istihdam olduğu aktarılmaktadır [17]. Ulusal düzeydeki

bu çalışmaysa, veteriner hekimlerin yalnızca %12'sinin özel sektörde çalıştığını göstermiştir *(Tablo 1)*. Can [2] veteriner fakültesi öğrencilerinin mezuniyet sonrası %47 oranında klinikleri, %33 oranında kamuyu, %17 oranında kendi besi/süt sığırcılığı işletmesini ve %3 oranında ilaç/yem sanayini tercih edeceklerini belirtmiştir. Veteriner hekimlerin özellikle kamu kurumları tarafından istihdam edilmesi, fakültelerin sayı ve kontenjana bağlı artışları ve kamudaki norm kadro uygulamaları dikkate alındığında, yakın gelecekte müteşebbis olmayı düşünmeyenler için iş olanakları ve ücretler/maaşlar açısından olumsuz bir ortamın oluşacağı öngörülmektedir. Her yıl yaklaşık 1.300 dolayında yeni mezunun [16] önümüzdeki 10-15 yıl içerisinde bugün faal olarak çalışan veteriner hekim sayısını bile geçebileceği göz ardı edilmemelidir.

Babası işveren olanların ücretli çalışanlardan; kendi işini kurma niyetinde olanların ise olmayanlardan daha yüksek bir skora sahip olmaları farklı çalışmalar tarafından da [2,30,31] desteklenmektedir. Klinisyenler kamudakilere göre $P<0.01$ düzeyinde daha yüksek bir girişimcilik skoruna sahipken, özel sektörle kamu arasında bu farklılığın bulunmaması dikkat çekicidir *(Tablo 2)*. Bu çalışmada cinsiyet açısından $P<0.05$ düzeyinde anlamlı bir farklılık bulunmamış olup, bu sonuç bazı çalışmalarla uyumludur [2,30,32]. Ancak bu çalışmada saptanan olasılık değerinin ($P=0.061$) biraz daha yüksek bir anlamlılık düzeyinde farklı yorumlanacağı unutulmamalıdır. Türkiye, ABD ve Avustralya'da pet hayvanları üzerine çalışan erkeklerin bayanlardan daha fazla gelir elde ettikleri [33] yani girişimciliğin farklı bir boyutu olan yönetimsel/iktisadi açıdan daha başarılı oldukları görülmektedir. Bununla beraber, Türkiye'de kadın girişimcilere yönelik toplumsal algının çok olumlu olmadığı ve erkeklere göre daha fazla sermaye sorunları [7] yaşadıkları unutulmamalıdır. Türkiye'nin doğu ve batı bölgeleri arasında gelişmişlik açısından önemli farklılıkların olduğu bilinmesine rağmen, bu çalışmada coğrafik bölgeler arasında anlamlı bir farklılık olmaması şaşırtıcıdır *(Tablo 2)*. Bu durum, çalışmada katılımcıların doğdukları veya uzun yıllar yaşadıkları bölgelerin (kent-kır/il-ilçe) yerine, yalnızca şu an çalıştıkları bölgelerin dikkate alınmasından kaynaklanmış olabilir.

Lojistik regresyon modeli, "yüksek lisans veya doktora eğitimi almayan", "özel sektörde çalışan" ve "yüksek girişimcilik skoruna sahip" veteriner hekimlerin müteşebbis olma olasılığının diğer gruplardan anlamlı biçimde daha yüksek olduğunu göstermiştir *(Tablo 4)*. Bazı çalışmalarla benzer biçimde [34-36] gelir düzeyinin GS ile anlamlı pozitif bir ilişkiye sahip olmasına rağmen, GN üzerinde etkili faktörlerden biri olmaması yani model içinde anlamlı çıkmaması dikkat çekicidir. Bu durum, mali gücün risk alma, fırsatları değerlendirme ve liderlik gibi girişimcilik bileşenlerini ön plana çıkarmasına rağmen, iş kurmak için geçerli/yeterli bir ölçüt olamayacağını göstermiştir. Eğitim düzeyi arttıkça GN olasılığının anlamlı biçimde azalması, yüksek lisans/doktora eğitiminin sektöre/pazara yönelik bir donanım kazanmaktan çok, kamuda bir makam veya ünvan kazanma amacı taşıması veya ilgili kişilerin hali hazırda çalışmakta olmaları ile açıklanabilir. Yine de, eğitimin girişimcilik niyetine etkisi yorumlanırken, bu çalışmadaki eğitim kategorilerinin sınırlı olduğu göz ardı edilmemelidir. Yalnızca Cumhuriyet Türkiye'sinde değil Osmanlı'da bile ailelerin çocuklarının geleceğinin girişimcilikte değil devlet memurluğunda gördüğü; bugün vasıflı işgücü ihtiyacına yönelik değerlendirmelerin neredeyse aynısının 1840'larda Osmanlı gazetelerinde de yer aldığı vurgulanmaktadır. Bu noktada, özellikle eğitim sistemi ile işgücü piyasası arasındaki uyumun daha iyi sağlanması ve genç meslektaşlara iş yaşamının gerektirdiği yetkinlik ve girişimcilik kültürünün kazandırılması [37] öne çıkmaktadır. Batılı ülkelerde, Türkiye'nin de kısmen aralarında bulunduğu doğulu kültürlerin aksine "bireyciliğin" kişileri daha özgür kılması ve ABD'li girişimcileri fırsatların, Türkleri ise zorunlulukların [12,38-40] harekete geçirmesi yukarıdaki ifadeleri destekler niteliktedir.

İsviçre ve Almanya'da akademisyenlerle yürütülen bir çalışmada genç bilim adamlarının diğer meslektaşlarına göre daha yüksek bir GN taşıdığı ve bayan akademisyenlerin girişimcilik niyetinin erkeklere göre daha zayıf kaldığı belirlenmiştir. Aynı çalışmada, akademisyenlerde GN ile yayın sayısı arasında negatif, özel sektöre danışmanlık yapma durumu arasında ise pozitif bir ilişki bulunmuştur [41]. Srilanka'da yapılan bir çalışmada ise akademisyenler için girişimci olmanın ikinci veya üçüncü bir tercih olduğu belirlenmiştir [42]. Türkiye'de avukat, hekim ve mühendislerinde aralarında bulunduğu çeşitli meslek grupları üzerinde yürütülen bir çalışmada, girişimci kişilik özelliği ile "yenilikçilik kapasitesi" ve "yenilikçiliğe destek" arasında pozitif ve anlamlı bir korelasyon saptanmıştır [43]. Yine ülkemizde yapılan bir çalışma "kesinlikle katılıyorum" ve "katılıyorum" yanıtları dikkate alındığında, genç nüfus içerisinde iş düşüncesini hayata geçirmek için aktif çaba gösterenlerin %45, başarılı bir girişimcilik için yenilikçi düşüncenin şart olduğunu düşünenlerin %84 dolayında olduğunu göstermiştir [44]. Almanya'da ücretli çalışanların durumunun incelendiği bir başka çalışmada, maaşların genel ortalamanın çok altında kaldığı düşüncesi ve algısının GN'ni artırdığı, adil bir ücret ve çalışma saatinin ise çalışanları mevcut işlerinde kalmaya yönelttiği belirlenmiştir [45].

Veteriner hekimlerin genellikle gerçekçi ve araştırmacı bir yapıda olmalarına karşın, sosyal ilişkiler ve girişimcilik konusunda zayıf kaldıkları; daha kaliteli ve başarılı bir hizmet sunumunun ise iletişim becerisi ve takım çalışması ile gerçekleşebileceği vurgulanmaktadır [20]. Bu noktada Can [2] veteriner fakültesi eğitim-öğretim programlarında girişimcilik dersleri ve bilimsel etkinliklerinin artırılmasını önerirken; Chadderdon ve ark.[20] veteriner fakültelerinde çok değişkenlik gösteren girişimcilik eğitim programlarının yönetim ve pazarlama konularına ağırlık da verilerek standardize edilmesini gerekli görmektedir. Günümüzde,

küresel boyutta hayvan refahı ve pet hayvanları bakımına ilgi artarken, çiftlik hayvanları hekimliğinde pazarın giderek daraldığı bilinmektedir. Örneğin İngiltere'de yüksek süt verimine karşın hayvan ve üretici sayısının giderek azaldığı, aile tipi hayvancılık işletmelerinin yerlerini daha büyük ölçekli anonim şirketlere bıraktığı ve bazı çiftliklerin hayvancılığı hobi veya tamamlayıcı bir gelir olarak görmeye başladığı belirtilmektedir [46,47]. Türkiye'de yakın gelecekte organik tarım/hayvancılık, gıda güvenliği ve sürü sağlığı konularının öncelik kazanacağı [29], klasik hekimlik anlayışının yerini alacak olan koruyucu hekimliğin ise işletme yönetimi bilgisini de gerektireceği vurgulanmaktadır. Ayrıca, mesleki alanda hayvan davranışları, yabani hayat, biyoteknoloji ve akvatik hayvanlarla [16] ilgili bakir çalışma alanları da dikkat çekicidir. Başarılı girişimciler tarafından Türk girişimcilerin özellikle "dünyadaki gelişmelere ayak uydurması" ve "yeniliklere açık olması" gerektiği belirtilirken; özgüven, dürüstlük/ticari ahlak ve risk alabilme en önemli kişisel özellikler olarak görülmektedir [5]. Türkiye'de işverenlerin güvenilir çalışanları bulma ve elde tutma konusunda güçlü bir arayış içinde oldukları [7] da unutulmamalıdır. Can [2] veteriner hekimliği mesleğinin daha rekabetçi ve yenilikçi bir yapı kazanmasında eğitim ve araştırmalarla fakültelere, kolaylaştırıcı mevzuatla da ilgili kurumlara görevler düştüğünü yinelemektedir.

Veteriner hekimlerin ekonomik açıdan tatmin edici ve sürdürülebilir bir hizmet için yüksek pazar şansı bulabilecek öncelikli alanlara yönelmeleri, tüketicilere yenilikçi ürün/hizmetler sunabilecek eğitim ve kültürü kazanmaları, hedeflerinde kararlı/istikrarlı olmaları ve ulusal/uluslararası gelişmeleri yakından takip etmeleri gerekmektedir. Gelecekte, GN üzerinde etkili olabilecek daha farklı değişkenlerin yanında, işveren olmayı başarmış örnek veteriner hekimlerin yaşadıkları fırsat ve zorlukların da ele alınacağı kapsamlı çalışmaların veteriner hekimliğinde mesleki girişimcilik literatürüne olumlu katkılar sağlayacağı düşünülmektedir.

Teşekkür

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KAYNAKLAR

1. Hisrich RD, Peters MP: Entrepreneurship. **In,** Entrepreneurship. 4th ed., Mass Irwin/McGraw-Hill, Boston, 1998.

2. Can MF: Veteriner fakültesi öğrencilerinin girişimcilik potansiyel ve eğilimlerinin analizi: Mustafa Kemal Üniversitesi örneği. *YYÜ Vet Fak Derg,* 25 (3): 81-86, 2014.

3. Müftüoğlu T, Ürper Y, Başak M, Tosunoğlu BT: Girişimcilik ve girişimcinin özellikleri. **In,** Girişimcilik. 6. Baskı, Eskişehir, 2009.

4. Keleş HN, Kıral Özkan T, Doğaner M, Altunoğlu AE: Önlisans öğrencilerinin girişimcilik düzeylerini belirlemeye yönelik bir araştırma. *Int J Econ Admin Stud*, 5 (9): 107-118, 2012.

5. Çetinkaya Bozkurt Ö, Kalkan A, Koyuncu O, Alparslan AM: Türkiye'de girişimciliğin gelişimi: Girişimciler üzerinde nitel bir araştırma. *SDÜ Sos Bil Enst Derg,* 15 (1): 1-19, 2012.

6. Kuratko DF, Hodgetts RM: Entrepreneurship. A Contemporary Approach. 2nd ed., Fort Worth Canada, 2001.

7. Benzing C, Chu HM, Kara O: Entrepreneurs in Turkey: A factor analysis of motivations, success factors, and problems. *J Small Buss Manage*, 47, 58-91, 2009. DOI: 10.1111/j.1540-627X.2008.00262.x

8. Karabulut AT: Üniversite öğrencilerinin girişimcilik özelliklerini ve eğilimlerini belirlemeye yönelik bir araştırma. *Marmara Üniv İİBF Derg*, 26 (1): 331-356, 2009.

9. Rideout EC, Gray DO: Does entrepreneurship education really work? A review and methodological critique of the empirical literature on the effects of university-based entrepreneurship education. *J Small Buss Manage,* 51, 329-351, 2013. DOI: 10.1111/jsbm.12021

10. Luchsinger V, Bagby DR: Entrepreneurship and intrapreneurship: Behaviors, comparisons, and contrasts. *SAM Adv Manage J,* 52 (3): 10-13, 1987.

11. Sharma P, Chrisman SJJ: Towards a reconciliation of the definitional issues in the field of corporate entrepreneursship. *Entrep Theor Pract,* 23, 11-27, 1999. DOI: 10.1007/978-3-540-48543-8_4

12. Çarıkçı IH, Koyuncu O: Bireyci-toplumcu kültür ve girişimcilik eğilimi arasındaki ilişkiyi belirlemeye yönelik bir araştırma. *Mehmet Akif Ersoy Üniv Sos Bil Enst Derg,* 3, 1-18, 2010.

13. Nicolaou N, Shane S, Cherkas L, Hunkin J, Spector TD: Is the tendency to engage in entrepreneurship genetic? *Manage Sci*, 54, 167-179, 2008. DOI: 10.1287/mnsc.1070.0761

14. Özgür A: Veteriner hekim terimi üzerine tarihsel bir araştırma. *Ankara Üniv Vet Fak Derg*, 44, 1-8, 1997.

15. WWA: Members. World Veterinary Association. http://www.worldvet.org /about.php?sp=members, *Accessed:* 06.05.2015.

16. TVHB: III. Türk Veteriner Hekimliği Kurultayı sonuç raporları. Türk Veteriner Hekimleri Birliği, 23-25 Nisan 2010. Ankara. http://tvhb.org.tr/sayfa/genel, *Accessed:* 30.04.2015.

17. Yiğit A, Aydın E, Cihan M: Evaluation of graduates of the Kafkas University Faculty of Veterinary Medicine in terms of several parameters. *Eurasian J Vet Sci,* 30, 166-173, 2014.

18. Resmi Gazete: Veteriner Hekimliği Mesleğinin İcrasına, Türk Veteriner Hekimleri Birliği ile Odalarının Teşekkül Tarzına ve Göreceği İşlere Dair Kanun. Sayı: 8661, 1954. http://www.mugla-vho.org/kanunlar/ *Accessed:* 18.04.2014.

19. Resmi Gazete: Veteriner Hizmetleri, Bitki Sağlığı, Gıda ve Yem Kanunu. Sayı: 27610, 2011. http://www.resmigazete.gov.tr/eskiler/2010/06/20100613-12.htm, *Accessed:* 18.04.2014.

20. Chadderdon LM, King LJ, Lloyd JV: The skills, knowledge, aptitudes, and attitudes of successful veterinarians: A summary of presentations to the NCVEI subgroup. *J Vet Ned Educ,* 28, 28-30, 2000.

21. Israel GD: Determining sample size. Fact Sheet PEOD-6, Institute of Food and Agricultural Sciences, University of Florida, US. November 1992.

22. TUİK: Nüfus istatistikleri. Yıllara göre il nüfusları. Türkiye İstatistik Kurumu. http://www.tuik.gov.tr/UstMenu.do?metod=temelist, *Accessed:* 19.04.2014.

23. Can MF, Yalçın C: Investigation of organizational responsibility and satisfaction level of the cattle producers in Turkey. *Kafkas Univ Vet Fak Derg*, 21, 711-717, 2015. DOI: 10.9775/kvfd.2015.13229

24. Demir MÖ: Çıkarım ve ilişki analizi. **In,** Sosyal Bilimlerde İstatistiksel Analiz. SPSS 20 Kullanım Kılavuzu. İkinci Baskı, Detay Yayıncılık, Ankara, 2012.

25. Can MF, Altuğ N: Socioeconomic implications of biosecurity practices in small-scale dairy farms. *Vet Quart,* 34, 67-73, 2014. DOI: 10.1080/01652176.2014.951130

26. Karagöz M: Parametrik olmayan testler. **In,** İstatistik Yöntemleri. 8. Baskı, Ekin Yayınevi, Bursa, 2011.

27. Cankurt M, Miran B, Şahin A: Sığır eti tercihlerini etkileyen faktörlerin belirlenmesi üzerine bir araştırma: İzmir ili örneği. *Hayvansal Üretim,* 51 (2): 16-22, 2010.

28. Hosmer DV, Hosmer T, Cessie L, Lmeshow S: A comparison of goodness of fit tests for the logistic regression model. *Stat Med,* 16 (9): 965-980, 1997.

29. Özen A, Doğan Ö, Gül Başağaç RT, Özkul T, Yüksel E: Türkiye'de veteriner hekimliği üzerine araştırmalar: III. İş fırsatları ve sektörel yönelimlere ilişkin görüş ve beklentiler. *Kafkas Univ Vet Fak Derg,* 18, 907-911, 2012. DOI: 10.9775/kvfd.2012.6018

30. Ishfaq A, Nawaz MM, Ahmad Z, Shaukat MZ, Usman A, Rehman W, Ahmed N: Determinants of students' entrepreneurial career intentions: Evidence from business graduates. *Eur J Soc Sci,* 15 (2): 14-22, 2010.

31. Tong XF, Tong DYK, Loy LC: Factors influencing entrepreneurial intention among university students. *Int J Soci Sci Human Stud,* 3 (1): 487-496, 2011.

32. Özden K, Temurlenk MS, Başar S: Girişimcilik eğilimi: Kırgızistan-Türkiye Manas Üniversitesi ve Atatürk Üniversitesi öğrencileri üzerine bir araştırma. *Rev Soc Econ Buss Stud*, 11, 1-20, 2008.

33. Aral Y, Cevger Y, Demir P, Aydın E: Ankara ili evcil hayvan veteriner kliniklerinin yönetimsel ve ekonomik açıdan değerlendirmesi. *Kafkas Univ Vet Fak Derg,* 16, 503-508, 2010. DOI: 10.9775/kvfd.2009.1285

34. Emsen ÖS, Yılmaz Ö, Çınar S: Genç nesilde mesleki eğilimler ve girişimcilik: Ampirik bir çalışma. *Verimlilik Derg*, 1, 153-176, 2011.

35. Kristiansen S, Indarti N: Entrepreneurial intention among Indonesian and Norwegian students. *J Enterprising Culture,* 12, 55-78, 2004. DOI: 10.1142/S021849580400004X

36. Şeşen H, Basım HN: Demografik faktörler ve kişiliğin girişimcilik niyetine etkisi*:* Spor bilimleri alanında öğrenim gören üniversite öğrencileri üzerine bir araştırma. *Ege Academic Review*, 12 (Special Isssue): 21-28, 2012.

37. Yıldırım K, Şahin L: Osmanlı'dan günümüze mesleki eğitimin gelişimi. *Çalışma ve Toplum,* 1, 77-112, 2015.

38. Aytaç Ö: Girişimcilik: Sosyokültürel bir perspektif. *Dumlupınar Üniv Sos Bil Enst Derg,* 15, 139-160, 2006.

39. Ersoy H: Kültürel çevrenin girişimcilik tercihine etkisi. *Organizasyon ve Yönetim Bilimleri Dergisi,* 2 (1): 71-77, 2010.

40. Friedman BA, Aziz N: Turkey and the United States: Entrepreneurship activity, motives, aspirations, and perceptions. *Int J Buss Soc Sci,* 3 (3): 96-107, 2012.

41. Moog P, Werner A, Houweling S, Gelner-Backes U: The impact of skills, working time allocation and peer effects on the entrepreneurial intentions of scientists. *J Technol Transf*, 40, 493-511, 2015. DOI: 10.1007/s10961-014-9347-x

42. Thrikawala S: The determinants of entrepreneurial ıntention among academics in Sri Lanka. *International Proceedings of Economics Development & Research,* 4, 454-468, 2011. http://ssrn.com/abstract=2333276, *Accessed:* 10.07.2015.

43. Bakan İ, Eğitmiş AM, Büyükbeşe T, Erşahan B: Kahramanmaraş'ta girişimcilik ve yenilikçilik: Profesyonel meslek gruplarında bir alan çalışması. *II. Bölgesel Sorunlar ve Türkiye Sempozyumu*. 1-2 Ekim 2012, s.260-267. Kahramanmaraş, Türkiye.

44. Öneren M: İç Anadolu bölgesindeki genç nüfusun girişimcilik eğilimleri üzerine bir araştırma. *Girişimcilik ve Kalkınma Dergisi*,7 (2): 7-28, 2012.

45. Werner A, Gast J, Kraus S: The effect of working time preferences and fair wage perceptions on entrepreneurial intentions among employees. *Small Bus Econ*, 43, 137-160, 2014. DOI: 10.1007/s11187-013-9528-2

46. DEFRA: Agriculture in the United Kingdom. Department for Environment Food and Rural Affairs. http://www.defra.gov.uk /evidence/statistics/foodfarm/general/auk/latest/index.htm, *Accessed:* 01.04.2015.

47. Henry C, Treanor N: The veterinary business landscape: Contemporary issues and emerging trends. http://cdn.intechopen.com/pdfs-wm/28664.pdf, *Accessed:* 10.03.2015.

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Research Article

Structural and Functional Properties of the Distal Muscles of Front and Hind Legs of Malakan Horses *(Equus caballus)* [1]

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Abstract

The purpose of this study was to determine the structural and functional properties of the distal muscles of front and hind legs of Malakan Horses. Thus, totally 10 Malakan horses (5 females and 5 males) were used in the study. From front and hind legs of the horses, the findings of totally 28 muscles were received. Accordingly, it is determined that the longest muscle in the distal of the front leg was m. flexor carpi ulnaris, the heaviest one was m. extensor carpi radialis and the muscle with the highest value in terms of parameters of tendon length and total muscle tendon length was m. extensor digitorum communis. From the distal muscles of the hind leg; it is observed that the longest one was m. tibialis cranialis, the heaviest one was m. gastrocnemius and the muscle with the highest value in terms of parameters of tendon length and total muscle tendon length was m. flexor digitorum superficialis. It was observed that while the difference determined between some of the values in the front and hind leg muscles had a statistical significance in the comparison performed between the genders ($P<0.05$), the differences had no statistical significance in the comparison performed based on the direction (right-left).

Keywords: *Muscle, Malakan horse, Tendon, Front and hind leg*

Malakan Atlarında *(Equus caballus)* Ön ve Arka Bacağın Distal'indeki Kasların Yapısal ve Fonksiyonel Özellikleri

Özet

Bu çalışma, Malakan Atlarında ön ve arka bacağın distal'inde bulunan kasların yapısal ve fonksiyonel özelliklerini belirlemek amacıyla yapıldı. Bu amaçla 5 dişi, 5 erkek olmak üzere toplam 10 Malakan Atı kullanıldı. Atların ön ve arka bacağından toplam 28 kasın bulgusu alındı. Buna göre; ön bacağın distal'inde bulunan kaslardan en uzununun m. flexor carpi ulnaris, en ağırının m. extensor carpi radialis, tendo uzunluğu ve toplam kas tendo uzunluğu parametreleri bakımından ise en yüksek değere sahip olan kasın m. extensor digitorum communis olduğu belirlendi. Arka bacağın distal kaslarından en uzun olanın m. tibialis cranialis, en ağır olanın m. gastrocnemius, tendo uzunluğu ve toplam kas-tendo uzunluğu bakımından ise en yüksek değere sahip olan kasın m. flexor digitorum superficialis olduğu görüldü. Cinsiyetler arası yapılan karşılaştırmada ön ve arka bacakta kasların bazı değerleri arasında belirlenen farkın istatistiksel önem taşıdığı belirlenirken ($P<0.05$), yöne (sağ-sol) göre yapılan kıyaslamada farkların istatistiksel anlam taşımadığı tespit edildi.

Anahtar sözcükler: *Kas, Malakan atı, Tendo, Ön ve arka bacak*

INTRODUCTION

Malakan Horse is an endemic horse breed which is raised in the north-eastern regions of Eastern Anatolia, can resist to extreme winter conditions and is used in carriage, draught, and horse riding. Its wide body and thick bone structure are remarkable. Also, the commonly seen types are the ones with black, gray, bay, and red coats [1-3]. There has been a serious literature deficit regarding this horse race in terms of basic sciences, clinical sciences

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and raising, husbandry and feeding in veterinary medicine.

Morphologic structure of the muscle determines its function [4-6]. While the muscle structure may vary between different muscles of the individual [7,8], the same muscles in different individuals can be different in terms of structure [9,10]. Also it is known that exercise [11], genetic structure and gender [12] are effective on the characteristics of the muscle structure. Burkholder [13] also add the muscle fiber volume, physiological characteristics, number of the fibers and their sequence to the factors affecting the structure of the muscle. Regarding the structural and functional properties of the muscles in Equidae family, Brown et al.[7] and Payne et al.[8] conducted a study on horses and Fayed [14] and Demiraslan and Özcan [15] conducted a study on donkeys. Also, the studies conducted on the muscle-tendon structure of four-leg animals is rather important in order to reveal the differences between the functions of front and hind legs [7,8].

The purpose of the study was to determine structural and functional properties of the distal muscles of front and hind legs of Malakan horses which are a native horse breed.

MATERIAL and METHODS

Totally 10 Malakan horses (5 males and 5 females) aged between 5 and 7 years were used in the study. The Malakan horses used had gray (3 males, 1 female), isabella (1 female), bay (2 males, 1 female), and red (2 females) coats. Average live weights of male and female Malakan horses were 314.00±5.47 kg and 287.80±7.69 kg, average cidago height was 142.40±3.04 cm and 138.40±1.14 cm, and average ridge height was 146.20±2.94 cm and 142.40±0.89 cm.

In this study, approval of the ethics committee was taken upon the decision no. 31 dated 20.02.2014 of Atatürk University Animal Experiments Local Ethics Committee regarding the usage of Malakan horses. The animals were taken to deep anesthesia according to the cadaver preparation techniques. Xylazine HCl (8 ml/100 kg, intravenous) and cloralhydrate (20 mg/kg intraperitoneal) were applied for this process [16]. After the blood of the animals taken to deep anesthesia was drained through arteria carotis communis, the front legs were cut from the art. humeri level and the hind legs were cut from the art. coxae level and separated as right and left. Then, in order to reduce the effect of rigor mortis that could occur in the muscles, the legs were kept in extension position in the refrigerator of Kafkas University Faculty of Veterinary Medicine Department of Anatomy at 4°C for 24 h and within 48 h, the distal muscles of the front and hind legs were dissected and required results were taken. In order to determine the study limits, only the distal muscles of the front and hind legs were preferred.

The dissected muscles were subject to a set of measurements and calculations in order to determine the structural and functional properties. For these measurements, Payne et al.[8] and Fayed [14] were taken as a reference. While the structural parameters in the study were determined as muscle length (KU cm), tendon length (TU cm), total muscle-tendon length (TKTU cm), muscle weight (KA g), tendon weight (TA g), total muscle-tendon weight (TKTA g), muscle volume (KH cm^3), tendon volume (TH cm^3), total muscle-tendon volume (TKTH cm^3), pennation angle (PA°), and muscle bundle length (KDU cm), the functional parameters were determined as the physiological cross-sectional area of the muscle (PCSA cm^2), maximum isometric force (FmaxMPa), structural index (AI), tendon cross-sectional area (TCSA cm^2), tendon pressure on the maximum isometric force (TSfmaxMPa) and the percentage of the distention ratio on the tendon pressure (TS%).

Functional parameters were calculated by using the following parameters.

a. PCSA = KH/KDU
b. Fmax = PCSA x 0.3 MPa
c. AI = KDU/KU
d. TCSA = TH/TU
e. TSfmax = Fmax/TCSA
f. TS% = TSfmax/1.5 GPa

While the distal front leg muscles evaluated in the study was examined as m. extensor carpi radialis (ECR), m. extensor digitalis lateralis (EDlat), m. extensor carpi obliquus (OEM), m. flexor carpi radialis (FCR), m. flexor carpi ulnaris (FCU), m. extensor carpi ulnaris (ECU), m. flexor digitorum superficialis (FDS) and mm. interossei (MIO); m. extensor digitorum communis (EDC) was investigated in two parts as superficial head, Thierness (Th) and deep head, Philips (Ph) and mm. flexores digitorum profundii (FDP) was observed in three parts as caput humerale (FDPH), ulnare (FDPU), and radiale (FDPR). While the distal hind leg muscles were examined as m. extensor digitorum longus (EDL), m. extensor digitorum lateralis (EDlat), m. peroneus tertius (PT), m. tibialis cranialis (Tcr), m. soleus (S), m. flexor digitorum superficialis (FDS), m. popliteus (P), m. extensor digitorum brevis (EDB), m. interosseus medius (lig. suspensorium - MIO), m. flexores digitorum profundi (FDP) was examined in three parts as m. flexor digitorum medialis (FDM), m. flexor digitorum lateralis (FDL), m. tibialis caudalis (Tcd) and m. gastrocnemius (G) was examined in two parts as caput lateralis (GL) and caput medialis (GM).

Data obtained from the study were standardized because the horses were at different ages (5-7) and different live weights (CA) [17]. Geometric similarity method was used in the study for standardization [18-20]. According to this method, data were calculated by using the following formulas.

a. KA/CA b. TA/CA c. TU/CA d.KDU/CA e. PCSA/CA

For the analysis of the descriptive values of the measurements and the calculations obtained from the study according to gender and direction (right/left),

2-t test (P≤0.05), a parametric test, was performed in SPSS statistical package software (16.0 version). In this study, terms in Nomina Anatomica Veterinaria [21] were taken as a basis.

RESULTS

Table 1, 2, 3, and *4* illustrates mean and standard deviation data of structural and functional properties obtained in the study.

Accordingly, it was observed that the muscle with maximum KU value in the front leg distal of Malakan horse was FCU and the muscle with minimum KU value was FDPR. When considered in terms of TU and TKTU parameters, it was observed that EDC had the maximum value. When KDU parameter was observed, it was determined that maximum and minimum values belonged to Th and FDS. ECR had the maximum KA and TKTA values and FDPH had the maximum TA value in the distal muscles of the front leg. When functional muscle properties were considered, it was observed that FDS and Th had the maximum and minimum PCSA and Fmax values in the distal of the front leg. In terms of TCSA, it was considered that the maximum value belonged to MIO. The muscles having the maximum and minimum values in terms of PA were determined as FCU and Th, respectively.

When the structural parameters of the distal muscles of front leg according to the gender were examined, it was found that FCR (12.50-10.13) and ECU (9.25-6.63) in terms of TU, FCU (117.27-85.97), ECU (147.94-113.22) and FDS (109.74-73.92) in terms of KA (g), ECR (15.14-11.36), FCR (2.62-1.55) and FDS (45.99-36.49) in terms of TA (g), FCR (83.69-76.72) in terms of TKTA (g), FCU (110.63-81.11), ECU (139.57-106.81) and FDS (103.53-69.74) in terms of KH (cm^3), ECR (13.52-10.15), FCR (2.34-1.38) and FDS (41.06-32.58) in terms of TH (cm^3), FCR (78.82-72.29) in

Table 1. *Structural parameters of distal muscle of front leg*
Tablo 1. *Ön bacağın distal kaslarına ait yapısal parametreler*

Muscles		KU (cm)	TU (cm)	TKTU (cm)	KDU (cm)	AI	KA (g)	TA (g)	TKTA (g)	PA (°)	KH (cm^3)	TH (cm^3)	TKTH (cm^3)
ECR	Mean	29.64	15.46	45.10	6.70	0.15	354.18	13.25	367.43	35.80	334.13	11.83	345.96
	SD	1.57	0.82	1.06	1.80	0.04	42.95	2.43	44.47	5.50	34.86	2.17	36.21
OEM	Mean	15.93	10.63	26.55	0.94	0.04	11.93	1.34	13.27	31.30	11.26	1.20	12.45
	SD	2.34	4.08	5.32	0.22	0.01	1.10	0.43	1.21	7.00	1.04	0.39	1.13
FCR	Mean	26.28	11.31	34.21	7.82	0.23	78.12	2.09	80.20	22.00	73.69	1.86	75.55
	SD	1.04	1.67	8.70	1.44	0.13	4.58	0.66	5.09	4.00	4.32	0.59	4.77
FCU	Mean	34.18		34.18	1.69	0.05	101.62		101.62	47.90	95.87		95.87
	SD	1.10		1.10	2.29	0.06	18.40		18.40	2.00	17.36		17.36
ECU	Mean	33.02	8.00	41.02	1.30	0.03	128.18	6.23	134.41	45.40	120.92	5.57	126.49
	SD	1.11	1.55	1.10	1.15	0.03	22.31	5.56	24.98	5.00	21.05	4.96	23.41
EDC	Mean	25.53	45.21	70.74	5.67	0.08	122.04	20.15	142.18	25.80	115.13	17.99	133.11
	SD	1.77	2.40	2.17	1.02	0.02	17.55	6.35	22.35	3.00	16.55	5.67	20.83
EDLat	Mean	23.24	33.50	56.74	2.56	0.04	23.89	6.60	30.49	21.20	22.54	5.89	28.43
	SD	1.83	2.99	2.33	0.36	0.01	2.34	1.27	2.63	2.00	2.20	1.13	2.46
Ph	Mean	23.24	33.50	56.74	2.56	0.04	23.89	6.60	30.49	21.20	22.54	5.89	28.43
	SD	1.83	2.99	2.33	0.36	0.01	2.34	1.27	2.63	2.50	2.20	1.13	2.46
Th	Mean	19.20		19.20	8.25	0.41	10.77		11.31	20.40	10.16		10.64
	SD	5.15		5.15	3.64	0.20	7.89		9.11	1.11	7.45		8.53
FDS	Mean	30.33	32.59	62.91	0.54	0.01	91.83	41.24	133.07	30.10	86.63	36.82	123.45
	SD	1.11	8.87	9.11	0.32	0.00	10.06	5.38	15.01	2.00	8.92	4.80	13.34
FDPH	Mean	31.90	37.20	69.10	5.76	0.08	272.63	50.87	323.50	39.70	257.20	45.42	302.62
	SD	1.16	2.46	2.25	2.06	0.03	50.51	3.52	52.57	3.00	47.65	3.14	49.48
FDPR	Mean	15.68	2.94	18.61	0.94	0.05	16.51	1.40	17.91	27.98	15.57	1.25	16.82
	SD	2.68	1.05	3.19	0.29	0.02	5.12	0.86	5.63	4.74	4.83	0.77	5.28
FDPU	Mean	20.08	16.21	36.29	2.54	0.07	41.55	1.90	43.45	29.20	39.20	1.70	40.89
	SD	2.11	4.09	3.43	0.63	0.02	7.98	0.73	7.84	2.00	7.52	0.65	7.40
MIO	Mean		27.15	27.15				41.01	41.01			36.62	36.62
	SD		0.95	0.95				4.52	4.52			4.04	4.04

Table 2. *Functional parameters of distal muscle of front leg*
Tablo 2. *Ön bacağın distal kaslarına ait fonksiyonel parametreler*

Muscles		PCSA (cm^2)	TCSA (cm^2)	Fmax (MPa)	TSFmax (MPa)	%TZ	KA/CA	KDU/$CA^{1/3}$	TA/CA	TKTA/CA	PCSA/$Ca^{2/3}$	TU/$CA^{1/3}$
ECR	Mean	54.46	0.76	16.34	21.03	14.02	1.18	1.00	0.04	1.23	1.21	2.32
	SD	11.72	0.13	4.52	2.30	4.20	0.48	0.27	0.01	0.48	0.69	0.11
OEM	Mean	12.51	0.12	3.75	33.22	22.15	0.04	0.14	0.01	0.04	0.28	1.59
	SD	2.72	0.03	0.81	11.09	7.39	0.00	0.03	0.00	0.00	0.07	0.60
FCR	Mean	9.74	0.16	2.92	18.39	12.26	0.26	1.17	0.01	0.27	0.22	1.69
	SD	2.01	0.03	0.60	4.22	2.82	0.01	0.22	0.00	0.01	0.04	0.23
FCU	Mean	103.16		30.95			0.34	0.26		0.34	2.31	1.69
	SD	44.00		13.20			0.04	0.35		0.04	1.01	0.23
ECU	Mean	124.91	0.63	37.47	81.59	54.39	0.43	0.19	0.02	0.45	2.82	1.20
	SD	15.16	0.45	13.55	16.76	17.84	0.05	0.17	0.02	0.06	1.05	0.21
EDC	Mean	20.76	0.40	6.23	16.28	10.85	0.41	0.85	0.07	0.48	0.46	6.78
	SD	3.95	0.14	1.19	3.36	2.24	0.05	0.16	0.02	0.06	0.08	0.37
EDLat	Mean	8.96	0.18	2.69	15.67	10.45	0.08	0.38	0.02	0.10	0.20	5.02
	SD	1.43	0.03	0.43	3.58	2.39	0.01	0.05	0.00	0.01	0.04	0.46
Ph	Mean	8.96	0.18	2.69	15.67	10.45	0.08	0.38	0.02	0.10	0.20	5.02
	SD	1.43	0.03	0.43	3.58	2.39	0.01	0.05	0.01	0.01	0.04	0.46
Th	Mean	3.73		1.12			0.04	1.24		0.04	0.08	1.59
	SD	7.34		2.20			0.02	0.55		0.03	0.16	0.60
FDS	Mean	184.27	1.27	55.28	47.78	31.85	0.31	0.08	0.14	0.44	4.15	4.89
	SD	21.57	0.64	8.47	8.81	2.54	0.05	0.05	0.01	0.06	1.39	1.31
FDPH	Mean	49.99	1.17	15.00	11.27	7.51	0.92	0.86	0.17	1.10	1.13	5.59
	SD	19.15	0.09	5.74	3.84	2.56	0.15	0.30	0.01	0.15	0.43	0.35
FDPR	Mean	17.20	0.43	5.16	14.90	9.94	0.06	0.14	0.00	0.06	0.39	0.45
	SD	5.48	0.21	1.64	10.48	6.99	0.02	0.04	0.00	0.02	0.13	0.14
FDPU	Mean	15.76	0.11	4.73	47.82	31.88	0.14	0.38	0.01	0.15	0.35	2.44
	SD	2.87	0.04	0.86	5.66	5.44	0.02	0.09	0.00	0.02	0.06	0.65
MIO	Mean		1.35						0.14	0.14		4.07
	SD		0.18						0.02	0.02		0.17

terms of TKTH (cm^3) were higher in males compared to females and the difference was statistically significant ($P<0.05$). It was determined that FDS (29.53-31.13) in terms of KU (cm) and Th (0.29-0.60) in terms of Yİ were higher in females compared to males and the difference was statistically significant ($P<0.05$).

When the functional parameters of the distal muscles of front leg according to the gender were examined, it was found that ECR (0.86-0.67) and FCR (0.19-0.14) in terms of TCSA (cm^2), FCU (0.37-0.31), ECU (0.47-0.40), and FDS (0.35-0.26) in terms of KA/CA, ECU (0.50-0.41) in terms of TKTA/CA, FCR (1.84-1.55) and ECU (1.36-1.01) in terms of TU/$CA^{1/3}$ were higher in males compared to females and the difference was statistically significant ($P<0.05$). It was determined that ECU (37.65-126.50) in terms of TSFmax (MPa) and FDS (4.81-4.96) in terms of TU/$CA^{1/3}$ had a higher value in females compared to males and the difference was statistically significant ($P<0.05$).

When the results regarding the distal muscles of the hind leg were considered, the muscles with maximum and minimum KU values were determined as Tcr and EDB. In terms of TU and TKTU, the muscles with high and low values were specified as FDS and EBD. It was observed that in the distal of the hind leg, maximum value in terms of the KDU parameter belonged to S and minimum value belonged to FDS. G had the maximum value in terms of KA and TKTA, and FDS had the maximum value in terms of TA. When the functional properties of the muscles were considered, it was observed that maximum and minimum values of PCSA and Fmax parameters belonged to FDL and S, maximum TCSA parameter belonged to G, maximum value in terms of PA belonged to FDS and minimum value belonged to FDM.

When the structural parameters of the distal muscles of hind leg according to the gender were observed, it was found that Tcr (30.88-28.85), GM (24.45-21.50), FDL

Table 3. *Structural parameters of distal muscle of hind leg*
Tablo 3. *Arka bacağın distal kaslarına ait yapısal parametreler*

Muscles		KU (cm)	TU (cm)	TKTU (cm)	KDU (cm)	AI	KA (g)	TA (g)	TKTA (g)	PA (°)	KH (cm^3)	TH (cm^3)	TKTH (cm^3)
EDL	Mean	25.31	45.65	70.96	5.82	0.08	197.61	26.44	224.05	29.60	186.42	23.61	210.03
	SD	1.51	3.12	3.91	0.82	0.01	27.01	3.79	20.22	3.00	13.22	2.31	15.00
EDlat	Mean	26.80	19.59	46.39	4.72	0.10	124.11	8.90	133.01	30.90	117.09	7.94	125.03
	SD	4.33	1.95	4.31	1.03	0.03	24.21	9.73	33.92	4.50	11.71	8.68	20.38
Tcr	Mean	29.86	8.84	38.70	6.07	0.18	147.71	4.65	152.35	35.40	139.35	4.15	143.50
	SD	1.34	0.72	1.21	0.62	0.02	18.47	0.54	18.56	3.00	17.42	0.49	17.50
PT	Mean		40.31	40.31				45.29	45.29			40.44	40.44
	SD		1.16	1.16				3.22	3.22			2.87	2.87
GL	Mean	25.13	16.80	37.73	3.02	0.08	451.98	29.41	474.05	36.30	426.41	26.26	446.10
	SD	0.99	1.76	7.67	0.54	0.01	22.10	1.25	28.12	4.00	20.84	1.12	26.10
GM	Mean	22.98		32.60	4.07	0.13	380.26		398.01	29.20	358.72		374.58
	SD	2.02		7.49	0.34	0.03	37.94		35.87	2.00	35.81		33.80
S	Mean	25.68	9.19	33.86	14.97	0.45	5.19	0.60	5.71	25.00	4.89	0.52	5.36
	SD	2.80	1.00	4.61	2.12	0.08	0.86	0.21	1.01	2.00	0.81	0.20	0.95
EDB	Mean	8.45	2.38	8.70	6.41	0.60	3.07	0.10	3.08	27.00	2.90	0.09	2.91
	SD	1.77	0.44	2.13	1.27	0.17	1.16	0.01	1.17	3.00	1.10	-	1.11
FDS	Mean	24.44	60.69	85.13	0.30	0.00	58.72	81.73	140.44	52.30	55.39	72.98	128.36
	SD	2.54	2.45	2.85	0.07	0.00	14.84	8.03	20.17	5.00	13.99	7.18	18.71
FDM	Mean	19.95	25.55	45.50	7.08	0.16	92.62	3.71	96.33	20.30	87.38	3.31	90.69
	SD	1.16	1.41	1.07	2.13	0.05	6.00	0.41	6.34	2.00	5.66	0.37	5.97
FDL	Mean	26.25	46.50	72.75	1.04	0.01	354.10	68.20	422.31	41.40	334.06	60.90	394.96
	SD	1.04	1.63	2.24	0.19	0.00	73.72	4.28	72.64	4.00	69.55	3.83	68.58
Tcd	Mean	21.28	8.89	30.16	4.64	0.15	91.67	1.10	92.77	29.00	86.47	0.99	87.46
	SD	1.38	1.17	1.45	0.32	0.01	25.34	0.41	25.62	2.00	23.90	0.37	24.16
P	Mean	19.50		19.50	3.50	0.18	173.30		173.30	43.70	163.50		163.50
	SD	1.51		1.51	0.75	0.03	7.01		7.01	6.00	6.61		6.61
MIO	Mean		30.75	30.75				40.78	40.79			36.42	36.42
	SD		1.56	1.56				7.83	7.84			7.00	7.00

(27.00-25.50), and Tcd (22.30-20.25) in terms of KU (cm), EDL (48.05-43.25) and PT (41.13-39.50) in terms of TU (cm), FDM (46.30-44.70) in terms of TKTU (cm), Tcr (0.19-0.16) in terms of Yİ, GM (405.90-354.62) in terms of KA (g), GL (30.07-28.10) and FDM (4.05-3.37) in terms of TA (g), GL(496.58-451.52) in terms of TKTA (g), GM (382.93-334.52) in terms of KH (cm^3), GL (26.85-25.08) and FDM (3.62-3.00) in terms of TH (cm^3), GL (466.95-425.25) in terms of TKTH (cm^3) were higher in males compared to females and the difference was statistically significant ($P<0.05$). It was observed that EDB (5.22-7.60) in terms of KDU (cm) and EDL (0.08-0.09) and EDB (0.44-0.76) in terms of Yİ were higher in females compared to males and the difference was statistically significant ($P<0.05$).

When the functional parameters of the distal muscles of hind leg according to the gender were observed, it was found that GM (98.26-78.88) and EDB (0.65-0.52) in terms of PCSA (cm^2), GM (29.48-23.67) and EDB (0.19-0.16) in terms of Fmax (Mpa), Edlat (39.60 20.66) in terms of TSFmax (MPa), Edlat (26.40 13.77) in terms of %TZ, GM (2.13-18.4) in terms of $PCSA/Ca^{2/3}$ were higher in males compared to females and the difference was statistically significant ($P<0.05$). EDL (0.78-0.97) in terms of $KDU/Ca^{1/3}$, S (1.31-1.45) in terms of $TU/CA^{1/3}$ were higher in females compared to males and the difference was statistically significant ($P<0.05$).

In the analysis performed according to the direction (right-left), no statistically significant difference was determined.

DISCUSSION

In the study, structural and functional properties of the distal muscles of front and hind legs of Malakan Horses and the possible differences of these properties between the right-left legs and females-males were determined. Since the obtained results have distinguishing characteristics

Table 4. *Functional parameters of distal muscle of hind leg*
Tablo 4. *Arka bacağın distal kaslarına ait fonksiyonel parametreler*

Muscles		PCSA (cm^2)	TCSA (cm^2)	Fmax (MPa)	TSFmax (MPa)	%TZ	KA/CA	KDU/$CA^{1/3}$	TA/CA	TKTA/CA	PCSA/$Ca^{2/3}$	TU/$CA^{1/3}$
EDL	Mean	33.55	0.51	10.06	23.56	15.71	0.66	0.87	0.09	0.75	0.75	6.84
	SD	3.52	0.26	2.06	4.97	3.98	0.22	0.14	0.04	0.26	0.29	0.36
EDlat	Mean	26.36	0.38	7.91	30.13	20.08	0.42	0.71	0.03	0.45	0.60	2.94
	SD	1.74	0.38	3.52	3.72	4.15	0.17	0.16	0.04	0.21	0.28	0.30
Tcr	Mean	23.26	0.47	6.98	14.91	9.94	0.50	0.91	0.02	0.51	0.52	1.33
	SD	4.39	0.06	1.32	2.73	1.82	0.08	0.09	0.00	0.08	0.11	0.12
PT	Mean		1.00						0.15	0.15		6.04
	SD		0.07						0.01	0.01		0.11
GL	Mean	145.42	1.58	43.63	25.35	16.90	1.52	0.45	0.10	1.59	3.28	2.64
	SD	26.99	0.15	8.10	3.81	2.54	0.07	0.08	0.01	0.05	0.69	1.67
GM	Mean	88.57		26.57			1.28	0.61		1.34	1.98	2.64
	SD	11.51		3.45			0.09	0.06		0.11	0.19	1.67
S	Mean	0.33	0.10	0.10	1.81	1.22	0.02	2.24	0.00	0.02	0.01	1.38
	SD	0.07	0.13	0.02	0.55	0.35	0.00	0.28	0.00	0.00	0.00	0.16
EDB	Mean	0.59	0.04	0.18	4.33	2.89	0.01	0.77	0.00	0.01	0.01	1.38
	SD	0.07	0.01	0.02	1.20	0.50	0.00	0.39	0.00	0.00	0.00	0.16
FDS	Mean	203.22	1.20	60.97	49.56	33.04	0.20	0.05	0.27	0.47	4.54	9.09
	SD	87.23	0.13	26.17	18.75	12.50	0.05	0.01	0.03	0.06	1.91	0.29
FDM	Mean	13.38	0.13	4.01	32.35	21.57	0.31	1.06	0.01	0.33	0.30	3.83
	SD	3.93	0.02	1.18	14.11	9.41	0.02	0.31	0.00	0.02	0.10	0.20
FDL	Mean	326.53	1.31	97.96	74.63	49.76	1.20	0.16	0.23	1.42	7.32	6.97
	SD	87.41	0.06	26.22	18.20	12.13	0.25	0.03	0.01	0.25	1.82	0.24
Tcd	Mean	18.62	0.11	5.59	53.40	35.60	0.31	0.69	0.00	0.31	0.42	1.33
	SD	5.18	0.04	1.56	14.82	9.88	0.08	0.04	0.00	0.08	0.11	0.18
P	Mean	48.33		14.50			0.58	0.52		0.58	1.09	1.33
	SD	8.75		2.62			0.03	0.10		0.03	0.22	0.18
MIO	Mean		1.19						0.14	0.14		4.61
	SD		0.25						0.03	0.03		0.25

compared to other animals (especially other horse breeds and donkeys), this study plays a key role in terms of making comparison and determining the locomotor activity.

Alexander et al.[22] argued that the decrease in the bundle length of the distal muscles in the legs of big animals can be tolerated by the tendons in this region which have a long and elastic structure. Shortening in the muscle bundles specified that the bundles can fit into a narrower area and number of fiber per unit area may increase. Thus, this situation caused an increase in PCSA and the force generated (see equipment and method). As a result, the short muscle fibers which can be packaged in a narrow area will be able to provide higher power generation by the increase of PCSA and able to increase the elastic energy quantity level which can be stored and released on the tendon via sufficient muscle-tendon movement [8]. Thus, the fact that muscles have small volume, short fibers and large PCSA shows that they have a high power generation capacity. Consequently, the force required for the muscle to be dynamic or static is produced by the muscles with a small volume and long tendons [7,8,23,24]. In the study, it was observed that the muscles in the distal of front and hind legs of Malakan horses generally had small volume, short fibers and high PCSA. The most significant muscles were determined as EDC, FDPH, FDS in the front leg and FDS and FDL in the hind leg.

Determination of the scaling and standardization of the findings obtained through the evaluation of muscle structure provides opportunity in the comparison of the animal types of results [22]. Alexander et al.[22] standardized various values (KA, TA, KDU, PCSA, TU) with the live body weight in their studies. Another standardization value in revealing the structural properties of the muscles is the structural index (KDU/KU). Structural index provides opportunity for the relatively comparison of the muscle

Table 5. *Some characteristics of hind leg distal muscle of horse, H: Horse (Payne et al.[8]), MH: Malakan Horse (Study), D: Donkey (Demiraslan, [26])*
Tablo 5. *At, Malakan Atı, ve merkep arka bacağı distal kaslarının bazı özellikleri, H: At (Payne et al.[8]), MH: Malakan Atı (Çalışma), D: Merkep (Demiraslan, [26])*

Muscles	KU (cm)			TU (cm)			KDU (cm)			KA (g)			TA (g)			PA (°)			PCSA (cm²)			TCSA (cm²)		
	H	MH	D	H	MH	D	H	MH	D	H	MH	D	H	MH	D	H	MH	D	H	MH	D	H	MH	D
EDL	27.1	25.31	18.06	47.2	45.65	35.51	8.1	5.82	3.65	462	197.61	60.08	59.6	26.44	12.65	29	29.6	25	54	33.55	15.5	1.13	0.51	0.32
EDlat	28.4	26.8	16.55	30.8	19.59	17.95	7	4.72	2.86	192	124.11	20.37	21	8.9	1.93	28	30.9	18	26	26.36	6.87	0.61	0.38	0.1
Tcr	32.6	29.86	21.48	9.2	8.84	7.59	4	6.07	3.36	309	147.71	37.82	26.9	4.65	2.74	41	35.4	33.35	73	23.26	10.52	2.61	0.47	0.33
PT				36.2	40.31	28.96							64.3	45.29	13.56							1.59	1	0.42
GL	26.2	25.13	17.62	24.4	16.8	14.87	5.6	3.02	2.25	808	451.98	92.95	90.7	29.41	12.2	34	36.3	37.8	137	145.42	40.25	3.32	1.58	0.73
GM	25.4	22.98	15.72				4.8	4.07	2.23	817	380.26	85.07				36	29.2	45.05	161	88.57	37.23			
S	15.5	25.68	17.47				12.1	14.97	6.05	6	5.19	1.81												
FDS	21.4	24.44	17.55	74.8	60.69	43.89	0.3	0.3	0.29	214	58.72	29.01	188.9	81.73	29.68	52	52.3	47.35	417	203.22	94.39	2.25	1.2	0.6
FDM	27	26.25	16.43	40.9	25.55	21.27	7	7.08	3.5	161	92.62	33.46	56.7	3.71	3.46	27	20.3	42.95	22	13.38	9.02	1.24	0.13	0.13
FDL	29.7	19.95	21.75	57.4	46.5	34.32	1	1.04	0.76	660	354.1	83.79	127	68.2	23.59	44	41.4	46.1	644	326.53	116.97	1.98	1.31	0.61
Tcd	24.2	21.28	15.95	13.1	8.89	10.42	5.7	4.64	2.43	224	91.67	19.27	6.1	1.1	0.91	31	29	13.7	37	18.62	7.61	0.42	0.11	0.08
P	23.5	19.5	16.19				3.8	3.5	1.84	280	173.3	54.91				42	43.7	38.3	70	48.33	28.69			
MIO				32.8	30.75	22.87							44.8	40.78	10.97							1.22	1.19	0.42

bundle length among the muscles. Fayed [14] specified that AI value in the distal muscles of front leg of the donkey varied between 0.024 and 0.759 and Demiraslan and Özcan [15] stated that AI value in the distal muscles of the hind leg of the donkey varied between 0.02 and 0.34. According to the results obtained in the study, it was observed that the structural index values of the muscles varied between 0.01 and 0.23 for the front leg and between 0.01 and 0.60 for the hind leg.

Tendons provide the muscles and bones engage each other and also increase the mobility of the leg bones in the distal of the leg [25]. However, it is notified that tendons act as an elastic energy storage [7]. The muscles evaluated in the study with maximum TSfmax and TZ% values were determined as ECU for the front leg and as FDL for the hind leg. In the study of Fayed [14], he evaluated the front leg distal muscles of the donkeys and the muscle with maximum TSfmax value belonged to the caput accessorium of EDC (Thiernesse muscle-Th). Fayed [14] specified that maximum TS% value belonged to FDPU which is one of the digital flexor muscles in the front leg.

In a study of Payne et al.[8], they remarked that the volumes of the distal leg muscles and mean KDU values between each other were similar. In the study, this similarity was also determined in the distal leg muscles of Malakan horses. Payne et al.[8] specified in the same study that m. gastrocnemius was volumetrically the largest distal muscle in hind leg of the horse and it ended with a durable and a common tendon. The results obtained in the study revealed that in parallel to the literature, the most volumetric muscle among the distal muscles of the hind leg was m. gastrocnemius and it reached to insertion point with a durable and common tendon.

While Fayed [14] specified that TA/CA value of m. interosseus medius was 0.65, the same value was specified as 0.14 in this study. According to these values, it can be asserted that m. interosseus medius rendered more volume in the front leg of donkey compared to the front leg of Malakan horse.

Energy storage capability or number of the tendon depends on the size of the tendon and the pressure applied on that tendon. In this case, the energy storage capacity of the tendon can be estimated by the elongation amount of the tendon and weight of the tendon and the length in dysfunctional (at rest) position [20]. When TU and TA were considered in the study, it was determined that the muscles having the longest tendon were EDC, FDS, and FDPH in the front leg and EDL, FDS, and FDL in the hind leg.

In the study of Fayed [14] regarding the front leg distal muscles of the donkeys, he determined the muscles with maximum value in terms of KDU, KU, TU, KA, TA parameters were FCR, FCU, FDPH, ECR, and FDPH respectively. In the study, this sequence was specified as Th, FDPH, EDC, ECR, and FDPH. In this case, it is remarkable that sequence of high muscle length is different in donkeys and Malakan horses.

In the study conducted by Brown et al.[7], they evaluated the structural and functional properties of front leg distal muscles of the horses and specified that high values for KU, TU, KH, PCSA, and KDU parameters belonged to FDPH, FCU, ECR, FDS, and FCU muscles. In our study, these muscles were determined as FDPH, EDC, ECR, FDS, and TH.

Table 5 illustrates comparatively the results obtained

from the hind leg distal muscles for the horses from the study of Payne et al.[8], for the donkeys from the study of Demiraslan [15] and for Malakan Horses from this study. When the muscles are generally observed according to the table, it is seen that high muscle structural and functional parameters are similar in horses and donkeys.

In the comparison performed between the genders; while it was specified that the difference observed between some values of the muscles in the front and hind legs had a statistical significance ($P<0.05$), the differences in the comparison performed according to the direction (right-left) had no statistically significance. Abe et al.[12] reported that gender is effective upon properties of the muscle structure. While the data obtained from our study support literature in terms of the differences between the genders, it also reveals that gender should not be neglected during the preparation of the anatomical or biomechanical muscle models in equidae.

In parallel to the results of the studies previously performed in the species of equidae family, it was determined for the Malakan horse that the regional muscles evaluated in the study had generally long tendons, pennate, and short muscle bundles. This situation is accepted as an indicator for the leg distal that acts as an elastic energy storage. Especially the extent of the effect of the differences between the species on structural and functional properties of the muscles are better understood at the end of the study. Thus, the thesis specifying that the differences between the species can be effective in developing a leg muscle model is supported by this study.

REFERENCES

1. Güleç E: Türk At Irkları. Anadolu At Irklarını Yaşatma ve Geliştirme Derneği, Ankara, 1995.

2. Güleç E: Ardahan Atı (Malakan Atı). Anadolu At IrklarınıYaşatma ve Geliştirme Derneği, Ankara, 1997.

3. Hendricks BL: International Encyclopedia of Horse Breeds. University of Oklahoma Press, Oklahoma State, 1995.

4. Sacks RD, Roy RR: Architecture of the hind limb of muscle of cats: Functional significance. *J Morphol,* 173, 185-195, 1982. DOI: 10.1002/jmor.1051730206

5. Lieber RL, Shoemaker SD: Muscle, joint, and tendon contributions to the torque profile of frog hip joint. *Am J Physiol,* 263, 586-590, 1992.

6. Lieber RL: Skeletal muscle anatomy. **In,** Lieber RL (Ed): Skeletal Muscle Structure and Function. 1-43, Williams and Wilkins, Baltimore, 1992.

7. Brown NA, Kawcak CE, McIlwraith CW, Pandy M: Architectural properties of distal forelimb muscles in horses, *Equus caballus*. *J Morph,* 258, 106-114, 2003. DOI: 10.1002/jmor.10113

8. Payne RC, Hutchinson JR, Robilliard JJ, Smith NC, Wilson AM: Functional specialisation of pelvic limb anatomy in horses *(Equus caballus). J Anat,* 206, 557-574, 2005. DOI: 10.1111/j.1469-7580.2005.00420.x

9. Abe T, Kumagai K, Brechue WF: Fascicle length of leg muscles is greater in sprinters than distance runners. *Med Sci Sports Exerc,* 32, 1125-1129, 2000.

10. Blazevich AJ, Gill ND, Zhou S: Intra- and intermuscular variation in human quadriceps femor is architecture assessed in vivo. *J Anat,* 209, 289-310, 2006. DOI: 10.1111/j.1469-7580.2006.00619.x

11. Blazevich AJ, Gill ND, Bronks R, Newton RU: Training specific muscle architecture adaptation after 5-wk training in athletes. *Med Sci Sports Exerc,* 35, 2013-2022, 2003. DOI: 10.1249/01.MSS.0000099092.83611.20

12. Abe T, Brechue WF, Fujita S, Brown B: Gender differences in FFM accumulation and architectural characteristics of muscle. *Med Sci Sports Exerc,* 30, 1066-1070, 1998. DOI: 10.1097/00005768-199807000-00007

13. Burkholder TJ, Fingado B, Baron S, Lieber RL: Relationship between muscle fiber types and sizes and muscle architectural properties in the mouse hind limb. *J Morph,* 22, 177-190, 1994.

14. Fayed MH: Architecture and functional specifications of the muscles of the antibrachium and manus regions of the African Ass *(Equus asinus). Adv Biol Res,* 4 (1): 45-64, 2010.

15. Demiraslan Y, Özcan S: Merkepte regio cruris ve pedis'i çevreleyen kasların yapısal ve fonksiyonel özellikleri. *Eurasian J Vet Sci,* 31, 1-7, 2015. DOI: 10.15312/EurasianJVetSci.201518470

16. Uçar Y: Yerli merkep'in *(Equus asinus)* gövde ve ard bacak iskelet kasları üzerinde makro-anatomik araştırmalar. *Ank Üniv Vet Fak Derg*, 27, 103-124, 1980.

17. Biewener AA: Biomechanical consequences of scaling. *J Exp Biol*, 208, 1665-1676, 2005. DOI: 10.1242/jeb.01520

18. Pollock CM, Shadwick RE: Allometry of muscle, tendon, and elastic energy storage capacity in mammals. *Am J Physiol,* 266, 1022-1031, 1994.

19. Bullimore S, Burn J: Scaling of elastic energy storage in mammalian limb tendons: Do small mammals really lose out? *Biol Lett,* 1, 57-62, 2005. DOI: 10.1098/rsbl.2004.0243

20. Payne RC, Crompton RH, Isler K,Savage R, Vereecke EE, Gunther MM, Thorpe SKS, Aount KD: Morphological analysis of the hind limb in apes and humans. II. Moment arms. *J Anat,* 208, 725-742, 2006. DOI: 10.1111/j.1469-7580.2006.00564.x

21. International Committee on Veterinary Gross Anatomical Nomenclature: General Assembly of the World Association of Veterinary Anatomists. Nomina Anatomica Veterinaria. 5th ed., Gent, 2012.

22. Alexander RM, Jayes AS, Maloiy GM, Wathuta EM: Allometry of the leg muscles of mammals. *J Zool,* 194, 539-552, 1981. DOI: 10.1111/j.1469-7998.1981.tb04600.x

23. Pasi BM, Carrier DR: Functional trade-offs in the limb muscles of dogs selected for running vs. fighting. *J Evol Biol,* 16, 324-332, 2003. DOI: 10.1046/j.1420-9101.2003.00512.x

24. Smith NC, Wilson AM, Jespers K, Payne RC: Muscle architecture and functional anatomy of the pelvic limb of the ostrich (*Struthio camelus*). *J Anat,* 209, 765-780, 2006. DOI: 10.1111/j.1469-7580.2006.00658.x

25. Williams SB, Payne RC, Wilson AM: Functional specialization of the pelvic limb of the hare (*Lepus europeus*). *J Anat,* 210, 472-490, 2007. DOI: 10.1111/j.1469-7580.2007.00703.x

26. Demiraslan Y: Yerli merkeplerde *(Equus asinus)* crus ve regio pedis'i çevreleyen kasların yapısal ve fonksiyonel özellikleri. *Doktora Tezi,* Kafkas Üniv. Sağlık. Bil. Enst., 2013.

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Research Article

The Effect of Fasting on the Plasma Disposition of Albendazole in Goats

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Abstract

This study was designed to investigate the effect of fasting on the plasma disposition of albendazole (ABZ) in goats following oral administration. A total of 10 goats, aged 5-6 months were used in this study. The animals were allocated into two groups (fasted and fed groups) of five animals each. ABZ was administered orally to animals in two groups at 10 mg/kg bodyweight. Heparinize blood samples were collected between 1 h and 144 h after treatment and the plasma samples were analysed by high performance liquid chromatography for ABZ, active albendazole sulphoxide (ABZSO) and inactive albendazole sulphone ($ABZSO_2$) metabolites. ABZ is not detected and ABZSO and $ABZSO_2$ metabolites were present in the samples of fed and fasted animals. Feeding was significantly enhanced the plasma concentration of the ABZSO and $ABZSO_2$ metabolites. The area under the curve (AUC) and half-life (t1/2) of both metabolites were significantly larger and longer in fed compared to fasted animals, respectively. Moreover the maximum plasma concentration (C_{max}: 1.06±0.17 µg/ml) of ABZSO2 was also significantly higher in fed group compared with the fasting group (0.72±0.20 µg/ml). The changes in plasma kinetics, reflecting an altered quantitative gastrointestinal absorption or metabolism, were reflected in increased availability of ABZ metabolites in the plasma of fed goats. This could be a strategy to extend the exposure of parasites to the more active metabolite of ABZ and thus to improve the efficacy in goats.

Keywords: *Benzimidazoles, Albendazole, Pharmacokinetics, Fasting, Goat*

Keçilerde Albendazolün Plazma Dağılımına Aç Bırakmanın Etkisi

Özet

Bu çalışma keçilere albendazolün (ABZ) ağız yolu ile uygulanmasını takiben aç bırakmanın etkisini araştırmak amacı ile yapılmıştır. Çalışmada 5-6 aylık yaşta 10 keçi kullanılmıştır. Hayvanlar her grupta (aç ve tok) beş keçi olacak şekilde iki gruba ayrılmıştır. Her iki gruptaki hayvanlara ABZ 10 mg/kg dozda ağız yolu ile uygulanmıştır. Heparinize kan örnekleri ilaç uygulanmasından sonra 1 saat ile 144. saat arasında toplanmıştır ve plazma örnekleri ABZ, aktif albendazol sülfoksit (ABZSO) ve inaktif albendazol sülfon ($ABZSO_2$) metabolitleri için yüksek basınçlı sıvı kromatografisi ile analiz edildi. Aç ve tok hayvanlardan toplanan örneklerde ABZ tespit edilememiş, ABZSO ve $ABZSO_2$ metabolitleri bulunmuştur. Besleme ABZSO ve $ABZSO_2$ metabolitlerinin plazma konsantrasyonunu anlamlı şekilde artırmıştır. Beslenen gruptaki hayvanlarda her iki metabolitin eğri altı alanı (EAA) ve yarılanma ömrü (t1/2) anlamlı bir şekilde geniş ve uzun bulunmuştur. $ABZSO_2$'un doruk plazma yoğunluğu (Y_{doruk}: 1.06±0.17 µg/ml) beslenen gruptaki hayvanlarda, aç bırakılan gruptaki hayvanlardan (Y_{doruk}: 0.72±0.20 µg/ml) anlamlı birşekilde yüksek bulunmuştur. Nicel gastrointestinal emilim yada metabolizmada ki değişimleri yansıtan plazma kinetiğindeki değişimler beslenen keçilerin plazmasında ABZ metabolitlerinin yararlanımının arttığının göstergesidir. Bu durum keçilerde ABZ'un aktif metabolitlerine parazitin daha fazla maruz kalması ve etkinliğinin düzeltilmesi için bir strateji olabilir.

Anahtar sözcükler: *Benzimidazol, Albendazol, Farmakokinetik, Aç bırakma, Keçi*

INTRODUCTION

Goats are one of the most important food-producing animal species in developing countries. However, their sensitivity to gastrointestinal nematode infestations causes disease and economic loss. Available benzimidazole anthelmintic derivatives such as albendazole (ABZ) are frequently used in adult and young animals as a primary strategy for controlling gastrointestinal nematodes. ABZ (methyl [5-(propylthio)-1H-benzimidazol-2-yl] carbamate), is highly effective against all stages of gastrointestinal nematodes including lungworms, cestodes and adult liver flukes in cattle, sheep and goats at different dose rates [1]. ABZ is a poorly water-soluble drug (0.2 mg/ml in water

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at 25°C) and, consequently, it is poorly and erratically absorbed from the gastro-intestinal tract. This property which is ideal for its use against luminal infections is a problem in the treatment of systemic helminthiasis [2].

Following oral administration, ABZ absorbed from the gastrointestinal tract is rapidly metabolized into its pharmacologically active metabolite, albendazole sulphoxide (ABZSO) and inactive metabolite albendazole sulphone ($ABZSO_2$) by the liver [3,4]. It is thought that the flavine-containing monooxygenase (FMO) is mainly responsible for sulphoxidation, whereas cytochrome-dependent mono-oxygenase is responsible for sulphonation [5].

Host-related factors, including biotransformation and absorption, affect the kinetic behaviour and resultant clinical efficacy of benzimidazole compounds in animal [6,7]. The gastrointestinal passage rate of digesta is affected by alteration in the quality and quantity of the feed consumed and this could confer variable absorption time and therefore bioavailability of drugs. Because of its poor water solubility, formulation of ABZ is restricted to oral administration. After oral administration of benzimidazole anthelmintics to ruminants, greater bioavailability is observed than in monogastric species because in ruminants, the relatively slower gut transit time conferred by the fore stomach reservoir means that benzimidazoles are more extensively absorbed than in monogastric animals [8].

Reduction of feed intake resulted in increased plasma availability of oxfendazole, albendazole and triclabendazole in sheep and goats [6,8-10]. In cattle, it was demonstrated that fasting or restricted feed intake increased the relative bioavailability of ABZ metabolites [11] whereas in the dog [12] and horse [13] feeding increased the bioavailability of benzimidazoles after oral administration. However, to the best of our knowledge, there is no published report concerning the relationship among feed restriction and plasma disposition of ABZ in goats, the present study was designed to investigate the influence of fasting on the plasma disposition of ABZ in goats following oral administration

MATERIAL and METHODS

The study was approved by the Animal Ethics Committee of Adnan Menderes University (HEK/2006-0010). A total of 10 goats, aged 5-6 months and weighing 16-20 kg were used in this study and identified by specific name tags attached to their ears. The animals were appropriately housed in single pens. Water was supplied *ad libitum*.

Treatments and Sampling

For experiments, the goats were weighted and randomly allocated into 2 groups; fed and fasted, each with 5 goats. Both fed group and fasted group were fed *ad libitum* hay plus a small concentrate ration (5% fat content) but the animals in fasted group were not fed prior 24 h and 8 h after drug administration. Drug amounts to be administered to the animals were prepared from a commercial formulation (Valbazen-K tablet, 76 mg albendazole/tablet, Pfizer) for delivery at a single dose of 10 mg/kg bodyweight.

Heparinized blood samples (5 ml) were collected via the catheter before and 1, 2, 4, 8, 12, 16, 20, 24, 32, 48, 72, 96, 120 and 144 h post-treatment. Blood samples were centrifuged at 3000 g for 20 min and plasma was transferred to plastic tubes. All the plasma samples were stored at -20°C until analysis and there were analysed within 30 days after collection

Analytical Procedures

The analytical procedures involved two phases of measurements with high performance liquid chromatography (HPLC) following a liquid-liquid phase extraction procedure, as described by Marriner and Bogan [14].

A stock solution (100 µg/ml) of pure standard compounds of ABZ, ABZSO and $ABZSO_2$ (SmithKline Beecham, West Sussex, UK) were prepared using acetonitrile as the solvent. The resulting stock was then further diluted to produce 0.5, 1, 5, 10 and 25 µg/ml solutions which were later utilized for the purposes of calibration or determining the recovery of the drug-free plasma samples.

The drug-free plasma samples (1 ml) were spiked with standards of ABZ, ABZSO and $ABZSO_2$ to reach final concentrations: 0.05, 0.1, 0.5, 1 and 2.5 µg/ml. FBZ (10 µg/ml) was used as an internal standard for ABZ analysis. After mixing for 15 s, 5 ml ethyl acetate was added. The tubes were shaken on a slow rotary mixer for 10 min. After centrifugation at 3.000 g for 5 min, the organic phase (3.5 ml) was transferred to a thin-walled 10 ml-conical glass tube and evaporated to dryness at 60°C in a sample concentrator (Maxi-dry plus, HetoLab. Equipment, Denmark). The dry residue was re-suspended with 250 µl mobile phase. Then the tubes were placed in an ultrasonic bath and finally, 35 µl of this solution was injected into the chromatographic system. For ABZ and metabolites, the mobile phase was a mixture of acetonitrile-water (93:07) to which glacial acetic acid was added (0.5%, v/v). It was pumped through the column (Macherey-Nagel, nucleosil C_{18} 4 µm, 250 mm x 4.6 mm, Duren, Germany) with nucleosil C_{18} guard column (Phenomenex, Cheshire, UK) in a linear gradient fashion changing from 93:07 (acetonitrile-water) to 70:30 for 12 min, 70:30 to 90:10 for 1.5 min and the last ratio (93:07) was maintained for 3.5 min. The flow rate was 1.1 ml/min. Samples were processed on a computerized HPLC system (1100 series, Agilent Technologies, GmbH, Germany) comprising a degasser, a quaternary pump (G1354A), an auto sampler (G1313), a column oven (G1316A) and diodearray detector (G1315B) set at 292 nm. The retention times were 7.58 min (ABZSO), 8.52 ($ABZSO_2$), 12.16 min (ABZ) and 12.77 min (FBZ).

Validation Procedures

The analytical method used for ABZ, ABZSO and $ABZSO_2$ in goat plasma was validated prior to the start of the studies. The analytes were identified with the retention times of pure reference standards. Recoveries of the three molecules under study were measured by comparison of the peak areas from spiked plasma samples with the areas resulting from direct injections of standards prepared in acetonitrile. The inter- and intra-assay precisions of the extraction and chromatography procedures were evaluated by processing replicate aliquots of drug-free goat plasma samples containing known amounts of the drugs on different days. Calibration graphs for three molecules were prepared (linear range 0.05-5.0 µg/ml). The slope of the lines between peak areas and drug concentrations were determined by least squares linear regression and showed correlation coefficient 0.999. The detection limits of the three molecules were established with HPLC analysis of blank plasma fortified with the standard, measuring the baseline noise at the retention time of the peak. The mean baseline noise at the peak retention time plus three standard deviations was defined as the detection limit (LOD). The mean baseline noise plus five standard deviations was defined as the limit of quantification (LOQ) [15].

Pharmacokinetics and Statistical Analysis of Data

The plasma concentration versus time curves of each molecules obtained after treatment in individual animal, were fitted with WinNonlin software program (version 5.2, Pharsight Corp., Mountain View, California, US). Pharmacokinetic parameters for each animal were estimated using non-compartmental model analysis extravascular input. The maximum plasma concentration (C_{max}) and time to reach maximum concentration (t_{max}) were obtained from the plotted concentration-time curve. The area under the curve (AUC) and mean residence time (MRT) from time zero to infinity were calculated by trapezoidal rule. Terminal half-life ($t_{1/2\lambda z}$) was calculated as:

$$t_{1/2\lambda z} = -\ln(2)/\lambda_z$$

where λ_z represents the first order rate constant associated with the terminal (log linear) portion of the curve.

The measurements for the above pharmacokinetic parameters were tabulated and statistically analyzed with Mann-Whitney U tests using a software package (SPSS ver. 17.0 for Windows - SPSS Inc., Chicago USA). Statistically significant differences were set at $P<0.05$.

RESULTS

The analytical method used to extract and quantify the plasma concentration of ABZ, ABZSO and $ABZSO_2$ by chromatographic analysis was validated before processing the experimental samples. This analysis yielded linear regression lines ranging from 0.05 to 5.0 µg/ml of ABZ, ABZSO and $ABZSO_2$ with a high correlation coefficient of 0.999. The limits of quantification (LOQ) of the assay were 0.02 for ABZ and 0.05 µg/ml for ABZSO and $ABZSO_2$. With six replicates of aliquots, the precision at each concentration level was lower than 15% of the coefficient of variation (CV), and the accuracy ranged from 85% to 98% for molecules. The inter-assay precision showed variation between 4.46 and 7.31%. The mean extraction recoveries were 75.4±6.3% for ABZ, 94.18±4.5% for ABZSO and 90.43±6.6% for $ABZSO_2$.

Clinically no adverse effects were observed in the goats following the oral administration of ABZ. After treatment ABZ were not detected parent molecule in plasma samples but ABZSO and $ABZSO_2$ metabolites were found over the detection limit level of fed and fasted animals. *Fig. 1* and *Fig. 2* shows the mean a (±SD) plasma concentration versus time curves and *Table 1* shows the mean (±SD) pharmacokinetic parameters of ABZSO and $ABZSO_2$. Feeding was significantly enhanced the plasma concentration of the sulphoxide and sulphone metabolite of ABZ. The area under the curve (AUC) and half-life ($t_{1/2}$) of both metabolites were significantly larger and longer in fed (AUC: 38.48±5.13

Fig 1. Comparative mean (n=5) plasma concentration profiles albendazole sulphoxide (ABZSO) in goats being offered of two different diets following an oral administration of albendazole to at 10 mg/kg bodyweight

Şekil 1. Keçilere Albendazolün 10 mg/kg dozda ağız yolu ile uygulanmasını takiben iki farklı diyet grubunda albendazol sülfoksitin (ABZSO) ortalama plazma konsantrasyonunun karşılaştırılması (n=5)

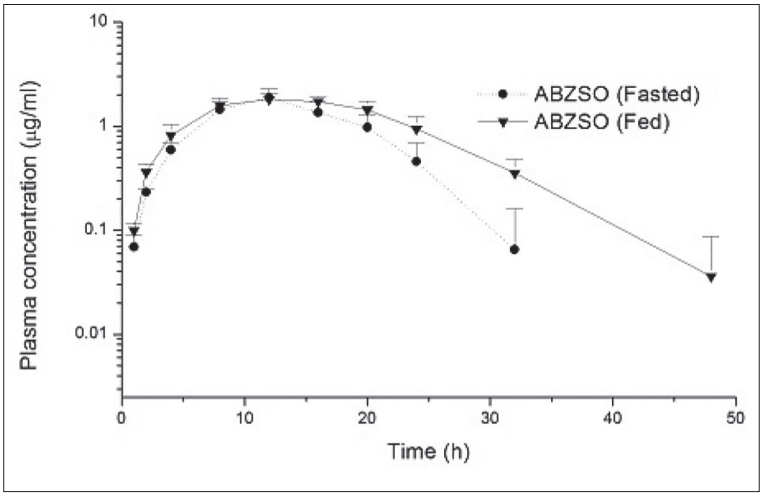

Fig 2. Comparative mean (n=5) plasma concentration profiles albendazole sulphone ($ABZSO_2$) in goats being offered of two different diets following an oral administration of albendazole to at 10 mg/kg bodyweight

Şekil 2. Keçilere Albendazolün 10 mg/kg dozda ağız yolu ile uygulanmasını takiben iki farklı diyet grubunda albendazol sülfonun ($ABZSO_2$) ortalama plazma konsantrasyonunun karşılaştırılması (n=5)

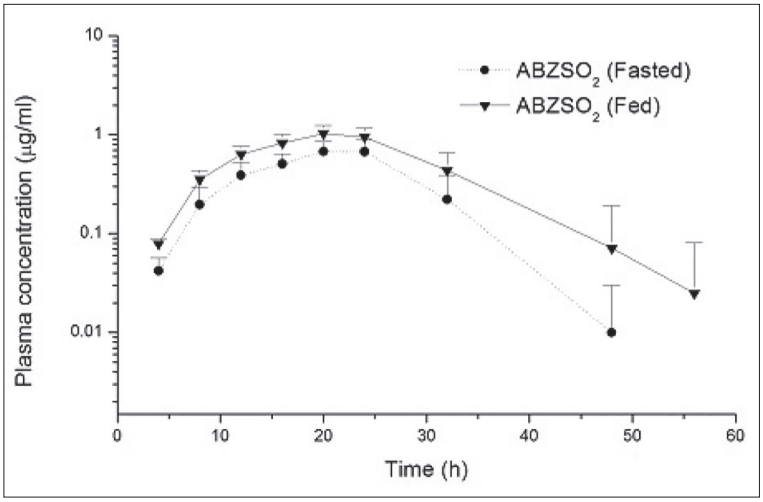

Table 1. *Mean (±SD) pharmacokinetic parameters of albendazole sulphoxide (ABZSO) and albendazole sulphone ($ABZSO_2$) in plasma following an oral administration of albendazole (10 mg/kg) to fasted and fed goats*

Tablo 1. *Aç ve tok keçilere Albendazolün (10 mg/kg) ağız yolu ile uygulanmasını takiben Albendazol sülfoksit (ABZSO) ve Albendazol sülfonun ($ABZSO_2$) ortalama (±SD) farmakokinetik parametreleri*

Parameters	ABZSO		$ABZSO_2$	
	Fasted	Fed	Fasted	Fed
T_{max} (h)	11.20±1.79	13.60±2.19	22.40±2.19	21.60±2.19
C_{max} (µg/ml)	1.89±0.40	1.90±0.22	0.72±0.20*	1.06±0.17
AUC_{last} (µg.h/ml)	27.00±7.09*	38.48±5.13	11.29±3.95*	22.48±5.59
$t_{1/2\lambda z}$ (h)	3.78±1.11*	6.73±0.62	4.30±1.06*	5.70±0.45
$AUMC_{last}$ (µg.h²/ml)	363.22±118.3*	611.26±151.7	216.93±89.5*	511.07±256.5
MRT_{last} (h)	13.25±1.03*	15.90±1.68	18.64±1.97	22.21±4.84

T_{max}: *time to reach peak plasma concentration,* ***C_{max}:*** *peak plasma concentration,* ***AUC_{last}:*** *area under the (zero moment) curve from time 0 to the last detectable concentration,* ***$t_{1/2\lambda z}$:*** *terminal half-life,* ***$AUMC_{last}$:*** *area under the moment curve from time 0 to t last detectable concentration,* ***MRT_{last}:*** *mean residence time; *The values in fasted group are significantly different from the values in fed group for both ABZSO and $ABZSO_2$ ($P<0.05$)*

µg.h/ml, $t_{1/2}$: 6.73±0.62 h for ABZSO; AUC: 22.48±5.59 µg.h/ml, $t_{1/2}$: 5.70±0.45 h for $ABZSO_2$) compared to fasted (AUC: 27.00±7.09 µg.h/ml, $t_{1/2}$: 3.78±1.11 for ABZSO; AUC: 11.29±3.95 µg.h/ml, $t_{1/2}$: 4.30±1.06 h for $ABZSO_2$) animals, respectively. The maximum plasma concentration (C_{max}: 1.06±0.17 µg/ml) of sulphone metabolite was also significantly higher in fed group compared with the fasting group (C_{max}: 0.72±0.20 µg/ml). Moreover, the mean residence time (MRT: 15.90±1.68 h) of albendazole sulphoxide in fed group were significantly different from those of fast group (MRT: 13.25±1.03 h).

DISCUSSION

The pharmacokinetics, bioavailability and activity of benzimidazoles are particularly influenced by the physico-chemical properties of drug, route of administration, sex, age, metabolic pathways of the active molecules, diet type and quality and quantity of diet. It was indicated that variations in the quality and quantity of diet could affect the bioavailability of benzimidazole anthelmintics in different animal species [8-11,16-20]. In ruminants, administration of benzimidazoles following a period of feed restriction greatly improves their bioavailability and this is thought to be associated with reduced absorbance to food and reduced reticulo-ruminal turnover and thus slower gut transit [10]. In addition, Singh et al.[20] observed reduced sulphoxide metabolite of albendazole in sheep fed compared to fast animals. The findings of the present study in goats differ substantially from those previous studies. In this study, the availability of ABZSO and $ABZSO_2$ in the plasma samples of fed goats was markedly greater compared to those of fasted goats, respectively. The C_{max} and AUC values of ABZ metabolites (ABZSO and $ABZSO_2$) in fed group are significantly higher and larger compared with those in fasted group. The apparent increased absorption in unfed goat is perhaps surprising since goats might be expected to behave like ruminants. The findings of the present study were suggested that feeding may delay gastric emptying in the goats thus slowing gut transport and improving absorption. These differences may be associated with prolonged absorption time of the drug from fed group compared with the fasted group. The physical form and quality of diet may affect the digestibility, flow rate into the gastrointestinal tract, the binding ability of content to the drug and the quality,

quantity and reductive capacity of the gastrointestinal microflora [21].

In the groups of this study, inter individual variations were observed in the plasma pharmacokinetic parameters. The origin of these variations was unclear. The animals were clinically healthy, possible chronic renal/hepatic problems may have contributed to these variations; since no biochemical tests were performed in the animals before starting the experiments in the present study. Albendazole, fenbendazole, triclabendazole and the pro-benzimidazoles (febantel and netobimin), which are commercially available thioether and sulphide benzimidazoles; commonly undergo microsomal sulphoxidation in liver. They are reversibly metabolised to their sulphoxide derivatives [13,22-24]. Irreversible sulphonation follows sulphoxidation and is a slower oxidative step resulting in a sulphone metabolite [25]. Sulphide and sulphoxide benzimidazoles are known to bind nematode tubulin [26] and therefore have activity against nematodes although sulphides exert inhibitory activity on tubulin at lower concentrations than sulphoxides.

In the present study, the parent ABZ was not determined, and ABZSO and $ABZSO_2$ were the main metabolites detected in the plasma samples during the experiment period following oral administration of ABZ. It is known that ABZ was rapidly and almost completely converted to ABZSO and $ABZSO_2$ in ruminant species. Although xenobiotic metabolism occurs mainly in the liver, extra-hepatic metabolism including gastrointestinal tract are also involved. The reductive environment of the gastro-intestinal tract of the ruminants could contribute to the metabolic reduction of the benzimidazole drugs. The reduction of netobimin into albendazole and oxidation of albendazole into albendazole sulphoxide were shown in an artificial rumen [27] and in the in vitro ruminal and intestinal fluids of sheep and cattle [28]. Furthermore, it was reported that the metabolism of oxfendazole to sulphide (fenbendazole) occurred in ruminal fluid in sheep and cattle [29].

In recent years, anthelmintic resistance to broad spectrum chemotherapeutic agents including benzimi-dazoles has become an increasing problem in domestic animals including goats, sheep, cattle, swine and horses throughout the world. Generally, irreversible resistance develops in helminths, usually within 5 years of introduction of the anthelmintic drugs [30]. There are many reports indicating that anthelmintic resistance to important nematodes in ruminants is emerging in many countries [31]. More intensive or often treatments with decreasing anthelmintic efficacy are likely to be served, which could increase the intensity the resistance problem. The progressive decline in the anthelmintic efficacy of BZD drugs because of the development of resistance in sheep and goats has forced to search new strategies: technics to increase efficacy of drugs available. Decreased drug availability in the systemic circulation and/or target tissues, resulting in a subtherapeutic exposure of those helminths having resistant genes, may facilitate the progressive development of anthelmintic resistance [32]. Modifying of diet before or after drug administration has been recommended to enhance the plasma concentration of active compounds thus improve the anthelmintic efficacy of those compounds and delay the development of anthelmintic resistance. It has been indicated that feed restriction caused increasing of the plasma levels of oxfendazole in sheep accounted for elevated efficacy of the drug against BZD-resistant strains of *Haemonchus contortus* and *Trichostrongylus colubriformis* [7,33].

It is concluded that the availabilities of ABZSO and $ABZSO_2$ in the plasma samples of fed goats were markedly greater than in those fasted goats. The changes in plasma kinetics, reflecting an altered quantitative gastrointestinal absorption or metabolism and increased availability of ABZ metabolites in the plasma of fed goats. This could be a strategy to extend the exposure of parasites to the more active metabolite of ABZ and thus to improve the efficacy and to delay the development of anthelmintic resistance in goats.

REFERENCES

1. Swarnkarn CP, Sanyal PK, Singh D, Khan FA, Bhagwan PSK: Comparative disposition kinetics of albendazole in sheep following oral and intraruminal administration. *Vet Res Commun*, 22, 545-551, 1998. DOI: 10.1023/A:1006145820818

2. Evrard B, Chiap P, DeTullio P, Ghalmi F, Piel G, Van Hees T, Crommen J, Losson B, Delattre L: Oral bioavailability in sheep of albendazole from a suspension and from a solution containing hydroxypropyl-b-cyclodextrin. *J Control Release*, 85, 45-50, 2002. DOI: 10.1016/S0168-3659(02)00270-5

3. McKellar QA, Scott E: The benzimidazole anthelmintic agents-review. *J Vet Pharm Ther,* 13, 223-247, 1990. DOI: 10.1111/j.1365-2885.1990.tb00773.x

4. Gokbulut C, Cirak VY, Senlik B: Plasma disposition and faecal excretion of netobimin metabolites and enantiospecific disposition of albendazole sulphoxide produced in ewes. *Vet Res Commun,* 30, 791-805, 2006. DOI: 10.1007/s11259-006-3336-y

5. Benoit E, Besse S, Delatour P: Effect of repeated doses of albendazole on enantiomerism of its sulphoxide metabolite in goats. *Am J Vet Res*, 53, 1663-1665, 1992.

6. Ali DN, Hennessy DR: The effect of feed intake on the rate of flow of digesta and the disposition and activity of oxfendazole in sheep. *Int J Parasitol*, 23, 477-484, 1993. DOI: 10.1016/0020-7519(93)90036-X

7. Ali DN, Hennessy DR: The effect of level of feed intake on pharmacokinetic disposition of oxfendazole in sheep. *Int J Parasitol*, 25, 63-7, 1995. DOI: 10.1016/0020-7519(94)E0054-Q

8. Gokbulut C, Boyacioglu M, Karademir U, Aksit D: The effect of fasting on the plasma disposition of triclabendazole following oral administration in goats. *Res Vet Sci*, 89, 415-417, 2010. DOI: 10.1016/j.rvsc.2010.04.009

9. Gokbulut C, Karademir Ü, Boyacıoğlu M, Akar F: The effect of diet type on the plasma disposition of triclabendazole in goats. *Res Vet Sci*, 82, 388-391, 2007. DOI: 10.1016/j.rvsc.2006.08.003

10. Taylor SM, Mallon TR, Blanchflower WJ, Kennedy DG, Green WP: Effects of diet on plasma concentrations of oral anthelmintics for cattle and sheep. *Vet Rec,* 28, 264-268, 1992. DOI: 10.1136/vr.130.13.264

11. Sanchez SF, Alvarez LI, Lanusse CE: Fasting induced changes to the pharmacokinetic behaviour of albendazole and its metabolites in calves.

J Vet Pharm Ther, 20, 38-47, 1997. DOI: 10.1046/j.1365-2885.1997.00810.x

12. McKellar QA, Galbraith EA, Baxter P: Oral absorption and bioavailability of fenbendazole in the dog and the effect of concurrent ingestion of food. *J Vet Pharm Ther*, 16, 189-198, 1993. DOI: 10.1111/j.1365-2885.1993.tb00163.x

13. McKellar QA, Gokbulut C, Benchaoui HA, Muzandu KM: Fenbendazole pharmacokinetics, metabolism and potentiation in horses. *Drug Metab Dispos*, 30, 1230-1239, 2002. DOI: 10.1124/dmd.30.11.1230

14. Marriner SE, Bogan JA: Pharmacokinetics of albendazole in sheep. *Am J Vet Res*, 41, 1126-1129, 1980.

15. Shrivastava A, Gupta VP: Methods for the determination of limit of detection and limit of quantitation of the analytical methods. *Chron Young Sci,* 2, 21-25, 2011. DOI: 10.4103/2229-5186.79345

16. Sanyal PK, Knox MR, Singh DK, Hennessy DR, Steel JW: Influence of diet type on the kinetic disposition of fenbendazole in cattle and buffalo. *Int J Parasitol,* 25, 1201-1205, 1995. DOI: 10.1016/0020-7519(95)00041-Y

17. Knox MR, Steel JW: Effects of diet and species on the pharmacokinetics of fenbendazole in cattle. *Vet Res Commun,* 21, 37-43, 1997. DOI: 10.1023/B:VERC.0000009699.60051.e1

18. Lifschitz A, Virkel G, Mastromarino M, Lanusse C: Enhanced plasma availability of the metabolites of albendazole in fasted adult sheep. *Vet Res Commun,* 21, 201-211, 1997. DOI: 10.1023/A:1005832412415

19. Oukessou M, Souhaili Z: The effect of feed quality on the kinetic disposition of orally administered triclabendazole in sheep. *Vet Res Commun,* 22, 257-263, 1998. DOI: 10.1023/A:1006095400309

20. Singh D, Sanyal PK, Swarnkar CP, Khan FA, Bhagwan PS: Influence of diet type and pretreatment fasting on the disposition kinetics of albendazole in sheep. *Vet Res Commun,* 23, 229-240, 1999. DOI: 10.1023/A:1006201226391

21. Bogan JA, Marriner SE: The rumen as a pharmacokinetic compartment. **In,** Ooms LAA, Degryse AD, Van Miert ASJPAM (Eds): Physiologial and pharmacological aspects of the reticulo-rumen, 53, Martinus Nijhoff, Dordrecht, 1987.

22. Marriner SE, Bogan JA: Pharmacokinetics of fenbendazole in sheep. *Am J Vet Res*, 42, 1146-1148, 1981.

23. Gyurik RJ, Chow AW, Zaber B, Brunner EL, Miller JA, Villani AJ, Petra LA, Parish RC: The metabolism of albendazole in cattle, sheep, rats and mice. *Drug Metab Dispos,* 9, 503-508, 1981.

24. Mohammed Ali NAK, Bogan JA, Marriner SE, Richards RJ: (1987): Pharmacokinetics of triclabendazole alone or in combination with fenbendazole in sheep. *J Vet Pharm Ther,* 9, 442-445, 1987. DOI: 10.1111/j.1365-2885.1986.tb00067.x

25. Averkin E, Beard C, Dvorak C, Edwards J, Fried J, Schiltz R, Kistner TP, Drudge JH, Lyons ET, Sharp ML, Corvin RM: Methyl 5(6)-phenylsulfinyl-2-benzimidazole carbamate: A new potent anthelmintic. *J Med Chem,* 19, 1164-1166, 1975. DOI: 10.1021/jm00245a029

26. Lacey E, Brady RL, Prichard RK, Watson TR: Comparison of inhibition of polymerisation of mammalian tubulin and helminth ovicidal activity by benzimidazole carbonates. *Vet Parasitol*, 23, 105-119, 1987. DOI: 10.1016/0304-4017(87)90029-X

27. Capece BP, Calsamiglia S, Castells G, Arboix M, Cristofol C: Effect of ruminal microflora on the biotransformation of netobimin, albendazole, albendazole sulfoxide, and albendazole sulfoxide enantiomers in an artificial rumen. *J Anim Sci*, 79, 1288-1294, 2001.

28. Lanusse CE, Nare B, Gascon LH, Prichard RK: Metabolism of albendazole and albendazole sulphoxide by ruminal and intestinal fluids of sheep and cattle. *Xenobiotica,* 22, 419-426, 1992. DOI: 10.3109/00498259209046653

29. Beretta C, Fadini L, Malvisi J, Montesissa C: *In vitro* febantel transformation by sheep and cattle ruminal fluids and metabolism by hepatic subcellular fractions from different animal species. *Biochem Pharmacol,* 36, 3107-3117, 1987. DOI: 10.1016/0006-2952(87)90619-8

30. Roos MH: The role of drugs in the control of parasitic nematode infections: Must we do without? *Parasitology,* 114, 137-144, 1997.

31. Coles GC, Jackson F, Pomroy WE, Prichard RK, Von Samson-Himmelstjerna, Silvestre A, Taylor MA, Vercruysse J: The detection of anthelmintic resistance in nematodes of veterinary importance. *Vet Parasitol,* 136, 167-185, 2006. DOI: 10.1016/j.vetpar.2005.11.019

32. Hennessy D: Modifying the formulation or delivery mechanism to increase the activity of anthelmintic compounds. *Vet Parasitol.* 72, 367-390, 1997. DOI: 10.1016/S0304-4017(97)00106-4

33. Ali D, Hennessy D: The effect of reduced feed intake on the efficacy of oxfendazole against benzimidazole resistant *Haemonchus contortus* and *Trichostrongylus colubriformis* in sheep. *Int J Parasitol*, 5, 71-74, 1995. DOI: 10.1016/0020-7519(94)E0055-R

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Research Article

Investigation of the Effects of Acupuncture Stimulation on the Size and Blood Flow of Corpus Luteum and Progesterone Levels in Dairy Cows [1] [2]

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Abstract

In this study, effect of acupuncture (AP) on the luteal size (LS), luteal blood flow (LBF) and progesterone (P4) levels were investigated in the presence of corpus luteum (CL) in cows. Seven days after 14-days interval PGF2α estrus synchronization protocol, CL positive animals were assigned either to a control group (AP–, n=10) or to an AP group (AP+, n=10) stimulated by using B22 and B23 sensitive acupoints. LS and LBF examinations were carried out before the stimulation (0h) and at 1st, 3rd, 6th hour on d7 and thereafter on d9, d10, d11, d12 and d13 following the AP stimulation in each group with a portable color Doppler ultrasonography. Blood samples for P_4 measurement were collected during each examination. There was no significant difference in LS, LBF, or P_4 mean values between groups. However, LBF significantly increased at 6h after stimulation ($P<0.05$) in AP+ group but it increased at d11 in AP– group ($P<0.05$). The significant increase in LS was observed earlier in AP+ group (on d9; $P<0.01$) than AP– (on d11; $P<0.05$). Serum P_4 concentrations increased at 3h, d9 and d10 in AP+ group ($P<0.05$), however a significant difference was only observed at 3h in AP– group ($P<0.05$). In conclusion, AP stimulation induces earlier increases in LS, LBF and P4 parameters in cows during luteal phase.

Keywords: *Acupuncture, Cow, Luteal size, Luteal blood flow, Progesterone*

Sütçü İneklerde Akupunktur Stimülasyonlarının Korpus Luteum Büyüklüğü, Kan Akımı ve Progesteron Değerleri Üzerine Etkilerinin İncelenmesi

Özet

Bu çalışmada akupunkturun (AP) ineklerde korpus luteum (CL) varlığında luteal büyüklük (LS), luteal kan akımı (LBF) ve progesteron (P4) seviyeleri üzerindeki etkisi incelendi. 14 gün aralıklı PGF2α östrus senkronizasyon protokolünden 7 gün sonra, CL tespit edilen hayvanlar kontrol (AP–, n=10) ve duyarlı B22 ve B23 kullanılarak stimüle edilen akupunktur gruplarına ayrıldı (AP+, n=10). LS ve LBF incelemeleri taşınabilir bir renkli Doppler ultrasonografi ile 7. günde stimülasyon öncesi (0. saat), izleyen 1, 3, 6. saatler ve 9, 10, 11, 12 ve 13. günlerde yapıldı. Her muayenede P_4 ölçümleri için kan örnekleri alındı. Gruplar arasında LS, LBF ve P_4 ortalama değerleri farklı değildi. Bununla birlikte, LBF AP+ grubunda stimülasyon sonrası 6. saatte ($P<0.05$) ancak AP– grubunda 11. günde arttı ($P<0.05$). LS'deki önemli artış ise AP+ grubunda (9. gün; $P<0.01$), AP– grubuna göre (11. gün; $P<0.05$) daha erken gözlendi. Serum P_4 konsantrasyonları AP+ grubunda 3. saat, 9. gün ve 10. günde artış gösterdi ($P<0.05$). Ancak AP– grubunda sadece 3. saatte önemli değişim gözlendi ($P<0.05$). Sonuç olarak, luteal faz sırasında akupunktur stimülasyonu LS, LBF ve P4 değerlerinde daha erken artışa neden olmaktadır.

Anahtar sözcükler: *Akupunktur, İnek, Luteal büyüklük, Luteal kan akımı, Progesteron*

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INTRODUCTION

Use of AP for treatment of various illnesses, which is an important part of Traditional Chinese Medicine (TCM), was started around 2200-3000 BC [1]. This method became popular in the 1970's, and in 1974, International Veterinary Acupuncture Society (IVAS) was found. Furthermore, in 1979 a symposium was held in Beijing by World Health Organization (WHO) [2,3]. The principle of AP was initially based on the balance of Ying and Yang in TCM but today it is mostly thought as sympathetic parasympathetic interaction [4]. According to this method there are meridian systems transporting the life energy which is thought to be regulating the body functions by connecting and communicating specific organs in the body and these meridians intersect in several points on the skin [5]. Most diseases and injuries are caused by problems in the flow and balance of the life energy. Therefore these disorders can be influenced by treating AP points. It is well known, that there are lots of neuroreceptors and nerve endings transmitting information, such as pain, heat or pressure, near or close to AP points [1,3,6]. By stimulating these points some alterations may occur in the related organs, which may bring out the activation of the natural healing process. These points are named as AP points and anatomical locations of these acupoints have been determined topographically [6].

AP can be utilized in large animal medicine both as therapeutics and as a diagnostic tool [7]. Specific AP points become sensitive (Ah-Shi points) or painful in case of certain pathological conditions. Applying pressure to these points and eliciting a painful or sensitive response may help to pinpoint areas of concern or disease. The techniques used include manual needle stimulation, electroacupuncture (EAP), moxibustion, injection-AP (liquid needle technique: aquapuncture), suture embedding and laser stimulation [8,9]. B meridian is the most used one of Bladder (B), Gallbladder (Gb) and Spleen (SP) meridians, which is used, in veterinary reproduction [10,11].

Effect of AP on fertility is explained with three different mechanisms: 1) AP may mediate the release of neurotransmitters to stimulate the secretion of the gonadotropin-releasing hormone (GnRH) 2) AP may stimulate blood flow to the uterus by inhibiting uterine central sympathetic nerve activity and 3) AP may stimulate the production of endogenous opioids, which may inhibit the central nervous system outflow and the biological stress response [12]. Thus this method has been used in treating different reproductive disorders such as; pseudopregnancy, silent heat, repeat breeder syndrome, cystic ovaries, uterine prolapse, retained fetal membranes [13], polycystic ovaries [14,15], induction of cyclicity in anestrus mares [16], induction of ovulation [17], and increasing the blood flow to uterus and ovaries [18].

AP stimulation of certain points historically associated with reproduction significantly alters plasma levels of sex hormones, such as Luteinizing hormone (LH), Follicle-stimulating hormone (FSH), estradiol (E_2) and progesterone (P_4) [19-21]. Luteal vascularization is the main factor in the formation and maintenance of pregnancy by the secretion of P_4. Moreover, there is an intensive molecular process happening during the formation and maintenance of luteal vascularization [22] and this process can be observed by colour Doppler ultrasonography that is a non-invasive tool [23]. Growth, development, and structural and functional maturity of the CL is completed in the middle of the luteal stage [24]. There have many studies investigating the effects of $PGF_2\alpha$ [25], GnRH, human chorionic gonadotropin (hCG) [26], oxytocin [27], estradiol 17β [28] and β carotene [29] on the blood flow of CL due to the importance of the maintenance of vascularization of this ovarian structure. However, to the best of our knowledge, during the literature search, we did not found any previous study regarding the induction of blood flow of CL with AP stimulation in cows. Hence, this study was designed to investigate the effect of AP stimulation on LBF, LS and the hormone profile during luteal phase in cows. It is aimed to present how AP stimulations form changes in LBF, LS and P_4 according to the AP– group.

MATERIAL and METHODS

This study was conducted on 20 Holstein Friesian cows in good general condition with no reproductive disorders in 50-70 days in milk (DIM), fed with TMR and water provided *ad libitum*. This study was carried out in a dairy herd located in Eregli/KONYA (TR) between November 2012 and November 2013. All cows in the study were synchronized by double injection of $PGF_2\alpha$ (Dinoprost, 25 mg/cow, i.m.; Enzaprost®, CEVA; Turkey) at 14 days interval. Seven days after second $PGF_2\alpha$ injection transrectal ultrasonography was performed to confirm the presence of the CL and animals having CL were assigned to either control group (AP–, n=10) or to AP group (AP+, n=10) and were stimulated by using B22 and B23 sensitive acupoints described by Kothbauer [9], stainless steel AP needles (Richter Pharma, Wels, Austria) which have silver rounded section at the tip with a 40 mm length were inserted 2 cm caudally at one hand lateral of the median dorsal line in right and left side of the cows between the transversal processus of vertebra lumbalis I-II and II-III, respectively for stimulation of acupoints. In 15 min time period needles were rotated four times around itself without penetrating or withdrawing them. In AP+ group, measurement of LS and LBF were done immediately before the stimulation at hour (h) 0 and at h1, h3 and h6 after the stimulation on day (d) 7 and also on d9, d10, d11, d12, and d13 after the second $PGF_2\alpha$ injection using a portable colour Doppler ultrasonography device equipped with a 10 MHz linear transducer. In AP–

group, no stimulation was applied. All other examination procedures were done same as AP+ group.

Ultrasonographic examinations and image collection were performed as described previously [26,29-31]. CL examination and measurement of the LS and LBF were done by transrectal ultrasonography using a portable colour Doppler device (LOGIQ Book XP, General Electric Healthcare, Solingen, Germany) equipped with a 10 MHz linear probe. During each ultrasonographic examination, the maximum cross-sectional area of CL was identified and stored for further off-line measurements. After morphological evaluation, the power mode was activated and LBF mapping in various transverse sections was conducted. To minimize the variations in recording, the settings of the Power Doppler system were fixed and the same was used for all examinations. The entire cross-sectional area of the CL was visible within the Power Doppler sample box. After recording at least six images, six images without flash artefacts and with the maximum number of coloured areas were stored. These images were exported in DICOM format into a computer. CL was measured on B-mode images using a computer-assisted image analysis program (Pixelflux, version 1.0; Chameleon-Software, Leipzig, Germany), and the mean value of six pictures was determined. The same software was also used to assess the total area of colour pixels within the luteal tissue. For this purpose the whole luteal structure and its blood flow area were chosen as the region of interest (ROI) and the coloured area within this ROI was calculated. The averages from four of the six images were used for further evaluation of LBF.

Blood samples for P_4 measurement were collected via coccygeal vein immediately before each examination. Serum samples were stored at -20ºC until analysis. Serum P_4 concentrations were measured by Electrochemi-luminescence immunoassay method in an accredited laboratory (Düzen Laboratory Group, Ankara, Turkey). Method was performed by autoanalisator (Cobas E601 modular device, Roche Diagnostics GmbH, Mannheim) with the described procedure of the manufacturer. The intra- and inter-assay coefficients of variation averaged 1.4 and 2.9%.

Data were executed by Post hoc procedure in SAS (2014) for distribution of data. Student t test were used to compare of independent groups with two-tailed distribution. Rank correlation coefficients were executed by Spearman correlation procedure. General linear Model methods with adapted in repeated ANCOVA procedure was using to compare to groups and to investigate for changing data over the exact time (independent regressors in groups). Data were summarized with group of the means and their standard error of means. All calculations and analysis was executed by SAS (2014).

RESULTS

No side effects were observed during the study period in the cows due to the stimulations. LS increased from 3.5 cm^2 to 4.6 cm^2 and from 4.4 cm^2 to 5.4 cm^2, respectively in AP+ and AP– groups between d0 and d13. When intergroup comparisons were evaluated after the stimulations, no significant difference was found in LS (P>0.05, *Table 1*). Increase in LS was observed in both groups after the stimulations but significant increase in LS according to h0 was observed earlier in AP+ group (on d9; P<0.01) compared with AP– (on d11; P<0.05) *(Table 1)*.

When AP+ and AP– groups were compared with each other, no significant difference was found in LBF at examination time points (P>0.05). Whereas, the increases in LBF within the different time points were significantly different in each group. In AP+ group, LBF significantly increased at 6h after stimulation (P<0.05). However, LBF remained bumpy and reached its minimal levels at d9 (5.3±0.4) and increased again to the starting levels at d11 in AP– group (P<0.05) *(Table 2)*.

Serum P_4 levels were not statistically different between AP+ and AP– animals both in h0 and also at all examination time points after stimulations (P>0.05). On the other hand serum P_4 concentrations significantly increased at 3h, d9 and d10 in AP+ group (P<0.05), however a significant difference was only observed at 3h in AP– group *(Fig. 1)*.

Correlations between LS and LBF (min: 0.379-max: 0.589, P<0.001) were found in all time points except d11 in AP+ cows. Similarly positive correlations between LS and LBF (min: 0.323-max: 0.594, P<0.001) were found in AP– too *(Table 3)*.

Table 1. *LS (cm^2) values after the application of AP*
Tablo 1. *Akupunktur uygulaması sonrası luteal büyüklük (cm^2)değerleri*

Group	Days									P
	7				9	10	11	12	13	
	h0	h1	h3	h6						
AP+	3.5±0.2[a]	3.3±0.3	3.7±0.2	3.7±0.2	4.1±0.2[b]	4.2±0.2	4.4±0.2	4.5±0.2	4.6±0.2	<0.01
AP-	4.4±0.2[*]	4.6±0.2	4.4±0.2	4.7±0.2	4.5±0.2	5.1±0.2	5.4±0.1[#]	5.2±0.2	5.4±0.2	<0.05
P	ns	ns	ns	ns	ns	ns	ns	ns	ns	

ns: *non-significant (P>0.05), Values with different superscripts [(a,b)] and asterisk [(*, #)] in the same line are statistically different*

Table 2. *LBF (mm^2) values after the application of AP*
Tablo 2. *Akupunktur uygulaması sonrası luteal kan akımı (mm^2) değerleri*

Group	Days									P
	7				9	10	11	12	13	
	h0	h1	h3	h6						
AP+	5.7±0.7[a]	5.9±0.5	6.9±0.5	7.0±0.5[b]	7.2±0.5	6.5±0.4	7.3±0.4	5.9±0.4	7.8±0.6	<0.05
AP-	7.4±0.7*	6.3±0.6	7.9±0.7	6.9±0.6	5.3±0.4[♯]	7.1±0.5	7.3±0.6	6.7±0.7	7.3±0.6	<0.05
P	ns	ns	ns	ns	ns	ns	ns	ns	ns	

ns: *non-significant (P>0.05), Values with different superscripts [(a,b)] and asterisk [(*, ♯)] in the same line are statistically different*

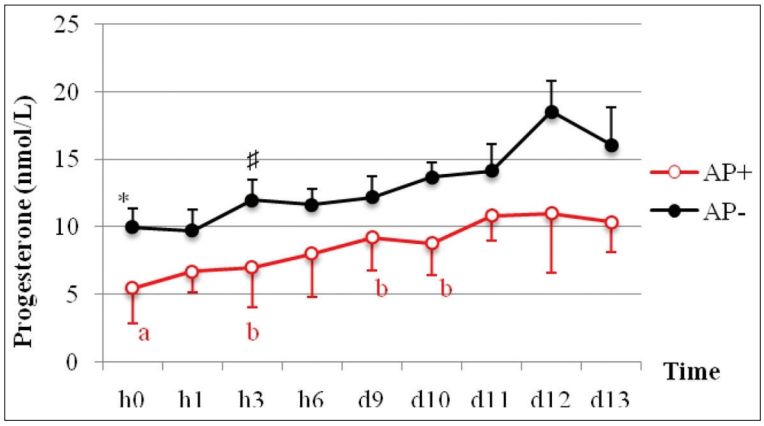

Fig 1. Mean P_4 (nmol/L) concentrations
Values with different superscripts [(a,b)] and asterisk [(*, ♯)] are statistically different (P<0.05)

Şekil 1. Ortalama P_4 (nmol/L) konsantrasyonları
Farklı harf [(a,b)] ve [(*, ♯)] işaretler istatistiki olarak farklıdır (P<0.05)

Table 3. *Correlations between LBF, LS and P_4 levels*
Tablo 3. *LBF, LS ve P_4 seviyeleri arasındaki korelasyonlar*

r_s	Group	Days								
		7				9	10	11	12	13
		h0	h1	h3	h6					
LBF/LS	AP+	0.435*	0.469*	0.589*	0.423*	0.417*	0.379*	0.061	0.583*	0.542*
	AP-	0.594*	0.459*	0.361*	0.516*	0.349*	0.477*	0.264	0.387*	0.323*
LBF/ P_4	AP+	0.830*	0.333	-0.079	0.127	0.214	0.024	0.617	-0.067	-0.406
	AP-	0.188	-0.535	-0.127	0.345	0.333	0.358	0.224	0.285	-0.126
LS/ P_4	AP+	0.236	0.176	-0.091	0.467	-0.048	0.261	0.617	0.200	0.200
	AP-	0.394	-0.231	-0.418	0.139	0.018	0.406	0.552	0.297	-0.167

** indicates the significance at 0.001 level*

DISCUSSION

Fertility/infertility and gynecology are the important topics of the AP that is used for many years in human and veterinary medicine [32]. AP–type stimuli influence reproductive organs and functions, including release of LH, FSH, P_4 and E_2 [33,34]. There are several studies on AP affecting the function of the CL [35,36].

B22 acupoint is used in cases of luteal insufficiencies, ovaritis, and small cystic degenerations of the ovary, anestrus, silent heat and sterility; B23 acupoint is used in cases of ovarian cycle disorders and sterility [9]. These acupoints were chosen and stimulated according to the above-mentioned indications.

As the CL matures, the majority of the steroidogenic cells establish contact with one or more capillaries [37], thus making the CL one of the most highly vascularized organs in the body [38]. Colour Doppler ultrasonography offers a useful, non-invasive approach to evaluate vascular function in the CL, allowing visual observation of local BF within the CL [39].

Maintenance of the pregnancy is dependent on the presence of the CL and continuous P_4 secretion capacity in domestic animals. There are several studies investigating the life cycle, size, blood flow of the CL and correlations between P_4 by using Doppler sonography [31,40,41]. Furthermore, CL grows rapidly during the development phase (ovulation- 5th day), reaches its maximum size in static phase

(5^{th}-16^{th} day) and regresses rapidly during luteolysis (16^{th}-17^{th} day) [40,41]. Life cycle and size of CL can be manipulated by several implementations during these phases. For example Ay et al.[29] reported additional β-carotene injections to the estrus synchronizations increased the size of the CL on day 7. Mann [42] demonstrated an increase in the LS between days 5-8 post ovulation very rapidly, and reported no significant increase after 8^{th} day. In our study, increase in LS according to the stimulation day (h0) occurred earlier in AP+ group (9^{th} day) than AP– group (11^{th} day) and continued until the end of the study.

It is known that CL is one of the most highly vascularized organs in the body [38]; therefore maintenance of the luteal function is dependent on the establishment and continuation of angiogenesis and vascularization [22]. LBF is more important than LS in the evaluation of luteal function [23,40]. It was demonstrated that follicular blood flow increased 1 h after hCG administration while it increased 6 h after GnRH injection in a study comparing the effects of GnRH and hCG injections in the presence of ovulatory follicle [26]. By the way, in our study, no significant differences ($P>0.05$) were reported in the LS and LBF between the groups on d9 and d12 after induction of ovulation. This study demonstrates effects of hormonal administrations on blood flow of the ovary in different ways. Although no significant differences were found between AP+ and AP– groups in the investigated parameters on the examination days, increases in these parameters were starting from h6 in AP+ group (except d10 and d12) and on d11 and d13 in AP– group.

Important hemodynamic changes occur during the follicular growth, ovulation and development of CL [22]. Angiogenic and vasoactive factors play vital role in the luteal life cycle in this period. Also it is known that changes in the systemic blood pressure are effective on the ovarian blood flow [43]. AP is effective on the regulation of the systemic blood flow, especially normalizing hypo and hypertension [44]. Studies investigating AP and blood flow of the genital organs were conducted on human or laboratory animals. He et al.[35] demonstrated AP stimulations were increased the ovarian expression of vascular endothelial growth factor (VEGF), VEGF mRNA and luteinizing hormone receptor (LHR) mRNA in rats. Besides EAP increased the blood flow of the target organs in genital tract [18,45].

AP shows this effect by reflecting the ovarian sympathetic nerves, and systemic circulation respectively [46]. Çakmak and Akpınar [18] reported that AP increased the blood flow of the testes in humans. Recurrent EAP stimulation causes increase in the uterine blood flow and it maintains for 10-14 days after the stimulation in women [46]. Although above mentioned studies were carried out in humans and rats they explain how AP stimulations cause earlier increase in LBF in this study. In addition AP stimulations cause not only earlier increase in LBF than AP– group; they also cause the increase to continue longer. This result supports that AP stimulations are effective on LBF for longer periods.

Angiogenic and vasoactive factors such as VEGF and basic fibroblast growth factor (bFGF) produced by the bovine CL regulates P_4 secretion, cell proliferation and angiogenesis in the developing CL [47]. The findings of our study indicate that AP stimulations can be effective on the activity of the angiogenic and vasoactive factors.

AP applications stimulate several neuropeptides such as serotonin, endogenous opioids and oxytocin in the central nervous system [48,49]. Because pituitary gland is regulated by the hormones originated by the central nervous system, it is thought that AP applications can regulate the endocrine system [49].

Hypothalamic endorphin has a tonic effect on GnRH pulse and the secretion of LH from hypophysis. By this it is in relation with hypothalamo-pituitary-ovarian axis [50,51]. Consequently AP can influence the reproductive hormones and activity by regulating the secretion of β-endorphin effecting the secretion of GnRH and gonadotropins [49].

Studies investigating the relation between AP and reproductive hormones are generally including treatment of reproductive problems or experimental studies. LH, P_4 and prolactin levels are increasing after AP in rats with implantation failure. AP causes this increase by upregulating the expression of the receptors of these hormones [35,52]. Lin and Wu [53] reported pulsatile LH secretion and P_4 levels were increased significantly 4-6 h after AP in gilts with pituitary responsiveness to GnRH. Stimulation of Pai-Hui and Wei-Ken acupoints cause decrease in LH levels within 2 h and increase in P_4 levels within 4-6 h in anestrus sows [21]. Despite no difference was found between the mean P_4 levels in this study conducted on cows, higher levels were achieved until d10 according to h0 in AP stimulated group ($P<0.05$). When increase in P_4 concentrations on d9 and d10 in this study was considered, it could be interpreted that P_4 secretion can be influenced by AP stimulations.

Different correlations between LBF, LS and P4 were reported in several studies [40,42]. A mild positive correlation was found in between LBF and LS except d11 in this study ($P<0.01$). However, no correlations were found between P_4 and LBF and P_4 and LS.

Studies on AP applications mentioned in this paper were especially carried out in abnormal cases or reproductive disorders. Besides AP whose mechanism of action is still not fully described, is based on the normalization of the defected physiology/function [6,9]. Important points in AP applications are finding the correct acupoint and giving the proper stimulation. This stimulation can be given by electric current, medicine, anesthetic agents or just by

twisting the needles such as in our study. But the frequency and the duration of the stimulation are important too.

In this study, effect of AP stimulations on the function of physiological CL in diestrus cows without reproductive problems was investigated. We could not find any similar study in our literature review. Although no significant difference between AP+ and AP– groups in LS, LBF and P_4 levels were found in this study, we conclude that increasing the frequency and duration of the stimulations may induce significant changes and earlier increases in LBF, LS and P_4 levels by considering the given information in the above mentioned studies. These increases indicate that AP can be effective on the activity of the CL similar to hormones and stimulation of the AP points besides hormonal injections (like GnRH) or injections of the hormones to the selected acupoints may have a positive effect on the life cycle of the CL especially in cows with luteal insufficiency.

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Conflicts of Interest

None of the authors have any conflicts of interest to declare.

REFERENCES

1. Yu C: Traditional Chinese Veterinary Acupuncture and Moxibustion. China Agriculture Press, Beijing, 1995.

2. WHO: Acupuncture: Review and analysis of reports on controlled clinical trials. 2002. http://apps.who.int/medicinedocs/pdf/s4926e/s4926e.pdf, *Accessed:* 10.11.2014

3. IVAS: What is veterinary acupuncture? https://www.ivas.org/about-ivas/what-is-veterinary-acupuncture/ *Accessed:* 11.10.2014.

4. Kaptchuk TJ: Acupuncture: Theory, efficacy, and practice. *Ann Intern Med*, 136, 374-383, 2002. DOI: 10.7326/0003-4819-136-5-200203050-00010

5. Fu HJ, He YQ, Gao Y, Man YC, Liu WK, Hao H: Acupuncture on the endometrial morphology, the serum estradiol and progesterone levels, and the expression of endometrial leukaemia-inhibitor factor and osteopontin in rats. *Evid Based Complement Alternat Med*, 1-9, 2011. DOI: 10.1155/2011/606514

6. Schoen A: Introduction to veterinary acupuncture: Scientific basis and clinical aplications. *Proceedings Annual Convention of the American Association of Equine Practioners*, 39, 1993.

7. Holt TN: Acupuncture: Treatment and Diagnosis, Food and Fiber Animal Medicine and Production 2012. http://c.ymcdn.com/sites/www.colovma.org/resource/resmgr/imported/Holt%20-%20Bovine%20acupuncture.pdf, *Accessed:* 13.1.2015.

8. Looney AL: Acupuncture in small animal patients. *Vet Med-Us,* 95 (8): 615-630, 2000.

9. Kothbauer O: Über die akupunktur und neuraltherapie bei fruchtbarkeitsstörungen des weiblichen Rindes. *Tierärztl Umschau*, 45, 225-236, 1990.

10. Kothbauer O, Meng A: Grundlagen der Veterinärakupunktur. Verlag, Wels, 1990.

11. Aslan S, Kılıçoğlu Ç: Akupunktur in der tieraerztliche geburtshilfe und gynaekologie. *Ankara Univ Vet Fak Derg*, 40, 38-48, 1993. DOI: 10.1501/Vetfak_0000001698

12. Al-Inany H: Acupuncture for infertility: A recently released evidence. *Middle East Fertil Soc J*, 13 (1): 67, 2008.

13. Lin JH, Chen WW, Wu LS: Acupuncture treatments for animal reproductive disorders. 2014. http://www.med-vetacupuncture.org/english/articles/lin99rep.html, *Accessed:* 10.12.2014.

14. Billhult A, Stener-Victorin E: Acupuncture with manual and low frequency electrical stimulation as experienced by women with polycystic ovary syndrome: A qualitative study. *BMC Complement Altern Med*, 12, 1472-6882, 2012. DOI: 10.1186/1472-6882-12-32

15. Feng Y, Johansson J, Shao RJ, Manneras-Holm L, Billig H, Stener-Victorin E: Electrical and manual acupuncture stimulation affect oestrous cyclicity and neuroendocrine function in an 5a-dihydrotestosterone-induced rat polycystic ovary syndrome model. *Exp Physiol*, 97, 651-662, 2012. DOI: 10.1113/expphysiol.2011.063131

16. Niemantsverdriet-Murton AS, Paccamonti D, Eilts BE, Pinto C, Seco EG, Costa L, Pettifer G: Use of acupuncture to induce cyclicity in anestrous mares. *J Equine Vet Sci*, 31, 97-102, 2011. DOI: 10.1016/j.jevs.2010.12.013

17. Mo X, Li D, Pu Y, Xi G, Le X, Fu Z: Clinical studies on the mechanism for acupuncture stimulation of ovulation. *J Tradit Chin Med*, 13 (2): 115-119, 1993.

18. Cakmak YO, Akpinar IN, Ekinci G, Bekiroglu N: Point- and frequency-specific response of the testicular artery to abdominal electro-acupuncture in humans. *Fertil Steril*, 90, 1732-1738, 2008. DOI: 10.1016/j.fertnstert.2007.08.013

19. Malven P, Bossut D, Diekman M: Effects of naloxone and electro-acupuncture treatment on plasma concentrations of LH in sheep. *J Endocrinol*, 101, 75-80, 1984. DOI: 10.1677/joe.0.1010075

20. Bossut DF, Stromberg MW, Malven PV: Electroacupuncture-induced analgesia in sheep: Measurement of cutaneous pain thresholds and plasma concentrations of prolactin and beta-endorphin immunoreactivity. *Am J Vet Res*, 47, 669-676, 1986.

21. Lin JH, Liu SH, Chan WW, Wu LS, Pi WP: Effects of electroacupuncture and gonadotropin-releasing hormone treatments on hormonal changes in anoestrous sows. *Am J Chin Med*, 16, 117-126, 1988. DOI: 10.1142/S0192415X88000182

22. Acosta TJ, Miyamoto A: Vascular control of ovarian function: Ovulation, corpus luteum formation and regression. *Anim Reprod Sci*, 82, 127-140, 2004. DOI: 10.1016/j.anireprosci.2004.04.022

23. Lüttgenau J, Bollwein H: Evaluation of bovine luteal blood flow by using color Doppler ultrasonography. *Reprod Biol*, 14, 103-109, 2014. DOI: 10.1016/j.repbio.2014.03.003

24. Lei Z, Chegini N, Rao CV: Quantitative cell composition of human and bovine corpora lutea from various reproductive states. *Biol Reprod*, 44, 1148-1156, 1991. DOI: 10.1095/biolreprod44.6.1148

25. Miyamoto A, Shirasuna K, Wijayagunawardane M, Watanabe S, Hayashi M, Yamamoto D, Matsui M, Acosta T: Blood flow: A key regulatory component of corpus luteum function in the cow. *Domest Anim Endocrinol*, 29, 329-339, 2005. DOI: 10.1016/j.domaniend.2005.03.011

26. Aslan S, Arslanbas D, Beindorff N, Bollwein H: Effects of induction of ovulation with Gnrh or hCG on follicular and luteal blood flow in Holstein-Friesian heifers. *Rerpod Domest Anim*, 46, 781-786, 2011. DOI: 10.1111/j.1439-0531.2010.01741.x

27. Brozos CN, Pancarci MS, Valencia J, Beindorff N, Tsousis G, Kiossis E, Bollwein H: Effect of oxytocin infusion on luteal blood flow and progesterone secretion in dairy cattle. *J Vet Sci*, 13, 67-71, 2012. DOI: 10.4142/jvs.2012.13.1.67

28. Pugliesi G, Beg M, Carvalho G, Ginther O: Effect of dose of estradiol-17β on prominence of an induced 13, 14-dihydro-15-keto-PGF 2α (PGFM) pulse and relationship of prominence to progesterone, LH, and luteal blood flow in heifers. *Domest Anim Endocrinol*, 41, 98-109, 2011. DOI: 10.1016/j.domaniend.2011.05.003

29. Ay SS, Kucukaslan I, Kaya D, Mulazimoglu SB, Emre B, Kacar C, Kalender H, Findik M, Bollwein H, Riegler M, Schafer-Somi S,

Scholbach J, Aslan S: The change in luteal blood flow and luteal size after beta carotene and Gnrh injections in early pregnant dairy cows. *Kafkas Univ Vet Fak Derg*, 18, 1035-1041, 2012. DOI: 10.9775/kvfd.2012.6959

30. Bollwein H, Prost D, Ulbrich S, Niemann H, Honnens A: Effects of a shortened preovulatory follicular phase on genital blood flow and endometrial hormone receptor concentrations in Holstein-Friesian cows. *Theriogenology*, 73, 242-249, 2010. DOI: 10.1016/j.theriogenology.2009.08.014

31. Acosta TJ, Hayashi K, Ohtani M, Miyamoto A: Local changes in blood flow within the preovulatory follicle wall and early corpus luteum in cows. *Reproduction*, 125, 759-767, 2003. DOI: 10.1530/rep.0.1250759

32. Maciocia G: Obstetrics and Gynecology in Chinese Medicine. Churchill Livingstone, Elsevier Health Sciences, 2011.

33. Aso T, Motohashi T, Murata M, Nishimura T, Kakizaki K, Kimura S: The influence of acupuncture stimulation on plasma levels of LH, FSH, progesterone and estradiol in normallly ovulating women. *Am J Chin Med*, 4, 391-401, 1976.

34. Yu J, Zheng H, Ping S: Changes in serum FSH, LH and ovarian follicular growth during electroacupuncture for induction of ovulation. *Zhong Xi Yi Jie He Za Zhi*, 9 (4): 199-202, 195, 1989.

35. He D, Huang G, Zhang M: Effects of acupuncture on the luteal function of rats with dysfunctional embryo implantation. *Zhongguo Zhen Jiu*, 29 (11): 910-913, 2009.

36. Nie GJ, Goodin AN, Braden TD, Wenzel JGW: Luteal and clinical response following administration of dinoprost tromethamine or cloprostenol at standard intramuscular sites or at the lumbosacral acupuncture point in mares. *Am J Vet Res*, 62, 1285-1289, 2001. DOI: 10.2460/ajvr.2001.62.1285

37. Reynolds LP, Grazul-Bilska AT, Redmer DA: Angiogenesis in the corpus luteum. *Endocrine*, 12, 1-9, 2000. DOI: 10.1385/ENDO:12:1:1

38. Wiltbank M, Dysko R, Gallagher K, Keyes P: Relationship between blood flow and steroidogenesis in the rabbit corpus luteum. *J Reprod Fertil*, 84, 513-520, 1988. DOI: 10.1530/jrf.0.0840513

39. Acosta TJ, Yoshizawa N, Ohtani M, Miyamoto A: Local changes in blood flow within the early and midcycle corpus luteum after prostaglandin F-2 alpha injection in the cow. *Biol Reprod*, 66, 651-658, 2002. DOI: 10.1095/biolreprod66.3.651

40. Herzog K, Brockhan-Lüdemann M, Kaske M, Beindorff N, Paul V, Niemann H, Bollwein H: Luteal blood flow is a more appropriate indicator for luteal function during the bovine estrous cycle than luteal size. *Theriogenology*, 73, 691-697, 2010. DOI: 10.1016/j.theriogenology.2009.11.016

41. Bollwein H, Lüttgenau J, Herzog K: Bovine luteal blood flow: Basic mechanism and clinical relevance. *Reprod Fertil Dev*, 25, 71-79, 2012. DOI: 10.1071/RD12278

42. Mann G: Corpus luteum size and plasma progesterone concentration in cows. *Anim Reprod Sci*, 115, 296-299, 2009. DOI: 10.1016/j.anireprosci.2008.11.006

43. Mitsube K, Zackrisson U, Brannstrom M: Nitric oxide regulates ovarian blood flow in the rat during the periovulatory period. *Hum Reprod*, 17, 2509-2516, 2002. DOI: 10.1093/humrep/17.10.2509

44. Longhurst JC, Tjen-A-Looi S: Acupuncture regulation of blood pressure: Two decades of research. *Int Rev Neurobiol*, 11, 1257-271, 2013. DOI: 10.1016/B978-0-12-411545-3.00013-4

45. Stener-Victorin E, Waldenstrom U, Andersson SA, Wikland M: Reduction of blood flow impedance in the uterine arteries of infertile women with electro-acupuncture. *Hum Reprod*, 11, 1314-1317, 1996.

46. Stener-Victorin E, Kobayashi R, Kurosawa M: Ovarian blood flow responses to electro-acupuncture stimulation at different frequencies and intensities in anaesthetized rats. *Auton Neurosci*, 108, 50-56, 2003. DOI: 10.1016/j.autneu.2003.08.006

47. Miyamoto A, Shirasuna K, Sasahara K: Local regulation of corpus luteum development and regression in the cow: Impact of angiogenic and vasoactive factors. *Domest Anim Endocrinol*, 37, 159-169, 2009. DOI: 10.1016/j.domaniend.2009.04.005

48. Andersson S, Lundeberg T: Acupuncture from empiricism to science: Functional background to acupuncture effects in pain and disease pain and disease. *Med Hypotheses*, 45, 271-281, 1995. DOI: 10.1016/0306-9877(95)90117-5

49. Stener-Victorin E, Jedel E, Manneråas L: Acupuncture in polycystic ovary syndrome: Current experimental and clinical evidence. *J Neuroendocrinol*, 20, 290-298, 2008. DOI: 10.1111/j.1365-2826.2007.01634.x

50. Jenkins PJ, Grossman A: The control of the gonadotrophin releasing hormone pulse generator in relation to opioid and nutritional cues. *Hum Reprod*, 2, 154-161, 1993. DOI: 10.1093/humrep/8.suppl_2.154

51. Genazzani AR, Genazzani AD, Volpogni C, Pianazzi F, Li GA, Surico N, Petraglia F: Opioid control of gonadotrophin secretion in humans. *Hum Reprod*, 2, 151-153, 1993. DOI: 10.1093/humrep/8.suppl_2.151

52. Xiong F, Gui J, Yang W, Li J, Huang GY: Effects of acupuncture on progesterone and prolactin in rats of embryo implantation dysfunction. *Chin J Integr Med*, 21, 58-66, 2015. DOI: 10.1007/s11655-014-1755-0

53. Lin JH, Wu LS, Dzang SH, Liu SH, Chan WW: Pituitary responsiveness to gonadotropin-releasing hormone in gilts treated with electroacupuncture. *18th Annual International Congress on Veterinary Acupuncture.* 16-20th September, Hawai, 1992.

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Research Article

Türkiye'de Deney Hayvanı Kullanmaya Yetkili Kişilerin Hayvan Kullanımına Yönelik Tutumları [1]

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Özet

Türkiye'de hayvan kullanılarak yapılan deneysel araştırmalarda, hayvan(lar) üzerinde uygulama yapacak kişinin "Deney Hayvanı Kullanım Sertifikası"na sahip olması zorunluluğu, "Hayvan Deneyleri Etik Kurullarının Çalışma Usul ve Esaslarına Dair Yönetmelik" ile 2006 yılında getirildi. Bu yönetmeliğe göre, bu kişilere 80 saatten oluşan bir eğitim verileceği bildirilmektedir. Çalışmada, deney hayvanı kullanmak üzere kurs programına katılan araştırmacıların hayvanların deneylerde kullanılmalarına ve kursun etkinliğine yönelik tutumlarının değerlendirilmesi amaçlandı. Çalışmanın materyalini, 2012-2015 yıllarında Türkiye'nin farklı illerinde ve farklı tarihlerde verilen kurs programına katılan 208 katılımcı oluşturdu. Çalışmada, kursiyerlere 2 bölümden oluşan bir anket uygulandı. Ankette 6 demografik soru ile hayvanların deneylerde kullanılmasına karşı tutumu ve kurs etkinliğini belirlemek amacıyla "Likert tipi" hazırlanan 14 soru yöneltildi. Anket uygulamasıyla elde edilen verilerin SPSS 20.0 istatistik programı ile sıklık dağılımları belirlendi. Veriler t-testi ve varyans analiz testlerine tabi tutuldu, P<0.05 olan değerler anlamlı kabul edildi. Katılımcıların %74'ü (n=154), araştırmalarda hayvan kullanımında denetlenebilirlik açısından "Hayvan Üretim ve/veya Barındırma Üniteleri"nin gerekliliği yönünde görüş bildirdi. İnsan çıkarının önceliği ile insan çıkarı için hayvan kullanımı konusunda cinsiyet açısından anlamlı bir fark bulundu. Kurs eğitiminin gerekli olduğu (%87, n=181) ve araştırmalarda bilinçli hayvan kullanımına katkısının olacağı (%87.5, n=182) ayrıca eğitim durumu ile çalışma deneyiminin bu görüşlere etkili olduğu saptandı. Sonuç olarak, deney hayvanı kullanımının denetlenmesini de kapsayan yasal düzenlemelerin araştırıcılar tarafından da kabul edildiği; her ne kadar insan-merkezci bir yaklaşım mevcutsa da, eğitimle bilinçlendirme ve yeterli denetleme yoluyla deney hayvanı kullanımının daha kabul edilebilir şekle kavuşturulabileceği söylenebilir.

Anahtar sözcükler: *Hayvan deneyleri, Hayvan kullanımı, Sertifika*

Attitudes Towards Using Animal of Authorized People for Use of Experimental Animals in Turkey

Abstract

In experimental researchs using animal in Turkey, the person who will do practice on animal will have to get "Certificate of Experimental Animal Use" has come into force by "Animal Experiments Regulation on Working Procedures and Principles of the Ethics Committees". According to this regulation it is reported that related person will be given a training consisting of up to 80 hours. It is aimed to evaluate that researches who attended in animal experiments research training program the attitudes towards training activities and use of animals in experiments. The material of the study is consisted of 208 participants who are from different cities and attended different training program between 2012 to 2015. In the study, participants were given a questionnaire consisting of two sections. Surveys to determine the course of six demographic questions and attitudes towards the use of animals in experiments with activity "Likert-type" prepared 14 questions were asked. The data were collected through questionnaires frequency distributions using SPSS 20.0 statistical software were determined. The data were subjected to t-test and analysis of variance test, P-value <0.05 was considered as a significant. In this research, 74% of respondents (n = 154) reported, in terms of accountability in the use of animals "Animal Production and/or Hosting Unit" in favor of the requirements. A significant difference in terms of gender in the use of animals for the benefit of people with the priority of human interests were found. Courses that require training (87%, n = 181) and will contribute to the conscious animal use in research (87.5%, n = 182) were found to be effective against this view of the experience of working with educational status. Consequently, the legal regulations issued in terms of monitoring the use of animals has been accepted by the investigators; although human-centered approach is present, the use of experimental animals can be said that given a more acceptable form through adequate supervision and awareness-raising by education.

Keywords: *Animal experiments, Animal use, Certificate*

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GİRİŞ

Hastalığın canlı (insan ya da hayvan) bireyde doğal seyrini görüp, buna yönelik tedavinin geliştirilmesi açısından bilimsel deneylere gereksinim vardır [1]. Hayvan deneyleri, *"sağlık ve hastalıklarla ilgili alanlarda çalışmalar yapmak için hayvanların kullanıldığı deneyler"* olarak tanımlanmaktadır [2]. Hayvan deneylerinin de yer aldığı deneysel, karşılaştırmalı yaklaşımlar M.Ö. 4-3. yy'da başlamış ve canlı üzerinde *(in-vivo)* yapılan deneysel çalışmalar daha da benimsenerek zaman içerisinde yaygınlaşmıştır. Hayvan deneylerinin yaygınlaşmasından itibaren günümüze kadar gelen süreçte bilim adamları bu denemelerin gerekliliğini savunurken, İngiltere'de 19. yy'ın sonlarında gelişmeye başlayan anti-viviseksiyonist hareket ile deneylerde hayvan kullanımının gereksizliği savunulmuş ve bu süreçte dünyanın ilk hayvanları koruma kanunu yürürlüğe girmiştir [3,4]. Bilim dünyasında bu tartışmalar sürerken bazı bilim adamlarınca geliştirilen alternatif yöntemlerin, hayvan deneylerinin yerini tutmaları amaçlanmasına rağmen [3], günümüzde yapılan tahminlere göre dünya'da her yıl hayvan deneyleri için yaklaşık 100 milyondan fazla hayvan kullanılmaktadır. Avrupa Birliği (AB) 2005 yılı verilerine göre deney hayvanlarının %60'ı biyomedikal araştırma-geliştirme çalışmalarında, %15'i ürünlerin üretim ve kalite kontrolünde, %8'i toksikoloji ve güvenlik testlerinde kullanılmaktadır [5].

İnsan ve hayvanlardaki hastalıkların kontrolü ve bunlarla mücadele edebilmek için deney hayvanı kullanımının gerektiğini bildiren Dünya Veteriner Hekimler Birliği (WVA), deneysel amaçlı kullanılacak hayvan kaynaklarının iyi bir şekilde düzenlenmesi, deneylerde hayvan kullanımının minimum seviyede tutulması, deneyin bilimsel nitelikte ve uygun şekilde planlanması, gereksiz tekrarlardan kaçınılması ve veteriner hekim kontrolü ve sorumluluğunda yapılması gerektiğine vurgu yapmıştır [6].

Russell ve Burch [7], "*3R prensibi*" (replacement, reduction, refinement) ile hayvan kullanımını ve hayvanlara acı çektirilmesini en aza indirmek için neler yapılması gerektiğini ortaya koymuş, bu prensip, dünyada olduğu gibi Türkiye'de de hayvan gönenci ile ilgili yasal düzenlemelerin temelini oluşturmuştur. Türkiye'de 3A (Alternatif arama, azaltma ve arındırma) olarak telaffuz edilen bu prensip [8], deney hayvanları kullanımında mümkünse alternatif metotların kullanılmasını; kullanılacak hayvanların sayısını minimize etmeyi ve maruz kalacakları/kalabilecekleri olumsuz koşullardan arındırmayı amaçlamaktadır [9].

Türkiye'de hayvan deneylerini içeren bilimsel araştırmaları denetlemek üzere oluşturulan araştırma etik kurulu ilk olarak 1986 yılında Hacettepe Üniversitesi Tıp Fakültesi bünyesinde oluşturulmuş; aynı yıl Gülhane Askeri Tıp Akademisi (GATA)'nde oluşturulan "*Bilim Kurulu*" da benzer görevi yürütmüş; daha sonra 1996 yılında Marmara Üniversitesi Tıp Fakültesi Deney Hayvanı Etik Kurulu, 1998 yılında Ankara Üniversitesi Veteriner Fakültesi Etik Kurulu, 1999 yılında GATA Hayvan Deneyleri Etik Kurulu kurulmuştur [10].

AB'nin Deneysel ve Diğer Bilimsel Amaçlar İçin Kullanılan Hayvanların Korunması amacıyla yayımladığı 86/609/EEC Sayılı Konsey Direktifine göre Tarım ve Köy İşleri Bakanlığı (Gıda Tarım ve Hayvancılık Bakanlığı-GTHB) tarafından çıkartılan *"Deneysel ve Diğer Bilimsel Amaçlar İçin Kullanılan Deney Hayvanlarının Korunması, Deney Hayvanlarının Üretim Yerleri ile Deney Yapacak Olan Laboratuarların Kuruluş, Çalışma, Denetleme, Usul ve Esaslarına Dair Yönetmelik*[1]*"*, Türkiye'de konu ile ilgili ilk yasal düzenlemedir [9].

Aynı yıl kabul edilip yürürlüğe giren "*Hayvanları Koruma Kanunu*[2]*"*nun 9 ve 17. maddelerine dayanılarak "*Hayvan Deneyleri Etik Kurullarının Çalışma Usul ve Esaslarına Dair Yönetmelik*[3]*"* çıkartılmış, bu Yönetmelik doğrultusunda oluşturulan *"Hayvan Deneyleri Merkezi Etik Kurulu"* ile *"Hayvan Deneyleri Yerel Etik Kurulları"*nın araştırmacılara deneylerinde kullanacakları hayvanlar için 3R prensibi doğrultusunda izin vereceği, bunun da hayvan gönenci açısından gerekli olduğu ifade edilmektedir [9]. Aynı Yönetmelik'te, bilimsel araştırmalarda hayvan(lar) üzerinde uygulama yapacak kişinin "*Deney Hayvanı Kullanım Sertifikası*"na sahip olması zorunluluğu getirilmekte; bunun için 80 saatten oluşan bir eğitim verileceği bildirilmektedir. Yönetmelikte "*deney hayvanı kullanıcısı*", "*Deney hayvanı kullanarak her türlü eğitim, araştırma, uygulama ve test yapmak isteyen veya bu programların yapılmasında deney hayvanlarına dokunarak ve gözlemleyerek katkıda bulunan öğrenciler, araştırmacılar, akademik, sağlık, teknik ve idari personel*" olarak tanımlanmaktadır [11,12]. Etik kurulların bilimin *"bürokratikleşmesi"*ne ve bilimsel çalışmaların yavaşlamasına neden olduğunu iddia edenler varsa da, birçok bilim insanı etik kurullar sayesinde gerçekleştirilen denetimlerin sadece laboratuar hayvanlarının koşullarını iyileştirmekle kalmadığını, bizzat araştırmaların kalitesinde de artışa neden olduğunu ifade etmektedirler [13]. Türkiye'de 2012 verilerine göre, bir hayvan deneyleri merkezi etik kurulu, çeşitli enstitü ve üniversitelerde toplam 94 adet hayvan deneyleri yerel etik kurulu mevcuttur [14].

Türkiye'de bilimsel araştırmalarda hayvan kullanımına karşı kısıtlı tutum analizi çalışması öğrenciler ve/veya öğretim elemanları [15-18] üzerinde gerçekleştirilmiştir.

Bu çalışmada, Sertifika Kurs Programına katılan araştırıcıların hayvanların deneylerde kullanımına ve kurs etkinliğine yönelik tutumlarının belirlenmesi amaçlandı.

MATERYAL VE METOT

"*Hayvan Deneyleri Etik Kurullarının Çalışma Usul ve Esaslarına Dair Yönetmelik*" kapsamında deney hayvanı kullanıcısı olarak ifade edilen kişilerden "*Deney Hayvanı Kullanım Sertifikası*" kursuna katılan araştırmacılar çalışma

[1] 16.05.2004 tarih ve 25464 sayılı Resmi Gazete (RG)
[2] 01.07.2004 tarih ve 25509 sayılı RG
[3] 06.07.2006 tarih ve 26220 sayılı RG. Aynı Yönetmelik, 15.02.2014 tarih ve 28914 sayılı RG'de yeni düzenlenmiş hali ile yürürlüğe girmiştir

kapsamında değerlendirildi. 2012-2015 yıllarında Türkiye'nin farklı illerinde (Burdur, Kars, Kayseri, Malatya ve Samsun) düzenlenen "*Sertifika Kurs Programı*"na katılan 208 araştırmacı çalışmaya dahil edildi.

Kurs bitiminde kursiyerlere yüz yüze görüşme yöntemiyle iki bölümden oluşan bir anket uygulandı. Deneylerde hayvan kullanımına ilişkin mevzuat göz önüne alınarak hazırlanan anket formunda altı demografik soru ile hayvanların deneylerde kullanılmasına karşı tutumu ve kurs etkinliğini belirlemek amacıyla "*Likert tipi*" hazırlanan 14 soru yöneltildi.

Anket uygulamasıyla elde edilen verilerin SPSS 20.0 istatistik programı ile sıklık dağılımları belirlendi. Veriler t-testi ve varyans analiz (ANOVA) testlerine tabi tutuldu. Varyans analizi uygulanan soruların grup içi karşılaştırması amacıyla Duncan testi uygulandı. P<0.05 olan değerler istatistikî olarak anlamlı kabul edildi [19].

BULGULAR

Çalışmaya ilişkin bulgular, demografik veriler, deneylerde hayvan kullanımına ilişkin tutum ve kursun etkinliği alt başlıkları ile tablolar halinde verildi.

Demografik Bulgular

Çalışmanın materyalini oluşturan ve Burdur, Kayseri, Kars, Malatya ve Samsun illerinde düzenlenen "*Sertifika Kurs Programı*"na dahil olan 208 katılımcının fakültelere göre düzenlenen demografik dağılımı *Tablo 1*'de sunuldu. Buna göre katılımcıların %65.4'ünü erkek (n=136), %34.6'sını kadın (n=72) araştırmacı oluşturmaktadır. Katılımcıların %82.7'si (n=172) lisansüstü eğitim dönemindedir. Katılımcıların %63'ü (n=131) daha önce araştırmalarda hayvan kullanmadığını ifade etmektedir.

Deneylerde Hayvan Kullanımına Yönelik Tutum

Katılımcıların %74'ü (n=154), araştırmalarda hayvan kullanımında denetlenebilirlik açısından "*Hayvan Üretim ve/veya Barındırma Üniteleri*"nin gerekliliği yönünde görüş bildirdi. İnsan çıkarının önceliği ile insan yararı için hayvan kullanımı konusunda cinsiyet açısından anlamlı bir fark bulundu *(Tablo 2)*.

Kurs Etkinliği

Kurs eğitiminin gerekli olduğu (%86.1, n=181) ve araştırmalarda bilinçli hayvan kullanımına katkısının olacağı (%87.5, n=182) ve kursun yeterliliği (%83.2, n=173) konusunda etkin bir görüşün olduğu belirlendi *(Tablo 3)*. Kurs etkinliğinin düzenlendiği il ve çalışılan kurum/çalışma alanı değerlendirmesinde istatistikî açıdan anlamlı sonuç olmadığı (P>0.05); kursun gerekliliği ile çalışma süresi açısından anlamlı bir fark olup (P=0.013), çalışma deneyimi düşük olanların (1-5 yıl) kursun gerekliliğine daha pozitif yaklaştıkları (%48.5, n=94) belirlendi *(Tablo 3)*. Daha önce deney hayvanı kullanımı ile kursun araştırmalarda bilinçli hayvan kullanımına katkı düzeyi arasında istatistikî açıdan anlamlı bir fark olduğu (P=0.043), daha önce deney hayvanı kullanmayanların daha pozitif tutum sergiledikleri belirlendi (%55.8, n=116).

TARTIŞMA ve SONUÇ

Hayvanların korunması konusunda Türkiye'deki yasal düzenlemelerin, uluslararası antlaşmalar bünyesinde ve AB mevzuatına da uygun hale getirilmesinin önemli olduğu; AB mevzuatında deneylerde hayvanların kullanılmalarında temel ilkenin en az sayıda hayvan kullanımı olduğu; Türkiye'de 1998 yılında kurulmaya başlanan etik kurullar yoluyla ve bunların yaygınlaşmasıyla beraber hayvanların (deneylerde kullanılmasında) korunması konusunda ivme kazanıldığı vurgulanmaktadır [10]. Araştırmacıların kullanacakları deney hayvanlarının bakım ve barınma şartları ile deney koşullarını düzenli tutup tutmamaları, hayvanların davranışı, fizyolojisi ve gönencinin yanı sıra araştırmanın sonucunu da doğrudan etkileyeceği ifade edilmektedir [20]. Çalışmada, yapılacak araştırmaların ruhsatlı "*Hayvan Üretim ve/veya Barındırma Üniteleri*"nde yapılması konusunda katılımcıların önemli bir kısmının hemfikir olması (%74, n=154) *(Tablo 2)* etik kurulların da güvencesi ile denetlenebilirliğin kabul edildiğinin ortaya konulması yukarıda sunulan literatür bilgileri destekleyici nitelikte olduğu ve bu şekilde hayvanların deneylerde kullanılmaları sırasında bakım ve barınma şartlarının daha düzenli hale getirilmesi ile haklarının korunması açısından etik kurullarının önemli bir rol üstlendiği söylenebilir.

İzmirli ve ark.[18], Avustralya ve Türkiye'deki veteriner fakültesi öğrencileri üzerinde yaptıkları bir çalışmada hayvanların deneylerde kullanılmasına genelde nötr bir yaklaşım olduğunu, ancak Türkiye'deki öğrencilerin hayvan kullanımında daha "*kabul edilebilir*" bir tutum sergilediklerini; Yerlikaya ve ark.[15] ile Özen ve Özen [16,17] veteriner fakültesi öğrencileri ile akademisyenlerinin alternatif yöntemlerin deney hayvanı kullanımına oranla pek çok yönden avantajlı olduğuna dair görüş bildirdiklerini belirlemişlerdir. Çalışmada, bilimsel araştırmalarda hayvan kullanımının zorunluluğu konusundaki tutumun yüksek olduğu (%69.2, n=144) ve çalışılan alan/mezun olunan fakülte değişkeni bakımından istatistikî açıdan anlamlı bir fark olduğu (P=0.01); alternatif yöntemler yerine deney hayvanı kullanımı konusunda Yerlikaya ve ark.[15] ile Özen ve Özen'e [16,17] paralel olarak negatif bir tutumun olduğu (%44.7, n=93) tespit edildi. Bununla birlikte il ile eğitim durumu yönünden istatistikî açıdan önemli bir fark olması (P=0.000 ve P=0.035) *(Tablo 2)* hayvan deneylerinin zorunluluğuna ilişkin genel görüşe rağmen, araştırmacıların aldıkları eğitim ve çalıştıkları alanın etkisiyle, alternatif seçenekleri de göz önünde tutma yönünde bir eğilim gösterdikleri şeklinde değerlendirilebilir.

Hayvan deneylerini savunanlar ile buna karşı olanlar

Tablo 1. *Katılımcıların fakülteleri esas alınarak düzenlenen demografik verilerin dağılımı*
Table 1. *The distribution of demographic data that organized on the basis faculties of participants*

Demografik Kriterler	Fakülte									
	Fen Fakültesi		Tıp Fakültesi		Veteriner Fakültesi		Diğer*		Toplam	
	Sayı	%	Sayı	%	Sayı	%	Sayı	%	Sayı	%
İl										
Burdur	2	1.0	3	1.4	7	3.4	2	1.0	14	6.7
Kars	18	8.7	37	17.7	17	8.1	7	3.4	79	38.0
Kayseri	1	0.5	34	16.3	5	2.4	9	4.3	49	23.6
Malatya	2	1.0	37	17.8	0	0.0	2	1.0	41	19.7
Samsun	0	0.0	18	8.7	1	0.5	6	2.9	25	12.0
Toplam	23	11.1	129	62.0	30	14.4	26	12.5	208	100.0
Cinsiyet										
Erkek	16	7.7	89	42.8	21	10.1	10	4.8	136	65.4
Kadın	7	3.4	40	19.2	9	4.3	16	7.6	72	34.6
Toplam	23	11.1	129	62.0	30	14.4	26	12.5	208	100.0
Eğitim Durumu										
Lisans	5	2.4	27	13.0	3	1.4	1	0.5	36	17.3
Y. Lisans	11	5.3	35	16.8	8	3.8	8	3.8	62	29.8
Doktora	5	2.4	38	18.3	13	6.2	11	5.3	67	32.2
Doktora üstü	2	1.0	29	13.9	6	2.9	6	2.9	43	20.7
Toplam	23	11.1	129	62.0	30	14.4	26	12.5	208	100.0
Ücretli Bir İşte Çalışma										
Evet	10	4.8	95	45.7	21	10.1	22	10.6	148	71.2
Hayır	13	6.2	34	16.3	9	4.3	4	1.9	60	28.8
Toplam	23	11.1	129	62.0	30	144	26	12.5	208	100.0
Çalışma Süresi										
1-5 yıl	18	9.3	64	33.0	14	7.2	9	4.6	105	54.1
6-10 yıl	1	0.5	22	11.3	3	1.5	3	1.5	29	14.9
11-15 yıl	1	0.5	27	13.9	2	1.0	6	3.1	36	18.6
16-20 yıl	0	0.0	7	3.6	5	2.6	2	1.0	14	7.2
20 yıldan >	1	0.5	5	2.6	2	1.0	2	1.0	10	5.2
Toplam	21	10.8	125	64.4	26	13.4	22	11.4	194	100.0
Hayvan Kullanma										
Evet	12	5.8	32	15.4	20	9.6	13	6.2	77	37.0
Hayır	11	5.3	97	46.6	10	4.8	13	6.2	131	63.0
Toplam	23	11.1	129	62.0	30	14.4	26	12.5	208	100.0

** Beslenme ve Diyetetik, Diş hekimliği, Eczacılık, Fizyoterapi, Hemşirelik, Mühendislik (Gıda ve Ziraat-Zootekni), Öğrenci, Öğretmen (Beden Eğitimi, Dershane)*

arasında orta bir yol olarak görülen hayvan gönenci bilimi, deneyler de dahil "*hayvanların bakımı, beslenmesi, barındırılması, yetiştirilmesi, nakliyesi, kesimi, tedavisi ya da bilimsel araştırmalarda kullanımı sırasında ağrı, acı ve ızdıraptan uzak, sağlık, mutluluk ve iyilik hallerinin sağlanması*" olarak tanımlanmaktadır [21,22]. Çalışmada hayvan deneylerinde insan çıkarının önceliği konusunda cinsiyet (P=0.002) yönünden anlamlı bir fark olduğu; ancak genel tutuma bakıldığında insanlara yararlı olacak bir amaç için hayvanların ağrı ve acı içeren deneylerde kullanılabileceği konusunda katılımcıların %36.6'sının (n=76) olumlu, %39.9'unun (n=83) ise olumsuz tutum sergilediği tespit edildi. Bu durumun denetlenebilirlik yönündeki tutum nedeniyle, katılımcıların hayvan gönencini göz ardı etmediği; insan çıkarının önceliği savunulsa da hayvanların, insanların yararı için ağrı ve acı veren deneylerde

Tablo 2. *Katılımcıların deneylerde hayvan kullanımına ilişkin görüşleri*
Table 2. *Participants' opinions on animal experiment*

Soru	Değişken		Kesinlikle Katılmıyorum		Katılmıyorum		Kararsızım		Katılıyorum		Kesinlikle Katılıyorum		İstatistik Önem Kontrolü	
			Sayı	%	Sayı	%	Sayı	%	Sayı	%	Sayı	%	X	
Hayvanların bilimsel araştırmalarda kullanılmaları zorunludur	Fakülte	Fen[a]	2	1.0	2	1.0	2	1.0	7	3.4	10	4.8	3.91	F= 3.141 p=0.026
		Tıp[a]	7	3.4	14	6.7	12	5.8	45	21.6	51	24.5	3.92	
		Veteriner[a]	5	2.4	4	1.9	5	2.4	9	4.3	7	3.4	3.30	
		Diğer*[a]	2	1.0	7	3.4	2	1.0	10	4.8	5	2.4	3.34	
		Toplam	16	7.7	27	13.0	21	10.1	71	34.1	73	35.1	3.75	
Hayvan deneylerinde insan çıkarları hayvan çıkarlarından önce gelmelidir	Cinsiyet	Erkek	20	9.6	17	8.2	29	13.9	41	19.7	29	13.9	3.30	t=3.099 p=0.002
		Kadın	16	7.7	14	6.7	20	9.6	18	8.7	4	1.9	2.72	
		Toplam	36	17.3	31	14.9	49	23.6	59	28.4	33	15.9	3.09	
İnsanlara yararlı olacak bir amaç için hayvanlar ağrı ve acı içeren deneylerde kulllanılabilir	Cinsiyet	Erkek	29	13.9	15	7.2	32	15.4	42	20.2	18	8.7	3.03	t=3.042 p=0.003
		Kadın	20	9.6	19	9.1	17	8.2	12	5.8	4	1.9	2.45	
		Toplam	49	23.6	34	16.3	49	23.6	54	26.0	22	10.6	2.82	
	Fakülte	Fen[ab]	6	2.9	2	1.0	7	3.4	6	2.9	2	1.0	2.82	F=2.806 p=0.041
		Tıp[a]	24	11.5	19	9.1	32	15.4	39	18.8	15	7.2	3.01	
		Veteriner[b]	12	5.8	6	2.9	6	2.9	3	1.4	3	1.4	2.30	
		Diğer*[ab]	7	3.4	7	3.4	4	1.9	6	2.9	2	1.0	2.57	
		Toplam	49	23.6	34	16.3	49	23.6	54	26.0	22	10.6	2.83	
Denetlenebilirlik açısından hayvanı içeren araştırmalar ruhsatlı "Hayvan üretim ve/ veya barındıma üniteleri"nde yapılmalıdır	İl	Burdur[a]	1	.5	0	.0	1	.5	4	1.9	8	3.8	4.28	F=2.538 p=0.041
		Kars[a]	11	5.3	9	4.3	11	5.3	14	6.7	34	16.3	3.64	
		Kayseri[a]	3	1.4	1	.5	2	1.0	14	6.7	29	13.9	4.32	
		Malatya[a]	3	1.4	2	1.0	6	2.9	9	4.3	21	10.1	4.04	
		Samsun[a]	3	1.4	0	.0	1	.5	6	2.9	15	7.2	4.20	
		Toplam	21	10.1	12	5.8	21	10.1	47	22.6	107	51.4	3.99	
	Fakülte	Fen[b]	5	2.4	3	1.4	5	2.4	3	1.4	7	3.4	3.17	F=3.491 p=0.017
		Tıp[a]	9	4.3	5	2.4	16	7.7	32	15.4	67	32.2	4.10	
		Veteriner[a]	4	1.9	2	1.0	0	.0	8	3.8	16	7.7	4.00	
		Diğer*[a]	3	1.4	2	1.0	0	.0	4	1.9	17	8.2	4.15	
		Toplam	21	10.1	12	5.8	21	10.1	47	22.6	107	51.4	3.99	
Hayvan sağlığı ve gönenci yararına alternatif yöntemler yerine hayvan kullanılmalıdır	İl	Burdur[b]	2	1.0	6	2.9	4	1.9	2	1.0	0	.0	2.42	F=5.274 p<0.000
		Kars[a]	13	6.2	10	4.8	11	5.3	25	12.0	20	9.6	3.36	
		Kayseri[b]	10	4.8	18	8.7	12	5.8	7	3.4	2	1.0	2.44	
		Malatya[b]	9	4.3	12	5.8	10	4.8	5	2.4	5	2.4	2.63	
		Samsun[b]	9	4.3	4	1.9	2	1.0	9	4.3	1	.5	2.56	
		Toplam	43	20.7	50	24.0	39	18.8	48	23.1	28	13.5	2.84	
	Eğitim Durumu	Lisans[b]	12	5.8	10	4.8	8	3.8	5	2.4	1	.5	2.25	F=5.170 p=0.035
		Y. Lisans[a]	7	3.4	18	8.7	14	6.7	17	8.2	6	2.9	2.95	
		Doktora[a]	13	6.2	15	7.2	11	5.3	16	7.7	12	5.8	2.98	
		Doktora üstü[a]	11	5.3	7	3.4	6	2.9	10	4.8	9	4.3	2.97	
		Toplam	43	20.7	50	24.0	39	18.8	48	23.1	28	13.5	2.84	

X: *Ortalama puan,* ***F:*** *Varyans değeri,* ***P:*** *Anlamlılık düzeyi, * Beslenme ve Diyetetik, Diş hekimliği, Eczacılık, Fizyoterapi, Hemşirelik, Mühendislik (Gıda ve Ziraat-Zootekni), Öğrenci, Öğretmen (Beden Eğitimi, Dershane),* ***t:*** *t testi değeri,* ***a.b:*** *Aynı sütunda farklı anlam taşıyan ortalama tutum değerleri arasındaki farklılık önemlidir (Her soruya ait değişken kendi içinde değerlendirilmiştir)*

Tablo 2. *Katılımcıların deneylerde hayvan kullanımına ilişkin görüşleri (Devam)*
Table 2. *Participants' opinions on animal experiment (Continued...)*

Soru	Değişken		Kesinlikle Katılmıyorum		Katılmıyorum		Kararsızım		Katılıyorum		Kesinlikle Katılıyorum		İstatistik Önem Kontrolü	
			Sayı	%	Sayı	%	Sayı	%	Sayı	%	Sayı	%	X	
Deney hayvanı kullanımı. et tüketimi için hayvan beslemekten daha insancıldır	İl	Burdur[b]	2	1.0	7	3.4	3	1.4	2	1.0	0	.0	2.35	
		Kars[a]	8	3.8	1	.5	14	6.7	28	13.5	28	13.5	3.84	
		Kayseri[b]	5	2.4	11	5.3	16	7.7	12	5.8	5	2.4	3.02	F=9.235
		Malatya[b]	9	4.3	11	5.3	8	3.8	4	1.9	9	4.3	2.82	p<0.000
		Samsun[b]	3	1.4	7	3.4	9	4.3	5	2.4	1	.5	2.76	
		Toplam	27	13.0	37	17.8	50	24.0	51	24.5	43	20.7	3.22	
	Fakülte	Fen[a]	0	.0	4	1.9	2	1.0	8	3.8	9	4.3	3.95	
		Tıp[b]	20	9.6	24	11.5	29	13.9	33	15.9	23	11.1	3.11	F=2.899
		Veteriner[b]	5	2.4	5	2.4	10	4.8	3	1.4	7	3.4	3.06	p=0.036
		Diğer*[b]	2	1.0	37	17.8	50	24.0	51	24.5	43	20.7	3.26	
		Toplam	27	13.0	37	17.8	50	24.0	51	24.5	43	20.7	3.22	

X: *Ortalama puan,* ***F:*** *Varyans değeri,* ***P:*** *Anlamlılık düzeyi, * Beslenme ve Diyetetik, Diş hekimliği, Eczacılık, Fizyoterapi, Hemşirelik, Mühendislik (Gıda ve Ziraat-Zootekni), Öğrenci, Öğretmen (Beden Eğitimi, Dershane),* ***t:*** *t testi değeri,* ***a.b:*** *Aynı sütunda farklı anlam taşıyan ortalama tutum değerleri arasındaki farklılık önemlidir (Her soruya ait değişken kendi içinde değerlendirilmiştir)*

Tablo 3. *Katılımcıların kursun gerekliliği ve bilinçli hayvan kullanımına sağlayacağı katkı konusundaki görüşleri*
Table 3. *Participants' opinions on requirement of the course and its contribution to the conscious animal use*

Soru	Değişken		Kesinlikle katılmıyorum		Katılmıyorum		Kararsızım		Katılıyorum		Kesinlikle katılıyorum		İstatistik Önem Kontrolü	
			Sayı	%	Sayı	%	Sayı	%	Sayı	%	Sayı	%	X	
Kurs programının gerekli olduğunu düşünüyorum	Çalışma Süresi	1-5 yıl[a]	5	2.6	2	1.0	4	2.1	26	13.4	68	35.1	4.42	
		6-10 yıl[a]	3	1.5	1	.5	1	.5	8	4.1	16	8.2	4.13	
		11-15 yıl[a]	2	1.0	1	.5	1	.5	5	2.6	27	13.9	4.50	F=3.276
		16-20 yıl[a]	0	.0	1	.5	2	.0	1	.5	10	5.2	4.42	p=0.013
		20 yıl ≥[b]	3	1.5	0	.0	1	.5	4	2.1	2	1.0	3.20	
		Toplam	13	6.7	5	2.6	9	4.6	44	22.7	123	63.4	4.33	
Kurs programının araştırmalarımda bilinçli hayvan kullanma konusunda yararlı olacağını düşünüyorum	Eğitim Durumu	Lisans[b]	1	.5	1	.5	1	.5	10	4.8	23	11.1	4.47	
		Y. Lisans[a]	1	.5	1	.5	3	1.4	17	8.2	40	19.2	4.51	F=2.949
		Doktora[a]	7	3.4	2	1.0	6	2.9	15	7.2	37	17.8	4.08	p=0.044
		Doktora üstü[a]	2	1.0	0	.0	1	.5	8	3.8	32	15.4	4.58	
		Toplam	11	5.3	4	1.9	11	5.3	50	24.0	132	63.5	4.38	
	Çalışma Süresi	1-5 yıl[a]	4	2.1	2	1.0	8	4.1	29	14.9	62	32.0	4.36	
		6-10 yıl[a]	3	1.5	0	.0	1	.5	8	4.1	17	8.8	4.24	
		11-15 yıl[a]	2	1.0	1	.5	0	.0	3	1.5	30	15.5	4.61	F=2.663
		16-20 yıl[a]	0	.0	0	.0	1	.5	2	1.0	11	5.7	4.71	p=0.034
		20 yıl ≥[b]	2	1.0	1	.5	0	.0	4	2.1	3	1.5	3.50	
		Toplam	11	5.7	4	2.1	10	5.2	46	23.7	123	63.4	4.37	
	Hayvan Kullanma	Evet	7	3.4	3	1.4	2	1.0	21	10.1	44	21.2	4.06	
		Hayır	4	1.9	2	1.0	9	4.3	28	13.5	88	42.3	4.13	t=2.037
		Toplam	11	5.3	5	2.4	11	5.3	50	24.0	132	63.5	4.10	p=0.043

X: *Ortalama puan,* ***F:*** *Varyans değeri,* ***P:*** *Anlamlılık düzeyi,* ***t:*** *t testi değeri,* ***a.b:*** *Aynı sütunda farklı anlam taşıyan ortalama tutum değerleri arasındaki farklılık önemlidir (Her soruya ait değişken kendi içinde değerlendirilmiştir)*

kullanımına yönelik tutum doğrultusunda daha çok çevremerkezci bir yönelim gösterdiği ileri sürülebilir.

İzmirli ve Phillips'in [23] hayvanlara karşı tutum konusunda kadınların daha sempatik bir tutum sergiledikleri görüşü, bilimsel araştırmalarda hayvan kullanımına yönelik Türkiye'de yapılan çalışmalarda da [15-17] ortaya konulmuştur. Literatür bilgilerle uyumlu olarak cinsiyet yönünden insan-hayvan çıkarı önceliği (P=0.002) ile insan yararı için hayvanların ağrı ve acı içeren deneylerde kullanılması (P=0.003) konusunda istatistikî açıdan anlamlı bir fark tespit edilmesi *(Tablo 2)*, hayvanlara karşı kadınların daha hassas tutum sergilemeleri, içgüdüsel olarak empati düzeylerinin yüksek olmasından kaynaklanmasının kuvvetle muhtemel olduğu söylenebilir.

İstanbul'da toplam 200 tıp hekimi ve veteriner hekimin deneylerde hayvan kullanımına yönelik bakış açılarını değerlendiren bir araştırma sonucuna göre hayvan deneylerinin insancıl olmadığını tıp hekimlerinin %85'i, veteriner hekimlerin ise %82'si kabul etmektedir [13]. Yıldırım ve Kadıoğlu [24], hayvanların öldürülmesi konusunda yaptıkları çalışmada, hayvanların et tüketimi için öldürülmesinin yüksek düzeyde, deneysel amaçlı hayvan kullanımının ise düşük düzeyde kabul edilebilir olduğunu ve tıp fakültesi öğrencilerinin laboratuar hayvanlarının kullanımına, mühendislik fakültesi öğrencilerinden daha olumlu baktıklarını belirlemiştir. Çalışmada, araştırmacıların et tüketimi için hayvan beslenmesine oranla deney hayvanı kullanımının daha insancıl olduğu yönünde bir tutum sergilediği (%45.2, n=94); kararsız tutum sergileyenlerin de önemli düzeyde (%24, n=50) olduğu saptandı *(Tablo 2)*. Hayvanların öldürülmesi işleminin sağladığı yararlılık ile benimsenebilirliği arasında doğru orantı olduğu görüşü [24], çalışma bulguları ile farklılık gösterse de, bu çalışmada araştırmacıların hayvan kullanımının gerekliliğini kabul etmeleri ve hayvan gönencine minimum düzeyde de olsa özen gösterdiklerini düşünüyor olmaları deney hayvanı kullanımının daha insancıl kabul edildiği sonucunu çıkarabilir.

Çalışmada, hayvanları koruma mevzuatı doğrultusunda zorunlu hale getirilen kurs programının gerekliliği katılımcılar tarafından önemli oranda desteklenmekte (%86.1, n=167), ancak bu tutumun çalışma deneyimi ile ters orantılı olduğu görülmektedir (P=0.013, *Tablo 3)*. Bu durumun, *Tablo 3*'te yer alan ve kurs programının araştırmalarda bilinçli hayvan kullanımına yönelik katkısı ile daha önce hayvan kullanma deneyimi ilişkisi verileri (P=0.043) doğrultusunda, rutin yapılan işlemlerin gereksiz/önemsiz görülmesinden kaynaklandığı ve kurs programının araştırmalarda bilinçli hayvan kullanımı konusunda genç araştırmacılar üzerine daha etkin bir rol oynadığı ileri sürülebilir.

Sonuç olarak, deney hayvanı kullanımının denetlenmesi açısından çıkarılan yasal düzenlemelerin araştırıcılar tarafından da kabul gördüğü; her ne kadar insan çıkarının önceliği kabul edilse de, hayvan deneylerinde ilgili tüm tarafların etik kuralların önemi ve gerekliliği konusunda eğitilmeleri, farkındalık ve duyarlılık kazanmalarıyla deney hayvanı kullanımının ideal kabul edilen 3R prensibine uygun hale kavuşturulabileceği söylenebilir.

Teşekkür

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KAYNAKLAR

1. Dunlop RH, Williams DJ: Veterinary Medicine: An Illustrated History. Mosby-Year Book, Inc. Missouri, USA, 1996.

2. Büken NÖ: Hayvan deneylerinde araştırmacı sorumluluğu ve deney hayvanlarının hakları. *Hacettepe Tıp Dergisi* 37, 1-5, 2006.

3. Çalgüner E, Diker Ş, Anadol E, Ergüven Kaya E: Dünden Bugüne Hayvan Deneylerinin Bilimdeki Yeri. *II. Ulusal Veteriner Hekimliği Tarihi ve Mesleki Etik Sempozyumu*, 24-26 Nisan, Konya, Bildiriler Kitabı, 359-361, 2008.

4. Bayne K, Howard BR, Kurosawa TM, Najera MEA: An Overview of Global Legislation, Regulatin and Policies. **In,** Hau J, Schapiro SJ (Eds): Handbook of Laboratory Animal Science. 3rd ed., pp.41, CRC Press, 2011.

5. Olsson AS, Robinson P, Sandoe P: Ethics of Animal Research. **In,** Hau J, Schapiro SJ (Eds): Handbook of Laboratory Animal Science. 3rd ed., pp.21, CRC Press, 2011.

6. WVA-World Veterinary Association: Policy statement on animal welfare, well-being and ethology. "Principles and Guidelines" *ILAR J*, 31, 29-30, 1989. DOI: 10.1093/ilar.31.4.29

7. Russell WMS, Burch RL: The Principles of Humane Experimental Technique, 1959, http://altweb.jhsph.edu/pubs/books/humane_exp/chap4d, *Accessed:* 18.03.2015.

8. Özen A: Hayvanlarla Olan İlişkimizi Düzenlemede Anahtar Tutum: "Hiç değilse…" *Türkiye'de Birinci Havan Refahı ve Veteriner Hekimliği Eğitim Konferansı,* Ankara, 75-82, 2005.

9. Yaşar A, İzmirli S: Türkiyede hayvan gönenci ile ilgili yasal düzenlemeler. *Vet Bil Derg,* 22 (3-4): 51-56, 2006.

10. Yaşar A, Yerlikaya H: Dünya'da ve Türkiye'de hayvan haklarının tarihsel gelişimi. *Vet Bil Derg,* 20 (4): 39-46, 2004.

11. İzmirli S, Aldavood SH, Yasar A, Phillips CJC: Introducing ethical evaluation of the use of animals in experiments in the Near East. *Altern Lab Anim,* 38, 331-336, 2010.

12. Güneli E: Uluslararası Laboratuvar Hayvanları Biliminde Kurslar. Laboratuvar Hayvanları Bilimi 3. Kongresi, 26-28 Eylül 2013, Erciyes Üniversitesi, Kayseri.

13. Coşkun AS, Elibol P, Coşkun M, Çalışkan E, Tükenmez MA, Yeğen BÇ: Hekimlerin ve veteriner hekimlerin araştırmalarda deney hayvanı kullanımına bakış açıları. *Cerrahpaşa Tıp Derg,* 38, 146-151, 2007.

14. Sinmez ÇÇ, Yasar A: An Evaluation on Turkey's Animal Experiments Center and Local Ethics Committees. *Medicres World Congress 2012 on Good Medical Research,* 1-4 June, Wien, Austria, p.36, 2012.

15. Yerlikaya H, Ozen A, Yasar A, Armutak A, Ozturk R, Bayrak S, Gezman A, Seker I: A survey of attitudes of Turkish veterinary students and educators about animal use in research. *Veterinární Medicína*, 49 (11): 413-420, 2004.

16. Özen R, Özen A: Erciyes Üniversitesi Veteriner Fakültesi öğretim elemanlarının bilimsel araştırmalarda hayvan kullanımı konusundaki yaklaşımları. *II. Ulusal Veteriner Hekimliği Tarihi ve Mesleki Etik Sempozyumu*, 24-26 Nisan, Konya, Bildiriler Kitabı, s.383-387, 2008.

17. Özen R, Özen A: Attitudes of Erciyes University students to the use of animals in research. *Kafkas Univ Vet Fak Derg*, 16, 477-481, 2010. DOI: 10.9775/kvfd.2009.1053

18. İzmirli S, Yiğit A, Phillips CJC: Attitudes of Australian and Turkish

students of veterinary medicine toward nonhuman animals and their careers. *Society Anim*, 22, 580-601, 2014. DOI: 10.1163/15685306-12341352

19. Büyüköztürk Ş: Sosyal Bilimlerde Veri Analizi El Kitabı. 167-82, Pegem Yayınevi, Ankara, 2011.

20. Koolhaas JM, Baumans V, Blom HJM, Von Holst D, Timmermans PJA, Wiepkema PR: Behavior, stress and well-being. **In,** Van Zutphen LFM, Bowmans V, Beynen A (Eds): Principles of Laboratory Animal Science. 75-99, Elsevier, Amsterdam, 1993.

21. Yaşar A: Selçuk Üniversitesi Veteriner Fakültesi'nde hayvan gönenci eğitimi. *Türkiye'de Birinci Hayvan Refahı ve Veteriner Hekimliği Eğitimi Konferansı*. 09-10 Haziran, Sunuş Özetleri s.5, Ankara, 2005.

22. Yaşar A: Tarihsel bir bakış açısıyla hayvan gönenci: veteriner hekimliği etiği, hayvan kullanım etiği, hayvan gönenci ve veteriner hekim ilişkisi. *Türkiye'de Birinci Hayvan Refahı ve Veteriner Hekimliği Eğitimi Konferansı*. 09-10 Haziran, Sunuş Özetleri, s.6, Ankara, 2005.

23. İzmirli S, Phillips CJC: Attitudes to animal welfare and rights throughout the world in the modern era: A review. *Eurasian J Vet Sci*, 28 (2): 65-68, 2012.

24. Yıldırım G, Kadıoğlu S: Medical school and engineering school students' view on animal killing: Cumhuriyet University sample. *Eurasian J Vet Sci*, 30, 174-180, 2014. DOI: 10.15312/EurasianJVetSci.201447373

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Research Article

Distributions of *CYP19, ERα* and *PGR* Allele Frequencies between Fertile and Subfertile Holstein-Friesian Heifers [1]

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Abstract

The aim of this study was to investigate the gene and genotype distributions of some mutations in the aromatase cytochrome P450 *(CYP19)*, estrogen receptor α *(ERα)*, and progesterone receptor *(PGR)* genes in fertile and subfertile Holstein-Friesian heifers using the PCR-RFLP method and comparing the distributions between groups. A total of 106 heifers were included the study, and the heifers that became pregnant after the first artificial insemination (n=51) were used as a fertile group. Heifers (n=55) with equal and more than 3 AIs were accepted as a subfertile heifers. Blood samples from all of the heifers were obtained for DNA isolation. While two alleles and three genotypes were found at the *PGR* and *ERα* loci, two alleles and two genotypes were detected at the *CYP19* locus. The A allele and AA genotype, G allele and GG genotype, and C allele and CT genotype were found to be predominant in *CYP19, ERα* and *PGR*, respectively. According to the chi-square test ($\chi 2$), two of the groups investigated were in Hardy-Weinberg equilibrium for all gene loci. There were no differences detected in allele or genotype frequencies between the fertile and subfertile heifers.

Keywords: *Holstein-Friesian, Heifer, Fertility, Subfertilty, Polymorphism, CYP19, PGR, ERα*

Fertil ve Subfertil Siyah Alaca Düveler Arasında *CYP19, ERα, PGR* Allel Frekanslarının Dağılımı

Özet

Bu çalışmanın amacı fertil ve subfertil Siyah Alaca düvelerde aromataz sitokrom P450 *(CYP19)*, östrojen reseptör α *(ERα)*, progesteron reseptör *(PGR)* genlerindeki bazı mutasyonların gen ve genotip frekanslarınınn PCR-RFLP yöntemi kullanılarak incelenmesi ve bu dağılımların fertil ve subfertil Siyah-Alaca düveler arasında karşılaştırılmasıdır. Çalışmaya toplam 106 düve dâhil edilmiş ve ilk tohumlamadan sonra gebe kalan düveler fertil düve grubu (n=51) olarak kullanılmıştır. Üç ve daha fazla tohumlama sayısına sahip düveler (n=55) subfertil olarak kabul edilmiştir. DNA izolasyonu için tüm düvelerden kan alınmıştır. *PGR* ve *ERα* genlerine ait lokuslarda iki allel ve üç genotip bulunurken, *CYP19* geninde incelenen lokusta iki allel ve iki genotip belirlenmiştir. *CYP19, ERα, PGR* lokuslarında sırasıyla A alleli ve AA genotipi, G alleli ve GG, C alleli ve CT genotipi predominat olarak bulunmuştur. Ki- Kare ($\chi 2$) test sonuçlarına göre incelenen her iki grupta tüm lokuslar bakımından Hardy- Weinberg dengesinde idi. Allel ve genotip frekansları bakımından fertil ve subfertil düveler arasında fark bulunamamıştır.

Anahtar sözcükler: *Siyah Alaca, Düve, Fertilite, Subfertilite, Polimorfizm, CYP19, PGR, ERα*

INTRODUCTION

In the dairy industry, most economically important traits depend on reproduction, which determines the economic value of dairy herds [1-3]. Reproduction is influenced by many environmental and genetic components, as are most complex traits. In contrast, long generation intervals and the low heritability of reproductive traits can cause limited success in the selection of these traits [1]. Intensive selection has been applied for 50 years in the dairy industry, resulting in increased milk production while leading to an important decrease in reproduction [3,4]. High producing dairy cows generally require more service per conception, with prolonged calving intervals and

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higher culling rates [2,3,5,6]. However, the fertility of heifers is greater than that of dairy cows due to the former's lack of milk production. The rate of pregnancy per artificial insemination (P/AI) is approximately 60-75% in heifers inseminated following estrus detection. Thus, heifers are expected to become pregnant after a maximum two AIs, and heifers with ≥3 AIs can be considered subfertile [7-11]. Subfertile heifers, like repeat breeder cows, cause economic losses in dairy herds because of increased insemination costs, later first calving ages and higher culling rates [5]. Subfertile heifers with increased age of first calving are not suitable for herds [10] because they are culled quickly due to low milk production and some health problems (dystocia, metritis, replacement of abomasum etc.). Subfertility in heifers is a multifactorial condition [7], and there is no accurate method for diagnosing the cause in most individuals. Although there are many causative factors, hormonal imbalance, genetic factors and the uterine environment are important as etiological factors in subfertile heifers [7,8]. Genetic markers might be helpful for selecting more appropriate individuals to keep in breeding herds [12,13].

In addition, long generation intervals and the low heritability of reproductive traits have caused limited success of selection for reproductive traits, such as fertility. Molecular genetics tools, which allow for the detection of genes, have major effects on complex traits, such as reproductive performance, and they can be used as selection criteria for these types of traits for genetic improvement [14]. There have been limited efforts to determine the major genes influencing reproductive traits.

Researchers have focused on the gene-regulated hormones that play critical roles in reproduction, such as aromatase cytochrome P450 enzyme *(CYP19)*, estrogen receptor α *(ERα)*, and progesterone receptor *(PGR),* which have been investigated [15-17] to evaluate their polymorphisms as selection criteria for these types of traits for genetic improvement. However, there have been limited efforts to determine the major genes influencing reproductive traits [14]. It is also important to determine the genes and genotype frequencies of candidate gene for quantitative traits. Various studies have been performed to determinate the gene and genotype frequencies affecting quantitative traits in cattle breeds reared in Turkey [12,13].

The aromatase cytochrome P450 enzyme catalyzes the conversion of androgens into estrogens and the biosynthesis of estrogen by aromatization [16]. This function of the hormone is important for controlling female reproduction. The aromatase cytochrome P450 enzyme is coded by the *CYP19* gene, which located on chromosome 10 in the bovine genome and has tissue-specific expression [18,19]. Placental expression of the *CYP19* gene is regulated by P1.1 promoter, and an A→G mutation at this region has been detected [19]. Subsequently, due to the critical role of this gene region where the mutation occurs, various studies have been undertaken to investigate the associations between alleles at this locus and different economically important traits, including reproductive traits [20-23].

The other important candidate gene is estrogen receptor α *(ERα)*. As with other nuclear receptors, estrogen receptors are transcription factors, and they regulate gene transcription [17]. Estrogens have also many functions in the critical process of the life cycle [24]. Due to their important roles, estrogen receptors and their genes are believed to be candidate markers for reproductive traits. There are two isoforms of estrogen receptor, called ERα and ERβ, which are coded by different genes located on chromosomes 9 and 10 of the bovine genome, respectively. Investigations of the functions of these two receptors have revealed that, while failure of ERα leads to infertility in both male and female mice, failure of ERβ has little effect on fertility [25]. All nuclear receptor genes, including *ER,* have a special structure at their 5'UTR region [26]. In addition to eight exons of *ERα,* there are additional exons encoding tissue-specific transcripts [26]. An A→G transition found at this region [25] has attracted attention for its possible effects on reproduction-related traits [26,27].

Progesterone plays a key role in the establishment and maintenance of pregnancy [15,28]. Because progesterone activity is regulated by progesterone receptor, the progesterone receptor gene *(PGR)* has been considered a good candidate for reproduction [27]. In the *PGR* gene, a G→C transversion and a T→C transition on introns 3 and 4 have been reported, respectively, and some associations have been indicated between these polymorphisms and reproductive parameters [27,29]. These polymorphisms were only recently reported, and there have been only a small number of studies performed on them to date.

Thus, the aim of this study was to evaluate and compare the frequency distributions of some mutations of the *CYP19*, *ERα*, and *PGR* genes with regard to fertility between fertile and subfertile Holstein-Friesian heifers.

MATERIAL and METHODS

Animals, Housing, and Sampling

This study was approved by the Ethics Committee of Uludag University (UÜHADYEK, Approval date: 04.06.2013; No: 2013-11/1). The study was performed at six different lactating dairy farms with an average of 400-750 milking cows, located in the Marmara region of the Turkey, and it was undertaken between January and December 2014. The first insemination age of the heifers was an average of 15 months old. Heifers were inseminated following estrus detection after spontaneous or $PGF_2\alpha$ -induced (one or two doses of $PGF_2\alpha$ apart from 14 days) estrus. Artificial

inseminations (AIs) were performed by farm veterinarians. The heifers were housed in a free-style barn, and they were fed total mixed rations, based on Natural Research Council [30] recommendations, and had unlimited access to water. Heifers (n=106) were selected and included in the study according to their AI numbers, and they were assigned to one of the two groups. Heifers (n=51) that became pregnant after the first AI were used as a fertile heifers group. First and second pregnancy checks were performed at 30 and 60 days after AI in the fertile heifers. If embryonic loss was detected at the 60 d pregnancy check, the heifers were excluded from the study. Subfertile heifers with ≥ 3 AIs (n=55) formed the study group. Blood samples were obtained from the coccygeal vein for DNA isolation.

DNA Isolation and PCR Amplifications

Total DNA was extracted using a genomic DNA purification kit (K0512, Fermentas, Lithuania) according to the instruction manual. Spectrophotometric methods were used to determine DNA quality and quantity. The primers and restriction enzymes used for PCR amplifications are given in *Table 1*.

PCR amplifications were performed in reaction mixtures of 25 µL containing 12.5 µL of 2× PCR Master Mix (K0172, Fermentas), 0.5 µM of each primer, and 25-75 ng of genomic DNA. Amplification was performed using a Techgene Thermal Cycler (Techne, Cambridge, UK). Restriction enzyme cuttings were performed according to the manufacturer's protocols. The restriction fragments were directly analyzed by electrophoresis in 2% and 2.5% agarose gels in 1× TBE buffer, stained with SafeView™ Classic (Applied Biological Materials Inc.) and visualized under UV light. Direct counting was performed to estimate the phenotype and allele frequencies of the genetic variants for all of the loci.

Statistical Analyses

Statistical analyses were conducted by using SAS [32]. Data were evaluated using PROC GLM, PROC COR and PROC REG in SAS. The effects of heifer ages, numbers of AIs, farm factor, *CYP19*, *ERα*, and *PGR* genes were included to the statistical models. The differences in numbers of AIs and ages between the fertile and subfertile heifers were determined using the PROC GLM. The PROC REG and PROC COR procedure were performed to determine the effect of heifer ages, farm factor, *CYP19*, *ERα*, and *PGR* genes on numbers of AIs.

The chi-square test (χ^2) was used to determine whether the populations were in Hardy-Weinberg equilibrium. All of the calculations and the χ^2 analyses were performed using PopGene32 software [33]. Differences in frequency distribution between the fertile and subfertile groups were tested by Fisher's exact test using Minitab software, version 15.0, and SPSS software, version 17.0. To categorize comparisons after the chi-square test (χ^2), the two-proportion z-test was used.

RESULTS

The numbers of AIs and the ages of the heifers were greater (P<0.01) in the subfertile heifers (4.6±0.16 and 19.9±0.42) than in the normal heifers (1±0.19 and 15.5±0.43, respectively). Although high correlation (r = 63.0%; P<0.001) was detected between age and AI numbers, no correlation was found between other factors. According to regression analyses, only age was effect on AI numbers, but farm factor, *CYP19*, *ERα*, and *PGR* genes did not effect on AI numbers (R^2 = 43.2%: AI = - 0.20 + 0.271 Age - 0.0948 Farm + 0.071 *CYP19* + 0.237 *PGR* -*0.702 ERα*).

When gene polymorphisms were evaluated, while two alleles and three genotypes were found at the investigated loci in the *PGR* and *ERα* genes, two alleles and two genotypes were detected at the investigated locus in the CYP19 gene. The GG genotype revealed two bands of 182 bp and 70 bp, while the AA genotype remained uncut for the investigated locus in *CYP19*. At the investigated locus in the *PGR* gene, TT genotype individuals had 515 bp, and CC genotype individuals had 398 bp and 117 bp. A homozygous individuals had only 242 bp uncut fragment, and G homozygous individuals had 182 bp and 60 bp fragments for the investigated locus in the *ERα* gene. Electrophorograms are presented in *Fig. 1*.

The A allele and AA genotype, the G allele and GG genotype, and the C allele and CT genotype were found to be predominant at the investigated loci in the *CYP19, ERα* and *PGR* genes, respectively. The frequencies of these predominant alleles were calculated for the whole population as 0.9623, 0.9198, and 0.6651 for investigated loci in the *CYP19, ERα* and *PGR* genes, respectively. The

Table 1. *Primers sequences and restriction enzymes used in the study*
Tablo 1. *Çalışmada kullanılan primer dizileri ve restriksiyon enzimleri*

Loci	Primers (5′ → 3′)	Enzym	References
PGR	CCCATCCCTTAGCATCTTCC TTACCAACGCTGACCCGAAG	*Eco*321	Yang et al.[28]
ERα	TTTGGTTAACGAGGTGGAG TGTGACACAGGTGGTTTTTC	*Bgl*I	Szreder and Zwierzchowski [29]
*CYP*19	CTCTCGATGAGACAGGCTCC ACAATGCTGGGTTCTGGACT	*Pvu*II	Vanselow et al.[31]

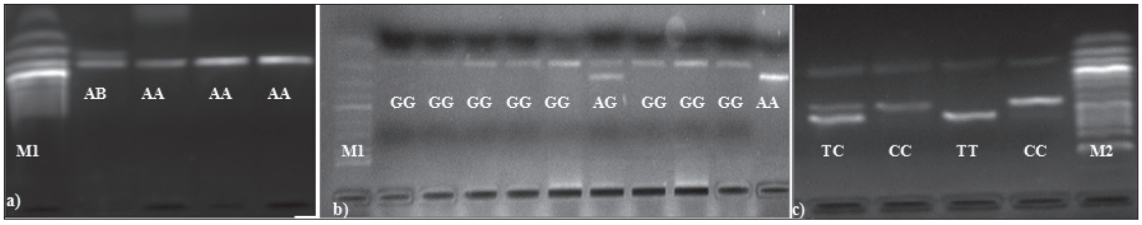

Fig 1. Illustration of PCR-RFLP fragments of loci investigated on agorose gels (a, b, c). **a-** Electroforetic results of CYP19 locus, **b-** Electroforetic results of *ERa*, **c-** Electroforetic results of *PGR;* M1: Bio Basic Inc., MSM 34, 100 bp DNA ladder, M2: BioLab Inc., N3236S, 50 bp DNA ladder

Şekil 1. Lokuslara ait PCR- RFLP parçalarının agoroz jellerdeki görünümü (a, b, c). **a-** CYP19 lokusuna ait elektroforetik sonuçlar, **b-** *ERa* lokusuna ait elektroforetik sonuçlar, **c-** *PGR* lokusuna ait elektroforetik sonuçlar; M1: Bio Basic Inc., MSM 34, 100 bp DNA markır, M2: BioLab Inc., N3236S, 50 bp DNA markır

Table 2. *Distributions of allelle and genotypes of CYP19, ER α and PGR genes in fertile and subfertil groups*
Tablo 2. *Fertil ve subfertil gruplarda CYP19, ERα ve PGR allel ve genotip frekanslarının dağılımları*

Lokus	Groups	n	Allel Frequencies		Genotype Frequencies*			χ^2(HWE)
CYP19	Subfertile	55	A	B	AA	AB	BB	
			0.955	0.045	0.909 (50)	0.091 (5)	-	0.098901^{ns}
	Fertile	51	0.971	0.029	0.940 (48)	0.06 (3)	- -	0.030921^{ns}
ER α	Subfertile	55	A	G	AA	AG	GG	
			0.064	0.936	- -	0.127 (7)	0.873 (48)	0.215877^{ns}
	Fertile	51	0.098	0.902	0.019 (1)	0.157 (8)	0.824 (42)	0.832404^{ns}
PGR	Subfertile	55	C	T	CC	CT	TT	
			0.636	0.364	0.400 (22)	0.473 (26)	0.127 (7)	0.008285^{ns}
	Fertile	51	0.696	0.304	0.431 (22)	0.529 (27)	0.04 (2)	2.992969^{ns}

ns: *non significant; * The numbers of animals carrying of each genotype shown in paranthesis*

allele and genotype frequency distributions between the two groups of heifers are given in *Table 2*.

According to the chi-square test (χ^2), the two groups investigated were in Hardy-Weinberg equilibrium. In addition, no differences were found in allele or genotype frequencies between fertile and subfertile heifers.

DISCUSSION

In cases of the detection of associations between markers and traits of interest, the marker can be used as a selection criterion for decreasing fertility problems. In this study, we investigated and compared polymorphisms in the genes regulating the main reproductive hormones between fertile and subfertile heifers.

Some researchers have been focused on determining the gene and genotype frequencies of candidate gene for subfertile animals [14,15]. However, there have been only limited efforts to determine the major genes influencing reproductive traits [12]. While in previous studies, polymorphisms in the *CYP19* [16,18-21,33], *ERα* [20,21,25,29,34], and *PGR* [15,28,35] genes were evaluated in cows, the present study is the first on these gene polymorphisms in subfertile heifers.

When the results of the present study were compared with those of others, the allele and genotype frequencies were similar *(Table 2)* to the frequencies observed in the previous studies performed in European cattle breeds [15,21,22,28,35,36]. There has been only one study of *ERα* [20] in which the A allele was predominant, in contrast with all the other studies. This contradiction might derive from the differences in nomenclature used in this study. There have been more studies performed on the relationships between *CYP19* and *ERα* polymorphisms [20-23,28] and some reproductive and productive traits, when considering them with *PGR* [28]. However, the results obtained from these studies were not in concordance with each other. While in some previous studies significant relationships have been found between polymorphisms in the *CYP19* and *ERα* genes and calving to conception intervals [20], calving difficulties [22,35] and milk

production, some of them did not find any associations with economically important traits, such as fertility [13] and milk production [36-38]. The gene and genotype frequency distributions for all of the loci did not differ between the fertile and subfertile groups. Although associations have been reported of the *CYP19, ERα* and *PGR* gene polymorphisms with some important traits in other studies [15,20,22,28,35,37], our findings did not support relationships between these mutations and pregnancy per AI. It was emphasized by previous studies that the allele frequency distributions in *CYP19* and *ERα* were not favorable for association analysis, as we found. To ensure the veracity of any associations, larger populations must be studied [14,38].

There have been few studies of the *PGR* gene supporting strong associations between the transferable number of ovaries and *PGR* polymorphisms. These studies have provided markers that can be used in embryo transfer [28,34], despite finding no differences in *PGR* genotypes between the groups' polymorphisms at this gene worth investigating further, because of both the limited numbers of studies of this gene and traits investigated. Association studies should be performed using a larger number of animals and more reproductive parameters.

In this study, we aimed to investigate the differences between fertile and subfertile heifers for polymorphisms in the *CYP19*, *ERα* and *PGR* genes. Despite the unavailable differences between groups, these polymorphisms should be investigated more intensively because they might be important to the improvement of reproductive traits with high heritability and repeatability, based on the critical roles that the genes play in these traits. Among these genes, the *PGR* polymorphism has been evaluated in subfertile heifers for the first time. Further investigations should be conducted with larger groups and more phenotypic data to exhibit more realistic results regarding possible associations.

REFERENCES

1. Pryce JE, Coffey MP, Brotherstone S: The genetic relationship between calving interval, body condition score and linear type and management traits in registered Holsteins. *J Dairy Sci*, 83, 2664-2671, 2000. DOI: 10.3168/jds.S0022-0302(00)75160-5

2. Royal M, Mann GE, Flint AP: Strategies for reversing the trend towards subfertility in dairy cattle. *Vet J*, 160, 53-60, 2000. DOI: 10.1053/tvjl.1999.0450

3. Lucy MC: Reproductive loss in high-producing dairy cattle: Where will it end? *J Dairy Sci*, 84, 1277-1293, 2001. DOI: 10.3168/jds.S0022-0302(01)70158-0

4. Butler WR: Review: Effect of protein nutrition on ovarian and uterine physiology in dairy cattle. *J Dairy Sci*, 81, 2533-2539, 1998. DOI: 10.3168/jds.S0022-0302(98)70146-8

5. Bartlett PC, Kirk JH, Mather E: Repeated insemination in Michigan Holstein-Friesian cattle: Incidence, descriptive epidemiology and estimated economic impact. *Theriogenology*, 26, 309-332, 1986. DOI: 10.1016/0093-691X(86)90150-0

6. Dobson H, Walker SL, Morris MJ, Routly JE, Smith RF: Why is it getting more difficult to successfully artificially inseminate dairy cows? *Animal*, 2, 1104-1111, 2008. DOI: 10.1017/S175173110800236X

7. Båge R, Bosu WTK, Rodríguez-Martínez H: Ovarian follicle apoptosis at the onset of standing estrus in virgin and repeat-breeder dairy heifers. *Theriogenology*, 56, 699-712, 2001. DOI: 10.1016/S0093-691X(01)00601-X

8. Båge R., Gustafson H, Lasson B, Forsberg M, Rodríguez-Martínez H: Repeat-breeding in dairy heifers: Follicular dynamics and estrus cycle characteristics in relation to sexual hormone patterns. *Theriogenology*, 57, 2257-2269, 2002. DOI: 10.1016/S0093-691X(02)00840-3

9. Singh B, Saravia F, Båge R, Rodríguez-Martínez H: Pregnancy rates in repeat-breeder heifers following multiple artificial inseminations during spontaneous oestrus. *Acta Vet Scand*, 46, 1-12, 2005. DOI: 10.1186/1751-0147-46-1

10. Wathes DC, Pollott GE, Johnson KF, Richardson H, Cooke JS: Heifer fertility and carry over consequences for life time production in dairy and beef cattle. *Animal*, 8, 91-104, 2014. DOI: 10.1017/S1751731114000755

11. Sood P, Zachut M, Dube H, Moallem U: Behavioral and hormonal patterns of repeat breeder cows around estrus. *Reproduction*, 149, 545-554, 2015. DOI: 10.1530/REP-14-0598

12. Oner Y, Pullu M, Akın O, Elmacı C: Investigation of beta-lactoglobulin (β-lg) and bovine growth hormone (bGH) genes polymorphisms by using HaeIII and MspI restriction enzymes in Brown Swiss and and Holstein breeds reared in Bursa region. *Kafkas Univ Vet Fak Derg*, 17, 371-376, 2011. DOI: 10.9775/kvfd.2010.3501

13. Korkmaz Ağaoğlu Ö, Akyüz B: Growth hormone gene polymorphism in four cattle breeds in Turkey. *Kafkas Univ Vet Fak Derg*, 19, 419-422, 2013. DOI: 10.9775/kvfd.2012.7961

14. Veerkamp RF, Beerda B: Genetics and genomics to improve fertility in high producing dairy cows. *Theriogenology*, 68, 266-273, 2007. DOI: 10.1016/j.theriogenology.2007.04.034

15. Driver AM, Huang W, Gajic S, Monson RL, Rosa GM, Khatib H: Effects of the progesterone receptor variants on fertility traits in cattle. *J Dairy Sci*, 92, 4082-4085, 2009. DOI: 10.3168/jds.2009-2013

16. Simpson ER, Mahendroo MS, Means GD, Kilgore MW, Hinshelwood MM, Graham-Lorence S, Amarneh B, Ito YJ, Fisher CR, Michael MD, Mendelson CR, Bulun SE: Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis. *Endocrinol Rev*, 15, 342-355, 1994. DOI: 10.1210/edrv-15-3-342

17. Metivier R, Petit FG, Valotaire Y, Pakdel F: Function of N-terminal transactivation domain of the estrogen receptor requires a potential α-helical structure and is negatively regulated by the A domain. *Mol Endocrinol*, 14, 1849-1871, 2000. DOI: 10.1210/mend.14.11.0546

18. Fürbass R, Kalbe C, Vanselow J: Tissue-specific expression of the bovine aromatase-encoding gene uses multiple transcriptional start sites and alternative first exons. *Endocrinology*, 138, 2813-2819, 1997. DOI: 10.1210/endo.138.7.5257

19. Vanselow J, Kühn C, Fürbass R, Schwerin M: Isolation of the bovine CYP19 promoter 1.2 and identification of genetic variants. *Anim Genet*, 31, 337-338, 2000. DOI: 10.1046/j.1365-2052.2000.00659.x

20. Szatkowska I, Grzesiak W, Jędrzejczak M, Dybus A, Zaborski D, Jankowiak D: An analysis of CYP19, CYP21 and ER genotypes in Polish Holstein-Friesian cows with regard to the selected reproductive traits. *Acta Vet Brno*, 80, 65-71, 2011. DOI: 10.2754/avb201180010065

21. Jędrzejczak M, Grzesiak W, Szatkowska I, Dybus A, Muszyńska M, Zaborski D: Associations between polymorphisms of CYP19, CYP21 and ER1 genes and milk production traits in Black-and-White cattle. *Turk J Vet Anim Sci*, 35, 41-49, 2011. DOI: 10.3906/vet-0911-205

22. Zaborski D, Grzesiak W: Detection of heifers with dystocia using artificial neural networks with regard to ERα-BglI, ERα-SnaBI and CYP19-PvuII genotypes. *Acta Sci Pol Zootechnica*, 10 (2): 105-116, 2011.

23. Zaborskia D, Grzesiaka W, Pilarczyka R: Detection of difficult calving's in the Polish Holstein-Friesian Black-and-White heifers. *J App Anim Res*, 2014. DOI: 10.1080/09712119.2014.987293

24. Eng FCS, Lee HS, Ferrara J, Willson TM, White JH: Probing the

structure and function of the estrogen receptor ligand binding domain by analysis of mutants with altered transactivation characteristics. *Mol Cell Biol,* 17 (8): 4644-4653, 1997.

25. Korach SK: Insights from the study of animals lacking functional estrogen receptor. *Science*, 266, 1524-1527, 1994. DOI: 10.1126/science.7985022

26. Kos M, Reid G, Denger S, Gannon F: Genomic organization of the human ERα gene promoter region. *Mol Endocrinol*, 15 (12): 2057-2063, 2001.

27. Gronemeyer H: Transcriptional activation by estrogen and progesterone receptors. *Annu Rev Genet*, 25, 89-123, 1991. DOI: 10.1146/annurev.ge.25.120191.000513

28. Yang WC, Tang KQ, Li SJ, Yang LG: Association analysis between variants in bovine progesterone receptor gene and superovulation traits in Chinese Holstein cows. *Reprod Dom Anim*, 46, 1029-1034, 2011. DOI: 10.1111/j.1439-0531.2011.01780.x

29. Szreder T, Zwierzchowski L: Polymorphism within the bovine estrogen receptor- α gene 5´-region. *J Appl Genet*, 45 (2): 225-236, 2004.

30. National Research Council: Nutrient Requirements of Dairy Cattle. 7th ed., National Academy of Sciences, Washington, DC, USA. 2001.

31. Vanselow J, Kühn C, Fürbass R, Schwerin M: Three PCR/RFLPs identified in the promoter region1.1 of the bovine aromatase gene (CYP19). *Anim Genet*, 30, 232-233, 1999. DOI: 10.1046/j.1365-2052.1999.00404-11.x

32. SAS 2002 User Guides: Version 9.2, Carry NC, USA, SAS Instute, Inc.

33. Yeh F, Yang RC, Boyle T: Popgene (v.1.32), Microsoft Windows-based freeware for Population Genetic Analysis, 2000. http://www.ualberta.ca/*fyeh/Pop32.exe, *Accessed: 13.04.2015*

34. Zahmatkesh A, Rahmani HR, Edriss MA, Sayed-Tabatabaei BE: Estrogen receptor α (ER-α) gene and bovine performance: Is there any relation? *Anim Sci Paper Rep,* 30 (4): 373-381, 2012.

35. Zahmatkesh A, Rahmani HR, Edriss MA, Sayed-Tabatabaei BE: Bovine ER-α polymorphism in relation with production traits. *International Conference on Food Engineering and Biotechnology, IPCBEE,* 9, 250-254, 2011.

36. Jędrzejczak M, Szatkowska I, Zych S, Grzesiak W, Czerniawska-Piątkowska E, Dybus A: Evaluation of associations of the polymorphism in the placenta-specific promoter 1.1 of the CYP19 gene in Black-and-White and Jersey cattle with milk production traits. *Arch Tierz Dummerstorf*, 49, 311-314, 2006.

37. Kowalewska-Łuczak I: Polymorphism of the CYP19 gene and milk production traits of dairy cattle. *Turk J Vet Anim Sci,* 34, 493-496, 2010. DOI: 10.3906/vet-0707-30

38. Kowalewska-Łuczak I, Michniewicz E, Kulig H: Effect of CYP19 SNPS on milk production traits of Jersey cows. *Acta Sci Pol Zootechnica*, 12 (1): 33-40, 2013.

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Research Article

Effect of Different Residual Variances on Genetic Parameters of Test Day Milk Yields

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Abstract

Heterogeneous residual variance effects on genetic parameters were examined for test day milk yields of Turkish Holsteins. A third order random regression models including the fixed, random additive genetic and permanent environmental effects were used. One of these models, RV10, residual variances is assumed to be different for each test day milk yields. The RV1 model has constant residual variance for each test day. Sequential (RV2 to RV9) and non-sequential (NRV2 to NRV9) groups of residual variances were also described in the models in order to compare estimates of variance components. The univariate analysis of milk yields for each test days was performed to define variance groups. The predicted residual variances ranged from 5.62 to 11.75 and from 5.61to 11.71 for RV and NRV models, respectively. Estimates of additive genetic variances changed between 0.55-6.76 for RV and 0.08-2.46 for NRV models. Permanent environmental variances were found between 2.36 and 18.60 for RV and 6.92 to 18.85 for NRV models. Heritability estimates varied from 0.02 to 0.43 for RV and 0.01 to 0.13 for NRV models. As a result, more accurate genetic parameter estimates are achieved by controlling the residual variances. RV10 model should be preferred to define details of the milk yield residual variances for each test day. However, RV5 model has been determined that an alternative model as compared with RV10.

Keywords: *Residual variance, Random regression model, Genetic parameter, Holstein Friesian*

Denetim Günü Süt Veriminin Genetik Parametre Tahminine Farklı Hata Varyanslarının Etkisi

Özet

Bu çalışmada Siyah Alaca'ların denetim günü süt verimine ait genetik parametre tahminine heterojen hata varyanslarının etkisi incelenmiştir. Bu amaçla sabit etkileri, şansa bağlı genetik ve kalıcı çevre etkilerini içeren üçüncü dereceden şansa bağlı regresyon modelleri kullanılmıştır. Bu modellerden RV10 modelinde hata varyansları her bir denetim gününde farklı kabul edilmiştir. RV1 modelinde ise hata varyansları tüm denetim günlerinde sabit kabul edilmiştir. Varyans bileşenleri tahminlerinin karşılaştırılmasında ardışık (RV2-RV9) ve ardışık olmayan (NRV2-NRV9) hata varyansı gruplarını içeren modeller kullanılmıştır. Söz konusu grupların belirlenmesinde denetim günü süt verimlerinin her biri için tek değişkenli analiz uygulanmıştır. Ardışık hata varyansı tanımlanan modellerde hata varyansı tahmini 5.62 ile 11.75 arasında ve ardışık olmayan hata varyansı tanımlanan modellerde ise 5.61 ile 11.71 arasında değişmiştir. Ardışık ve ardışık olmayan hata varyanslı modellerde eklemeli genetik varyanslar sırasıyla 0.55 ile 6.76 ve 0.08 ile 2.46 arasında tahminlenmiştir. Kalıcı çevre varyansları ise ardışık modellerde 2.36 ile 18.60 ve ardışık olmayanlarda 6.92 ile 18.85 arasında tahminlenmiştir. Kalıtım derecesi tahminleri de ardışık modeller için 0.02 ile 0.43 arasında ve ardışık olmayan modeller için 0.01 ile 0.13 arasında elde edilmiştir. Sonuç olarak, hata varyanslarının kontrol altına alınmasıyla daha güvenilir genetik parametre tahminlerine ulaşılmıştır. Her bir denetim günündeki hata varyansının tanımlamasında RV10 modelinin kullanımı tavsiye edilmiştir. Bunun yanı sıra, RV5 modelinin RV10 modeline göre alternatif bir model olduğu belirlenmiştir.

Anahtar sözcükler: *Hata varyansı, Şansa bağlı regresyon modeli, Genetik parametre, Siyah Alaca*

INTRODUCTION

In animal science, repeated measures such as milk yield, body weight and food intake have been analyzed with different models [1]. Genetic evaluation of milk production traits in dairy cattle can be improved by using test day models instead of aggregated 305 day production records [2-4]. In recent years, many countries have commonly used random regression models for production traits to improve the efficiency of the selection programs.

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Assuming homogeneous residual variance is a common approach in these models. However, this assumption may lead to lower or higher impact on the evaluation for different parts of the lactation. Several studies have shown that the residual variance changes over time because of herd management, weather conditions, lactation number, age at calving, month of calving, days in milk, pregnancy status, medical treatments and milking times etc. [5-9]. Generally, these mentioned environmental factors make the residual variances larger and more variable at the beginning and at the end of the lactation in comparison with those at the middle part. Therefore, information coming from each part of the lactation where the residual variance is actually larger than the assumed homogeneous value will has lower weight than it really has [10]. In addition, Olori et al.[6] conveyed that constant residual variance assumption causes residual variances to be underestimated and heritabilities to be overestimated in early stages of lactation. Instead of constant residual assumption, heterogeneous of residual variance can be included in the models. Different approaches have been proposed based on heterogeneous residual variance during lactation. In some previous studies, lactation divided into different periods with assuming homogeneity of residual variance within the period and heterogeneity between them [6,11,12]. This approach is easy to implement and can be useful in the sense that it provides more useful information on the expected pattern of residual variance which changes over lactation. However, defining the different arbitrary classes of heterogeneous of residual variance is more important for a good model. If the identification of the classes in terms of residual variance is not accurate, the proposed model will not be correct [10]. Olori et al.[6,7] reported that a correct estimation of residual variance in each class depends on which lactation stages are assumed to have the same residual variance.

Few earlier studies have been proposed heterogeneity of residual variance in random regression model for the estimation of genetic parameters for test day milk records. Olori et al.[6] investigated constant and varied residual variances in the scope of random regression models. They found that constant residual variance structure in random regression models alters estimates of residual variance in early lactation but has no significant effect on the additive genetic and permanent environmental variances. Olori et al.[7] also modeled third, fourth and fifth order random regression models with varying residual variance groups (constant, 4, 10 and 37 measurement error classes) for estimates of variance components. They obtained declined estimates of residual variances as the order of fit of the additive genetic, phenotypic and permanent environmental variances increased. Moreover, Rekaya et al.[11] compared alternative models to analyze test day yields in the Spanish Holstein Friesian population. They adopted two random regression models with have constant residual variance and allowed 30 residual variance classes. They estimated smaller residual variances under heterogeneous residual variance model. In their study larger residual variances showed lower heritability estimates at the beginning and end of the lactation. Lo'pez-Romero et al.[10] considered homogeneous and heterogeneous of residual variance with three and 30 arbitrary measurement error classes of different length in random regression models with third and fifth order additive genetic and permanent environmental effects. Residual variances obtained from 30 arbitrary intervals were constant between 70 and 300 days of lactation and tended to increase towards beginning and end of the lactation. The assumptions on the residual variance pattern did not affect the estimates of the daily additive genetic variance and only affect the estimates of daily permanent environmental variance in the first part of the lactation. Fujii and Suzuki [13] estimated genetic parameters for milk yield by using random regression models under heterogeneous residual variances and tested residual variances in three models with linear and quadratic exponential functions of calving year and also divided residual variances into five groups according to calving year. They estimated permanent environmental variances larger than additive genetic variances and the pattern of the permanent and additive genetic variances were very similar in different models. But estimates of residual variances were increased along lactation.

Heterogeneous residual variance assumptions have not been adequately highlighted in the literature. In this study, therefore, several random regression models under homogeneous and heterogeneous residual variance assumptions with sequential residual variance classes of test days were employed for analyses of variance components. Unlike previous studies, random regression models under non-sequential residual variance classes of test days for analyses of variance components were also investigated with this study. All models were compared based on their fitting performance and estimates of genetic parameters for milk yields of Turkish Holstein Friesians.

MATERIAL and METHODS

Data

Holstein milk records from different farms who are the members of Isparta province Cattle Breeders Association in Turkey were the material of this study. Milk yields were collected at monthly periods (TD1-TD10) from 2001 through 2011. Test day milk records less than 5 kg were excluded from the analyses. Only records collected between DIM 5 and DIM 307 were included. Age at first calving was also limited between 20 and 51 month. In the final, data set total of 43206 test day milk records from 6085 Turkish Holstein Friesian cows in 248 herds were analyzed. The descriptive statistics of final data set were given in *Table 1*.

Table 1. *Descriptive statistics of the final data*
Tablo 1. *Veri setine ilişkin tanımlayıcı istatistikler*

Item	No.
Records	43206
Mean TD records per cow	18.78
Herds	248
Herd-year-season level	3809
Animals with records	6085
Sires with progeny records	667
Dams with progeny records	4241

Method

The random regression models under different residual variance structure were applied to first lactation test day milk yield records of Turkish Holsteins. Third order Legendre polynomial model was preferred to obtain the best fit [7,14-19]. The used random regression model is as follows:

$$y_{ijk} = HYS_i + \sum_{m=1}^{5} \beta_m X_m(t_{jk}) + \sum_{m=1}^{3} \alpha_{jm} \phi_m(t_{jk}) + \sum_{m=1}^{3} p_{jm} \phi_m(t_{jk}) + e_{ijk}$$

where y_{ijk} is the k^{th} test day milk yield of the cow j at i^{th} herd-year-season, HYS_i is the i^{th} herd-year-season effect, β_m is the m^{th} fixed regression coefficients associated with the m^{th} covariate, t_{jk} is the k^{th} test day of the cow j, $X_m(t_{jk})$ is the m^{th} covariates (X_1: Age at first calving, c=305, X_2=DIM/c, $X_3=(X_2)^2$, X_4=ln(c/DIM), $X_5==(X_4)^2$ depending on DIM=t, of the Ali and Schaeffer [20] function evaluated at t_{jk}), α_{jm} is the m^{th} additive genetic random regression coefficients for cow j, p_{jm} is the m^{th} permanent environmental random regression coefficients for cow j, ϕ_m is the m^{th} polynomial and e_{ijk} is the random residual effect with $e_{ijk} \sim N(0,\sigma^2 e_{ijk})$.

In this random regression model several residual variance groups were designed. Residual variance was firstly assumed constant (RV1 model) through lactation. On the contrary to RV1, residual variance was assumed different for each test day with RV10 model. Then univariate analysis of milk yields for each test days was performed with the univariate models as given below:

$$y_{ijk} = HYS_i + \alpha_j + e_{ijk}$$

where y_{ijk} is the k^{th} test day milk yield of the cow j at i^{th} herd-year-season, HYS_i is the i^{th} herd-year-season effect, α_j is the additive genetic effect for cow j and e_{ijk} is the random residual effect.

Sequential (RV) and non-sequential (NRV) groups for residual variance were described according to results of this preliminary analysis *(Fig. 1)*. Residual variance patterns for RV and NRV groups were shown in *Fig. 1*. For example, in RV2, two residual variance groups were described with 1 (for the first five test days) and 2 (for the last five test days). However, in NRV2 the two groups were not described sequentially as RV2 *(Fig. 1)*.

Analyses were performed using DFREML statistical package [21]. The goodness of fit of the model was investigated by Akaike's information criterion, AIC [22]. This likelihood based criterion has been calculated as: AIC= -2 × LogL + 2 × p where p denotes the number of parameters estimated. The model which gives the lowest AIC values was chosen as the better approximating model [10,23]. Furthermore, residual variance structures were compared Likelihood ratio test-LRT [24] within sequential and non-sequential residual variance groups. LRT for model i and j was LRT_{ij}= -2 × ($LogL_i$-$LogL_j$). In the LRT, the Log Likelihood (LogL) differences were tested using Chi-square (χ^2) test with the degree of freedom determined as the number of the parameter differences between the models [25].

RESULTS

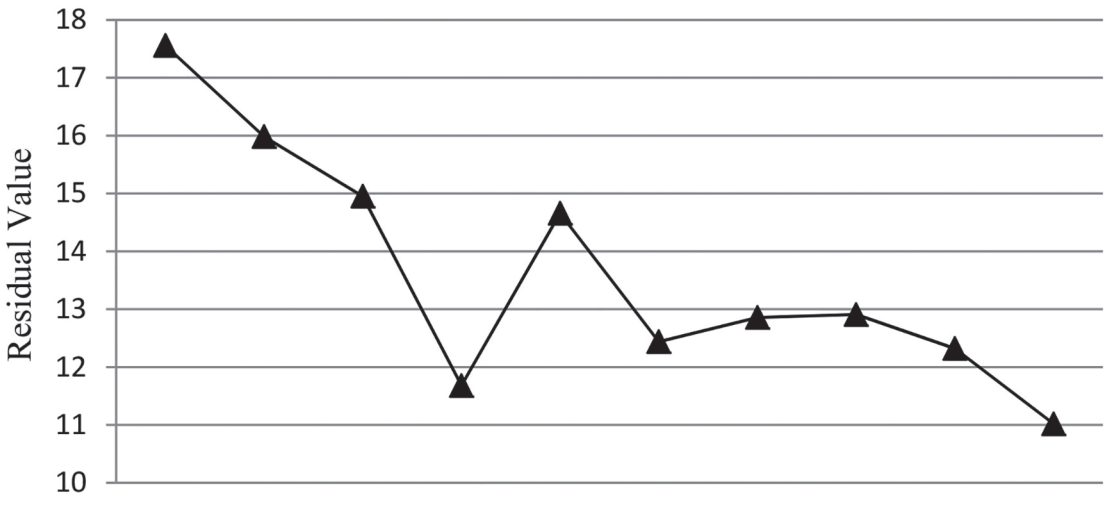
Fitting Performance of Models

The fitting performance of the RV and NRV models were presented in *Table 2*. However, only the comparisons of the models with previous one were given in the last column of *Table 2*. Fitting performance of all RV and NRV models

Table 2. *LogL and AIC values of the RV and NRV models*
Tablo 2. *RV ve NRV modellerinde LogL ve AIC değerleri*

RV Group	Parameter No.	AIC	LogL Values	LRT
Constant RV	13	111088	-55525	-
RV2	14	140279	-70119	-29188*
RV3	15	140286	-70122	-6*
RV4	16	140191	-70074	96*
RV5	17	140154	-70054	40*
RV6	18	140218	-70085	-62*
RV7	19	140550	-70250	-330*
RV8	20	140546	-70247	6*
RV9	21	140210	-70078	338*
RV10	22	140143	-70043	70*
Constant RV	13	111088	-55525	-
NRV2	14	140209	-70084	-29118*
NRV3	15	140194	-70076	16*
NRV4	16	140699	-70327	-502*
NRV5	17	140147	-70050	554*
NRV6	18	140590	-70271	-442*
NRV7	19	140164	-70057	428*
NRV8	20	140148	-70048	18*
NRV9	21	140232	-70089	-82*
RV10	22	140143	-70043	92*

** Values within a column differ significantly at P<0.05*



	TD1	TD2	TD3	TD4	TD5	TD6	TD7	TD8	TD9	TD10
RV1	1	1	1	1	1	1	1	1	1	1
RV2	1	1	1	1	1	2	2	2	2	2
RV3	1	1	1	1	2	2	3	3	3	3
RV4	1	2	2	3	3	4	4	4	4	4
RV5	1	2	2	3	4	5	5	5	5	5
RV6	1	2	2	3	4	5	5	5	5	6
RV7	1	2	2	3	4	5	5	6	6	7
RV8	1	2	2	3	4	5	6	6	7	8
RV9	1	2	2	3	4	5	6	7	8	9
RV10	1	2	3	4	5	6	7	8	9	10
NRV1	1	1	1	1	1	1	1	1	1	1
NRV2	1	1	1	2	1	2	2	2	2	2
NRV3	1	1	1	3	1	2	2	2	2	3
NRV4	1	1	2	3	2	4	4	4	4	3
NRV5	1	2	3	4	3	5	5	5	5	4
NRV6	1	2	3	5	4	6	6	6	6	5
NRV7	1	2	3	5	4	6	6	6	6	7
NRV8	1	2	3	7	4	5	5	5	6	8
NRV9	1	2	3	8	4	5	6	5	7	9
NRV10	1	2	3	4	5	6	7	8	9	10

Fig 1. Patterns of RV and NRV groups according to univariate analysis of residual variances of each test day

Şekil 1. Denetim günü hata varyanslarının tek değişkenli analizine göre RV ve NRV grupları

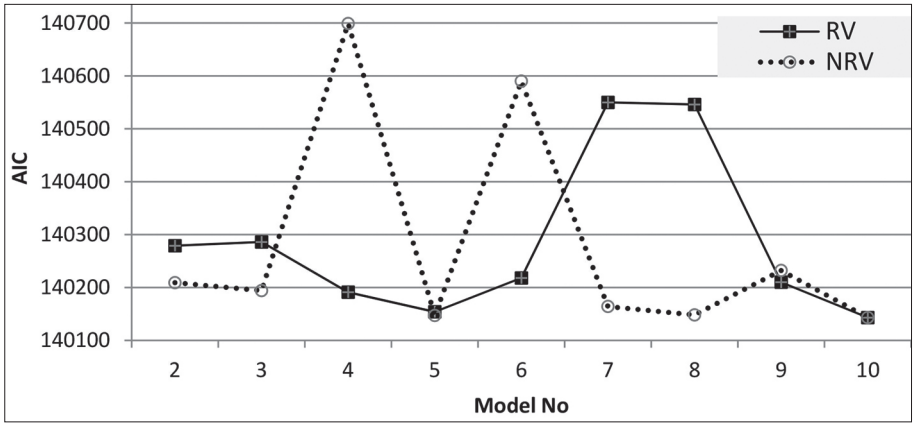


Fig 2. Changes of AIC values for all RV models

Şekil 2. RV modellerinde AIC değerlerinin değişimi

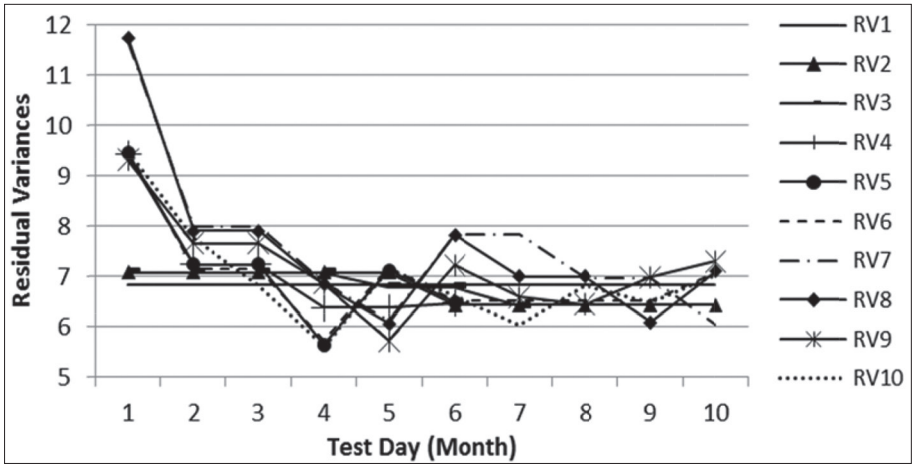
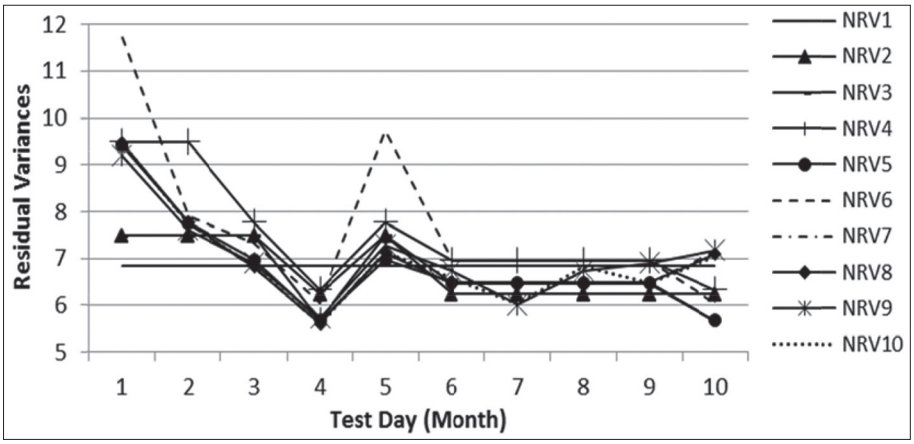


Fig 3. Change of residual variances of the test day milk yields under RV models

Şekil 3. RV modellerinde denetim günü süt verimi hata varyansının değişimi

Fig 4. Change of residual variances of the test day milk yields under NRV models

Şekil 4. NRV modellerinde denetim günü süt verimi hata varyansının değişimi



were found significantly different due to LRT results. On the other hand, the RV1 model which has the least number of parameter and constant residual variance, showed a better performance with the lowest AIC value. AIC values of other models were closer each other compared with RV1. But nevertheless, there are still some differences among models for AIC values *(Table 2)*. Especially RV5, RV10 and NRV5 had a better performance. Among them the RV5 model defines residual variance structure in detail at the beginning and peak of lactation while RV10 model separately evaluates residual variances of each test day milk yields *(Fig. 2)*. Similar fitting structure is also valid for the LogL values.

Residual Variances

The variability of the residual variances was explored by plotting the estimated residuals from the random regression RV and NRV models in *Fig. 3* and *Fig. 4*, respectively. Residual variances changed from 5.62 to 11.75 for RV models and from 5.61 to 11.71 for NRV models. The higher residual variance was obtained at the first test day and then decreased to 5-8 test days for the next test days *(Fig. 3, Fig. 4)*. When compared all RV and NRV models, NRV4 and NRV6 models gave higher residual variance estimates *(Fig. 4)*. In fact, these are the models that have the worst results in model fitting *(Table 2)*.

Estimates of Other Variance Components and Genetic Parameters

Estimates of additive genetic variances varied from 0.55 to 6.76 for RV and 0.08 to 2.46 for NRV models. Changes of the permanent environmental variances were found between 2.36 to 18.60 for RV and 6.92 to 18.85 for NRV models. The heritability estimates for test day milk yields from RV and NRV models were summarized in *Table 3*. The estimates ranged from 0.02 to 0.48 for RV and 0.01 to 0.17 for NRV models. In terms of heritabilities, there is a similarity among models except the RV1, RV6 and RV9 models.

The RV1 model gave higher estimates of heritability at beginning and end of lactation compared with the models specified for the study *(Table 3, Fig. 5-a)*. However, it has similar heritability estimates ($h^2 \sim 0.10$) with other models in the middle of lactation. On the other hand, permanent environmental effect did not change during lactation for the RV1 model. Variability of heritability estimates during lactation can be explained by variability in the genetic variances.

When compared all models, in the middle of the lactation heritability estimates from the RV9 model is two times ($h^2 \sim 0.20$) and RV6 model is four times

Table 3. *Heritability estimates and sum of absolute differences for RV and NRV models*
Tablo 3. *RV ve NRV modellerinde kalıtım derecesi tahminleri ve mutlak sapmalar toplamı*

Model	TD										SAD[1]
	1	2	3	4	5	6	7	8	9	10	
Constant RV	0.32	0.22	0.14	0.11	0.10	0.12	0.17	0.26	0.37	0.48	1.37
RV2	0.10	0.12	0.13	0.13	0.12	0.12	0.11	0.10	0.08	0.05	0.06
RV3	0.10	0.12	0.13	0.13	0.12	0.12	0.11	0.10	0.08	0.05	0.06
RV4	0.09	0.12	0.12	0.13	0.13	0.12	0.11	0.10	0.07	0.05	0.06
RV5	0.09	0.11	0.12	0.13	0.12	0.12	0.11	0.09	0.07	0.04	0.02
RV6	0.02	0.11	0.23	0.38	0.41	0.43	0.36	0.23	0.10	0.02	1.45
RV7	0.10	0.11	0.10	0.11	0.11	0.10	0.10	0.11	0.11	0.12	0.25
RV8	0.07	0.09	0.10	0.11	0.12	0.11	0.11	0.10	0.10	0.08	0.19
RV9	0.04	0.09	0.14	0.19	0.19	0.19	0.17	0.14	0.09	0.04	0.4
RV10	0.09	0.11	0.13	0.14	0.12	0.12	0.11	0.09	0.07	0.04	0
NRV2	0.09	0.10	0.11	0.12	0.11	0.12	0.12	0.11	0.10	0.07	0.15
NRV3	0.10	0.11	0.12	0.13	0.11	0.12	0.11	0.09	0.07	0.05	0.05
NRV4	0.07	0.08	0.10	0.12	0.11	0.12	0.11	0.10	0.09	0.08	0.18
NRV5	0.09	0.11	0.12	0.13	0.12	0.11	0.11	0.10	0.09	0.08	0.1
NRV6	0.07	0.09	0.10	0.12	0.10	0.12	0.11	0.11	0.09	0.09	0.2
NRV7	0.05	0.10	0.15	0.17	0.13	0.10	0.04	0.01	0.01	0.06	0.36
NRV8	0.09	0.11	0.12	0.13	0.12	0.12	0.11	0.09	0.07	0.05	0.03
NRV9	0.07	0.10	0.12	0.13	0.12	0.12	0.12	0.11	0.09	0.07	0.13

[1] ***SAD:*** *Sum of absolute differences of heritability estimates for test day milk yields of the model from estimates of RV10 model*

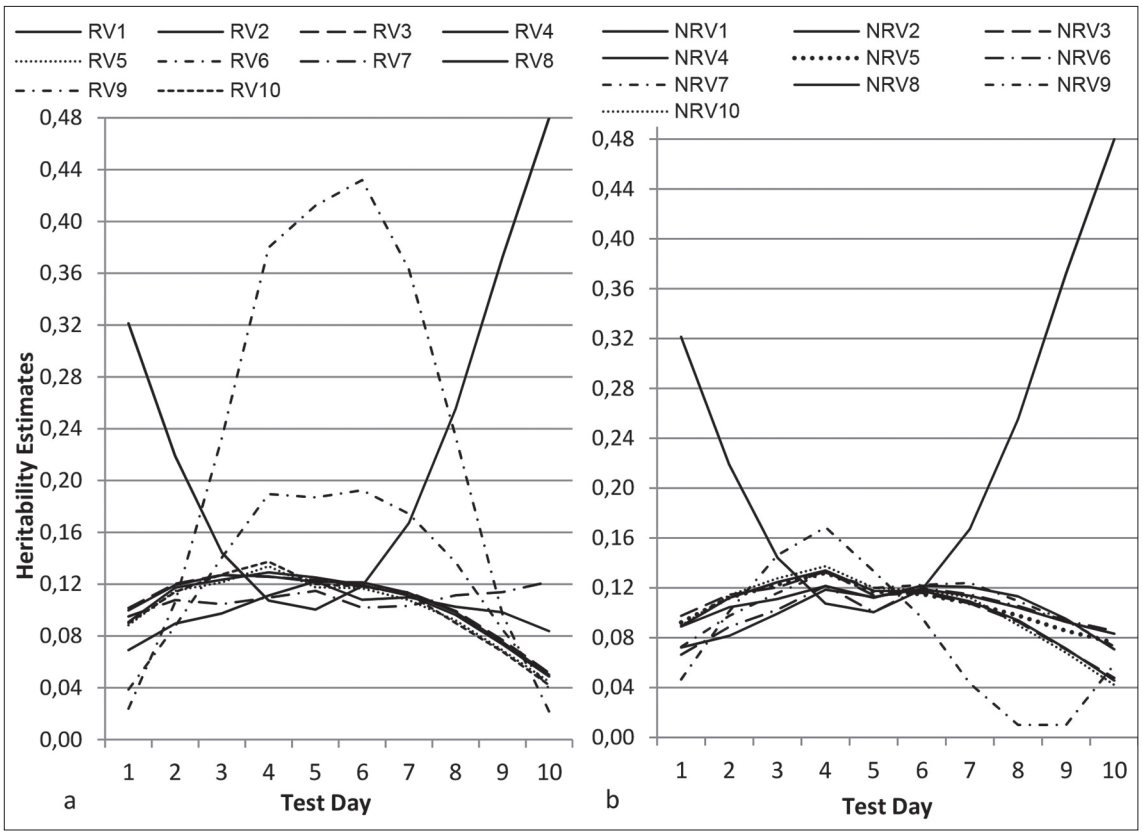


Fig 5. Changes of heritability estimates over lactation from RV models *(left)* and NRV models *(right)*
Şekil 5. RV *(sol)* ve NRV modellerinde *(sağ)* kalıtım derecesi tahminlerinin değişimi

(h^2 ~ 0.40) greater than the estimates from other models *(Fig. 5-a)*. Considering sum of absolute differences of heritability estimates of each test days in the model from the estimates obtained in RV10 model, RV5 model has the least deviation. Heritability estimates from NRV models were found closer each other than estimates of RV models *(Fig. 5-b)*. Only NRV7 model had different and fluctuating pattern. This model had lower heritability estimates at the beginning of the lactation and then the estimate increased up to fourth test day, then it fell rapidly approaching to zero at the eight test day and then increased again at the end of lactation *(Fig. 5-b)*. The models evaluated in the present study exhibited similar results for estimates of genetic and permanent environmental effects.

DISCUSSION

Regarding to fitting performance, RV and NRV models have not been found similar in majority. The LRT results were almost different among models. The RV1 model was the best model which was commonly used in literature [6,7,17,26]. However, the RV5, RV10 and NRV5 models recommended because of their detail descriptions on residual variances. The estimated residual variances were found higher at the beginning of the lactation and decreased along the lactation as expected. These estimates ranged from 5.62 to 11.75 for the RV models and ranged from 5.61 to 11.71 for the NRV models. Similar patterns and closer results of the residual variances have been reported by Olori et al.[6]. On the other hand, these estimates were higher than those estimated by Rekaya et al.[11] and Fujii and Suzuki [13].

We found that the estimates of additive genetic variances varied from 0.55 to 6.76 for the RV models. The estimates of additive genetic variances illustrated fluctuating values according to sequential RV models. In the model RV1 that error variance assumed constant, the additive genetic variances were higher at the beginning and end of the lactation, but lower at middle of the lactation. However genetic variances were estimated higher at middle of the lactation for the RV6 and RV9 models. Moreover, estimates of additive genetic variances were 0.08 to 2.46 for the NRV models. The estimates of additive genetic variances for both RV and NRV models were lower when compared with the findings of previous studies [6,7,10,11,13].

Permanent environmental variances were between 2.36 to 18.60 for RV and 6.92 to 18.85 for NRV models. In previous studies [7,12] higher permanent environmental variances were obtained but Rekaya et al.[11] reported lower estimates in their studies when compared with the present study. In addition, all models had same tendency with higher permanent environmental variances at the start and end of lactation, but lower at the middle of the lactation. This result has been obtained by other authors [6,12,13] under different RV models.

In this study, heritability estimates ranged from 0.02 to 0.43 and from 0.01 to 0.13 for RV and NRV models, respectively. For all models specified under the study, heritability estimates were lower at beginning and late part of lactation as estimates of additive genetic variances. Particularly, the RV6 and RV9 models overestimated heritabilities during middle lactation. This was probably the cause of the high additive genetic variances and low estimates of residual variances. Although the pattern of heritabilities was the same but our estimates were lower than those estimated by Olori et al.[6,7]; Rekaya et al.[11] and Lo'pez-Romero et al.[12].

The model under constant residual variance assumption gave opposite estimates for additive genetic variances and heritability values when compared to other models considered. Thus it can be seen that more accurate parameter estimates are achieved by controlling the residual variance with heterogeneous residual variance assumption. However, defining the classes of heterogeneous residual variances is more important for the accurate parameter estimates. Besides sequential pattern, the heterogeneous residual variance groups can be classified as non-sequential pattern. When it is asked to define details of the milk yield residual variances for each test day, RV10 model should be preferred. However, if there are problems due to model complexity for residual variances the model which defines residual variances in detail at beginning, peak of the lactation, and evaluates together other parts of the lactation, should be used. In this study RV5 is the alternative model especially for parameter estimates and its simplicity as compared to RV10.

Acknowledgements

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REFERENCES

1. Orhan H, Eyduran E, Akbaş Y: Defining the best covariance structure for sequential variation on live weights of Anatolian merinos male lambs. *J Anim Plant Sci,* 20 (3): 158-163, 2010.

2. Ptak E, Schaeffer LR: Use of test day yields for genetic evaluation of dairy sires and cows. *Livest Prod Sci*, 34, 23-34, 1993. DOI: 10.1016/0301-6226(93)90033-E

3. Jamrozik J, Schaeffer LR, Dekkers JCM: Genetic evaluation of dairy cattle using test day yields and random regression model. *J Dairy Sci*, 80, 1217-1226, 1997. DOI: 10.3168/jds.S0022-0302(97)76050-8

4. Druet T, Jaffrézic F, Ducrocq V: Estimation of genetic parameters for test day records of dairy traits in the first three lactations. *Genet Select Evol*, 37, 257-271, 2005. DOI: 10.1186/1297-9686-37-4-257

5. Perochon L, Coulon JB, Lescourret F: Modelling lactation curves of dairy cows with emphasis on individual variability. *Anim Sci*, 63, 189-200, 1996. DOI: 10.1017/S1357729800014740

6. Olori VE, Hill WG, Brotherstone S: The structure of the residual error variance of test day milk yield in random regression models. *Interbull Bullet*, 20, 103-108, 1999.

7. Olori VE, Hill WG, Mcguirk BJ, Brotherstone S: Estimating variance components for test day milk records by restricted maximum likelihood with a random regression animal model. *Livest Prod Sci*, 61, 53-63, 1999. DOI: 10.1016/S0301-6226(99)00052-4

8. Jaffrezic F, White IMS, Thompson R, Hill WG: A link function approach to model heterogeneity of residual variances over time in lactation curve analyses. *J Dairy Sci*, 83, 1089-1093, 2000. DOI: 10.3168/jds.S0022-0302(00)74973-3

9. Swalve HH: Theoretical basis and computational methods for different test day genetic evaluation methods. *J Dairy Sci*, 83, 1115-1124, 2000. DOI: 10.3168/jds.S0022-0302(00)74977-0

10. Lo'pez-Romero P, Carabaño MJ: Comparing alternative random regression models to analyse first-lactation daily milk yield data in Holstein-Friesian cattle. *Livest Prod* Sci, 82, 81-96, 2003. DOI: 10.1016/S0301-6226(03)00003-4

11. Rekaya R, Carabaño MJ, Toro MA: Use of test day yields for the genetic evaluation of production traits in Holstein-Friesian cattle. *Livest Prod Sci*, 57, 203-217, 1999. DOI: 10.1016/S0301-6226(98)00181-X

12. Lo'pez-Romero P, Rekaya R, Carabaño MJ: Assessment of homogeneity vs. heterogeneity of residual variance in random regression test-day models in a Bayesian analysis. *J Dairy Sci*, 86, 3374-3385, 2003. DOI: 10.3168/jds.S0022-0302(03)73941-1

13. Fujii C, Suzuki M: Comparison of homogeneity and heterogeneity of residual variance using random regression test-day models for first lactation Japanese Holstein cows. *J Anim Sci*, 77, 28-32, 2006. DOI: 10.1111/j.1740-0929.2006.00316.x

14. Van Der Werf JHJ, Goddard ME, Meyer K: The use of covariance functions and random regressions for genetic evaluation of milk production based on test day records. *J Dairy Sci*, 81, 3300-3308, 1998. DOI: 10.3168/jds.S0022-0302(98)75895-3

15. Pool MH, Meuwissen THE: Prediction of daily milk yields from a limited number of test days using test day models. *J Dairy Sci,* 82, 1555-1564, 1999. DOI: 10.3168/jds.S0022-0302(99)75383-X

16. Veerkamp RF, Thompson R: A covariance function for feed intake, live weight, and milk yield estimated using a random regression model. *J Dairy Sci*, *82*, 1565-1573, 1999.

17. Kettunen A, Mantysaari EA, Pösö J: Estimation of genetic parameters for daily milk yield of Primiparous Ayrshire Cows by random regression test day models. *Livest Prod Sci*, 66, 251-261, 2000. DOI: 10.1016/S0301-6226(00)00166-4

18. Strabel T, Szyda J, Ptak E, Jamrozik J: Comparison of random regression test-day models for production traits of dairy cattle in Poland. *Interbull Bullet*, 31,197-200, 2003. DOI: 10.4141/cjas87-067

19. Takma Ç, Akbaş Y: Comparison of fitting performance of random regression models to test day milk yields in Holstein Friesians. *Kafkas Univ Vet Fak Derg*, 15, 261-266, 2009. DOI: 10.9775/kvfd.2008.138-A

20. Ali TE, Schaeffer LR: Accounting for covariances among test day milk yields in dairy cows. *Can J Anim Sci*, 67, 637-644, 1987.

21. Meyer K: DFREML 3.0α Program Package and User Notes. Animal Genetics and Breeding Unit, University New England, Armidale, New South Wales, Australia, 1998.

22. Akaike H: Information theory as an extension of the maximum likelihood principle. 2nd International Symposium on Information Theory. Akademiai Kiado, Budapest, Hungary, 1973.

23. Apiolaza LA, Gilmour AR, Garrick DJ: Variance modeling of longitudinal height data from a *Pinus radiata* progeny test. *Can J For Res*, 30, 645-654, 2000. DOI: 10.1139/x99-246

24. Rao CR: Linear Statistical Inference and Its Applications. 2nd ed., John Wiley&Sons, New York, NY, USA, 1973.

25. Huelsenbeck JP, Bull JJ: A likelihood ratio test to detect conflicting phylogenetic signal. *Syst Biol*, 45, 92-98, 1996. DOI: 10.1093/sysbio/45.1.92

26. Pool MH, Janss LLG, Meuwissen THE: Genetic parameters of legendre polynomials for first parity lactation curves. *J Dairy Sci*, 83, 2640-2649, 2000. DOI: 10.3168/jds.S0022-0302(00)75157-5

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Research Article

Effects of Using Transglutaminase and Fat Replacer on Functional Properties of Non-Fat Yoghurt [1]

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Abstract

The aim of this study was to investigate the effectiveness of microbial transglutaminase (mTGase, EC 2.3.2.13) as compared with using of a commercial fat replacer (Dairy-Lo) in the manufacture of non-fat set yoghurt. For this purpose, two types of non-fat yoghurt supplemented with mTGase and Dairy-Lo and control non-fat yoghurt without additive as a control sample were produced and stored at 4°C for 20 days. Physical properties of the non-fat yoghurt were improved by mTGase during 20-day storage, moreover; addition of mTGase did not have any effect on the acetaldehyde content of yoghurt. While the sample supplemented with Dairy-Lo showed the lowest serum separation, the gel strength of this sample was weaker than those made with mTGase. Sensory results indicated that non-fat yoghurt with mTGase had taste and aroma similar to that of the control yoghurt. In addition, the incorporation of Dairy-Lo had negative effect on sensory properties of non-fat yoghurt. According to the results obtained, the use of mTGase could be suggested for the production of non-fat yoghurt with reduced dry matter content without adversely affecting the textural properties of the end product.

Keywords: *Acid gels, Enzymatic modification, Fat replacer, Non-fat yoghurt, Transglutaminase*

Transglutaminaz ve Yağ İkame Maddesi Kullanımının Yağsız Yoğurdun Fonksiyonel Nitelikleri Üzerine Etkileri

Özet

Bu çalışmanın amacı yağsız yoğurt üretiminde mikrobiyal transglutaminaz (mTGase, EC 2.3.1.13) ile ticari bir yağ ikame maddesinin (Dairy-Lo) etkinliğini karşılaştırmalı olarak araştırmaktı. Bu amaçla, mTGase ve Dairy-Lo ilavesi ile iki tip yağsız yoğurt ve herhangi bir katkı ilave edilmeksizin kontrol yağsız yoğurt üretilmiş ve +4°C'de 20 gün süreyle depolanmıştır. Yağsız yoğurt örneklerinin fiziksel özellikleri mTGase ile 20 günlük depolama süresince gelişmiş, ilaveten; mTGase ilavesinin yoğurdun asetaldehit içeriği üzerine olumsuz herhangi bir etkisi olmamıştır.Duyusal analiz sonuçları da mTGase ile yağsız yoğurdun kontrol yoğurdununkine benzer tat-aroma profiline sahip olduğunu desteklemektedir. Dairy-Lo ilavesi edilen yoğurtta en düşük serum ayrılması görülürken, aynı örnekte pıhtı sıkılığı mTGase ilavesi ile üretileninkinden daha düşük olmuştur. İlavaten, Dairy-Lo ilavesi yağsız yoğurdun duyusal özelliklerini olumsuz yönde etkilemiştir. Elde edilen sonuçlara göre, mTGase kullanımı, son ürünün tektürel özelliklerine olumsuz etkisi olmadan düşük kurumaddeli yağsız yoğurt üretimi için tavsiye edebilir.

Anahtar sözcükler: *Asit jeller, Enzimatik modifikasyon, Yağ ikame maddesi, Yağsız yogurt, Transglutaminaz*

INTRODUCTION

In recent years, low fat and non-fat dairy products including yoghurt have gained popularity because of consumer awareness about health concerns related to decreasing the risks connected with obesity and coronary heart diseases [1]. However, the partial or total removal of fat from yoghurt decreases the overall quality perceived by the consumers [2]. It was reported that reduction of fat content in yoghurt resulted in lower gel strength and firmness than full fat yoghurt, as a consequence of lower number of fat globules embedded in the protein network [3].

To improve textural and functional properties of non-fat yoghurt, the use of additives has been widely investigated [4]. Fat replacers can be successfully used in

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the manufacture of reduced fat dairy products such as cheese, ice cream and yoghurt [5,6]. Fat replacer is an ingredient that can be used to provide some or all of the function of fat, yielding fewer calories [7]. Also, fat replacers can be used to solve some physical and textural problems originating from low-fat level in the dairy products. Dairy-Lo is a protein-based fat replacer which has a GRAS (Generally Recognized as Safe) status derived from whey protein concentrate [5,8].

Enzymatic cross-linking of protein by mTGase modifies the techno-functional properties of proteins and is reported as an innovative way of producing novel milk gels [9-11]. The mTGase which catalyzes the acyl-transfer (acyl donour) reaction between the γ-carboxyamid group of peptide or protein-bound glutaminyl residues and primary amines (acyl acceptor), is a transferase [12-14]. MTGase catalyzes the reactions which cause to the formation of cross-links in food proteins [15,16]. In this way, intermolecular cross-linking of proteins results in high molecular weight polymers which have different functional properties to improve the techno-functional properties of foods [10,17,18]. Milk proteins, especially caseins, are good substrates for cross-linking with mTGase [18-21]. The effect of cross-linking of milk proteins on various functional properties has been investigated [9,10]. It was reported that cross-linking of the proteins in milk improved gels firmness and reduced serum separation of acid-induced milk gel, mainly set-type yoghurts [17,22-25]. Özer et al.[26] also expressed that the mTGase added into milk may be an alternative method instead of addition of extra protein and stabilizer in non-fat yoghurt.

The present study was carried out to examine the effects of TGase and commercial fat replacer (Dairy-Lo) on some chemical, microstructural and textural properties of non-fat yoghurts.

MATERIAL and METHODS

Materials

Raw cow's milk was obtained from the Ankara University, Agricultural Faculty Dairy Farm. The raw milk contained 11.5 g/100 g total dry matter, 3.65 g/100 g protein and 3.5 g/100 g fat. Lyophilized-mixed yoghurt culture containing *Streptoccocus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* coded Bulk Set Y 502 (Danisco Deutschland GmbH, Niebüll, Germany) was used as starter culture. The mTGase was supplied by Ajinomoto Co. (Japan, with declared activity of 100 units/g ActivaMP) at an enzyme/substrate ratio of 1 unit/g milk protein. The commercial protein based fat replacer Carbelac Dairy-Lo (Carbery Group, Ireland) was used at a level of 1.5% (w/v) as recommended by the manufacturer. The chemicals which were supplied from Merck Chemicals Ltd. (Merck, UK) were of analytical grade.

Production of Yoghurt

Raw milk was standardized to maximum 0.15% fat content and then divided three parts. First part was used for the production of control yoghurt (sample A) without any additive. Second part (sample B) was incubated with mTGase at 50°C for 60 min after pasteurization (85°C for 15 min) in order to improve so that the gel strength and to decrease the syneresis in yoghurt [25]. Dairy Lo was added to the third part of milk at the ratio of 1.5% before homogenization (sample C). Yoghurt production is outlined in *Fig. 1*.

Milk samples were inoculated with commercial yoghurt starter culture (2%, v/v), and then, were incubated at 43°C until pH 4.6 was attained. After incubation, yoghurt samples were cooled down to room temperature and kept in refrigerator at 4°C for 20 days. Samples were analysed at the 1st, 10th and 20th day of storage. Total dry matter contents of the samples A, B and C were 10.18%, 10.21% and 10.98%, respectively.

Chemical and Physical Analysis

The acidity of yoghurts was determined by titration and expressed as SH [27]. The pH was measured by a digital pH meter (MP 225, Mettler-Toledo GmbH, Giessen, Germany). Fat contents of the samples were determined by the Gerber method, while dry matter and protein contents were detected by oven drying and Kjeldahl methods, respectively [27]. For determination of tyrosine value, *spectrophotometric* method was used as reported by Hull [28].

Viscosity measurements were carried out using a viscometer (181/VTR 24, Thermo Haake GmbH, Karlsruhe, Germany) at +4°C. Gel firmness was measured by using a penetrometer (Model 17310-0, *Stanhope*-Seta Ltd., Surrey, *England)* which equipped with a 25 g conical (45°C) probe. Penetration depth of the probe into the yoghurt gel within 1 s of duration was referred to the value of penetrometer as millimeter.

Serum separation was measured by transferring twenty five gram of yoghurt samples into a funnel with filter paper placed on a flask. The volume of serum collected after draining at 4°C for 2 h was measured as serum separation value [29].

Determination of Carbonyl Compounds

Carbonyl compounds (i.e. acetaldehyde, acetone and diacethyl) were determined by headspace method using the procedure reported by Ulbert [30]. Five grams of yoghurt samples transferred into headspace vials (Agilent, made in USA, 20 mL flat bottom) and capped using crimper. Samples were kept at −18°C until the analysis was conducted. Prior to analysis, frozen samples were held at 70°C for 20 min in an oven. Then, the gas sample in

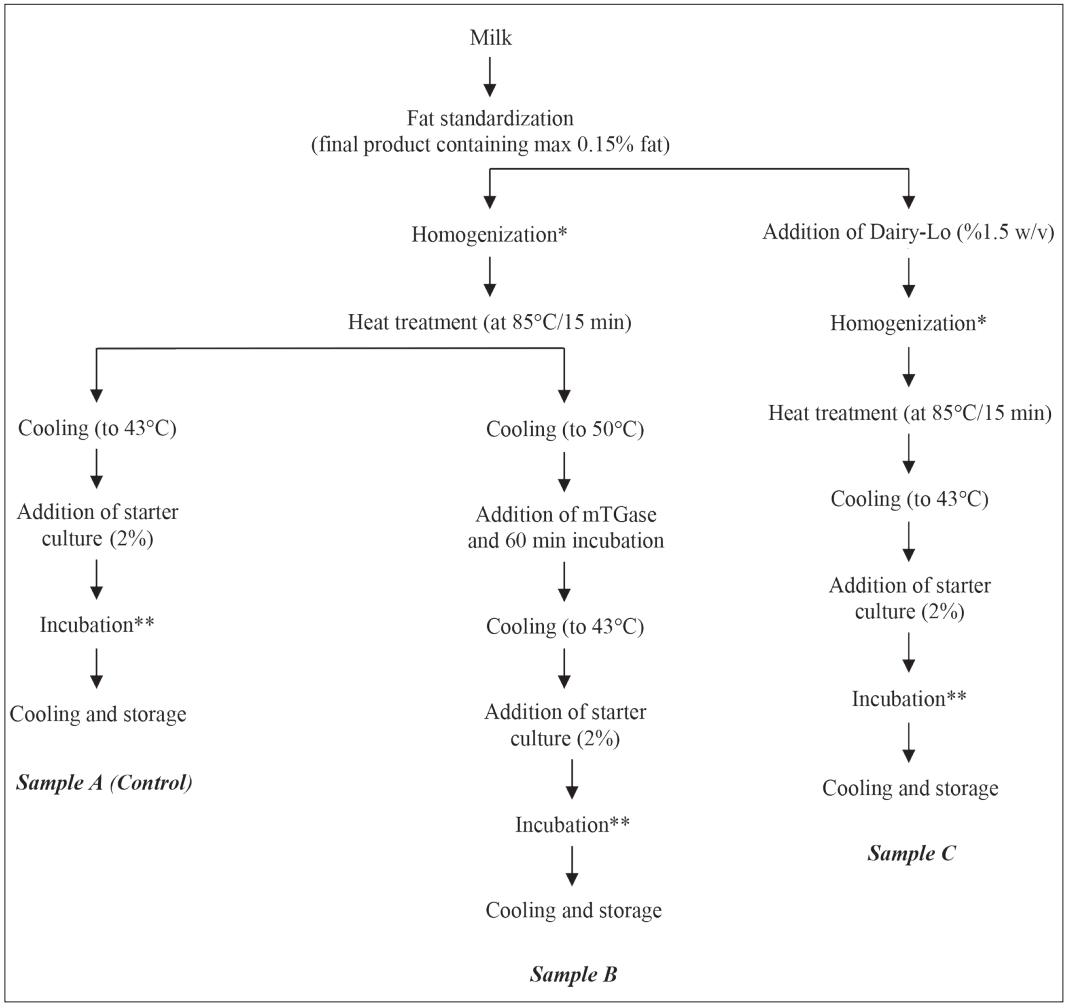

Fig 1. Flow diagram of yoghurt samples (*50°C 175 bar; ** at 43°C until pH 4.6)
Şekil 1. Yoğurt üretim şeması (*50°C 175 bar; ** 43°C'de pH 4.6'ya kadar

headspace of vial was injected with a gas-tight syringe (1.000 µL) to GC equipped with a FID detector and Innowax polyethylene glycol capillary column (30 m long, 320 µm in diameter, 0.25 µm film thickness) (Agilent Technologies Inc., CA, USA). Operation conditions: temperature; injection block 80°C and FID 250°C; flow rates (mL min^{-1}); make-up gas (nitrogen) 30 mL/min, hydrogen 40 mL/min, air 400 mL/min and carrier gas (nitrogen) 0.7 mL/min. The programme of oven temperature was as follows: raised to 50°C for 0.5 min followed by increasing to 60°C at a rate of 4°C/min then kept for 0.5 min, increased to 70°C at a rate of 4°C/min and kept for 0.5 min, then increased to 180°C at a rate of 20°C/min and kept for 0.2 min.

Concentrations of the standard mix solutions for each carbonyl compound were 25, 50, 75 and 100 ppm. Calibration curves were prepared by plotting the peak area against the mass of each carbonyl compound.

Sensory Analysis

Sensory analysis of the yoghurt samples was performed by 10 experienced panelists using a 0-5 point scale for appearance, consistency, odor and taste on 1, 10 and 20 days of analysis [31]. The yoghurt samples in 200 g plastic cups which were coded with three digit numbers were tempered to 10°C before serving to assessors.

Determination of Microstructural Properties of Yoghurts

Microstructure of the yoghurt samples was determined by scanning electron microscope. Samples were prepared by fixing on stapes by using carbon coated bands according to the method proposed by Skriver et al.[32] and Hayat [33]. Samples were then coated with pure gold using Polaron SC 502 sputter coater (Quo-rum Technologies, New Haven, UK) and examined with a Jeol JSM 6060 LV scaning electron microscope (*Jeol* Ltd, Tokyo, Japan).

Statistical Analyses

Data were analyzed using Minitab 13.0 statistical software (Minitab INC., PA, USA). The comparison of differences between the samples were determined by one-way analysis of variance (ANOVA) at $P<0.01$ [34].

RESULTS

The titratable acidity (SH) and pH values of the yoghurts are shown in *Table 1*. Sample supplemented with Dairy-Lo had significantly higher acidity level than the other samples (P<0.01).

The viscosity and penetrometer values (consistency) of the yoghurt samples are given *Table 1*. The sample treated with mTGase had significantly higher viscosity and consistency values than the other samples (P<0.01). These results can be attributed to the formation of cross-linking of milk proteins induced by mTGase which leads to the decrease in gel permeability, resulting in more stable and firm structure [17,25,26]. However, viscosity and consistency of the yoghurt sample added with Dairy-Lo was similar to the control yoghurt.

One of the factors affecting the acceptance of yoghurt by consumer is serum separation [37]. A gel formed with ϵ-(γ-glutamyl)lysine bonds improves water holding capacity of set type of yoghurt made from milk treated with mTGase, which results in reduction in serum separation [21]. These results confirmed that serum separation of the yoghurt sample treated with mTGase was significantly (P<0.01) reduced compared with the control sample. However, the lowest level of serum separation was determined in the sample added with fat replacer.

Scanning electron micrographs (SEM) (x2.500 and x 5.000) of yoghurt gels are shown in *Fig. 2*. Microstructure of the non-fat yoghurts consisted of a protein network composed by chain and aggregates of fused casein micelles, where the streptococci and lactobacilli are easily distinguished *(Fig. 2-A2)*. The protein network of the control sample *(Fig. 2-A2)* was less dense and more open as a consequence of smaller fused casein micelles aggregate, and probably absence of fat globules. Besides, SEM related to the protein matrices of the sample treated with mTGase (B1 and B2) was relatively more compact than the control sample *(Fig. 2 A1* and *A2)*. This result was in an agreement with the results obtained from Lorenzen et al.[17], Faergemand and Qvist [18], Şanlı et al.[25].

Sensory evaluation results of yoghurts are given in *Table 2*. Significant differences were observed in consistency and appearance of the yoghurt samples during storage period. Consistency scores were the highest in the

Table 1. *Physical and chemical characteristics of non-fat yoghurt samples during storage period*[1]
Tablo 1. *Depolama sürecinde yağsız yogurt örneklerinin fiziksel ve kimyasal karakteristikleri*[1]

Parameters	Days	A	B	C
Titratable acidity (SH)	1	35.03±1.51^{c}	39.03±1.51^{b}	45.05±2.39^{a}
	10	38.14±1.89^{b}	40.00±1.01^{b}	52.36±1.01^{a}
	20	42.05±2.39^{b}	43.02±0.2^{b}	52.09±0.00^{a}
pH	1	4.37±0.00^{a}	4.11±0.07^{b}	4.06±0.04^{b}
	10	4.15±0.08^{a}	3.89±0.03^{b}	3.85±0.04^{b}
	20	4.03±0.08	3.88±0.00	3.85±0.01
Tyrosine (g/5 g)	1	0.51±0.17^{b}	0.46±0.08^{c}	0.57±0.00^{a}
	10	0.60±0.01^{b}	0.53±0.00^{c}	0.61±0.01^{a}
	20	0.71±0.06	0.70±0.08	0.74±0.01
Penetrometer value (mm/s)	1	46.05±0.42^{a}	33.75±0.63^{b}	45.15±1.20^{a}
	10	46.30±0.56^{a}	30.25±1.34^{b}	45.95±0.28^{a}
	20	40.05±0.21^{b}	27.80±1.27^{c}	44.58±1.94^{a}
Whey separation (mL/25 g)	1	9.12±0.17^{a}	7.25±0.00^{b}	4.00±0.00^{c}
	10	8.00±0.35^{a}	5.12±0.17^{b}	2.75±0.35^{c}
	20	7.75±0.35^{a}	5.62±0.53^{b}	3.25±0.35^{c}
Viscosity (Pas)	1	7.75±3.54^{b}	19.75±3.54^{a}	8.00±0.00^{b}
	10	11.00±0.00^{b}	27.00±1.41^{a}	12.00±1.41^{b}
	20	11.50±7.00^{b}	28.50±7.04^{a}	13.50±7.07
Acetaldehyde (ppm)	1	82.11±2.90	84±42±6.97	86.54±1.08
	10	55.71±3.37^{b}	61.10±1.27^{b}	72.56±1.99^{a}
	20	55.04±0.96	64.35±4.70	65.75±6.51
Diacethly (ppm)	1	18.96±1.65	18.59±0.29	16.30±0.50
	10	19.81±2.13	19.63±1.63	20.45±1.46
	20	18.95±3.44	20.45±1.46	18.88±4.31
Acetone (ppm)	1	4.31±0.08^{a}	4.58±0.13^{a}	6.44±0.13^{b}
	10	4.62±0.17^{a}	4.00±0.20^{a}	6.40±0.83^{b}
	20	4.93±0.29^{a}	5.38±0.004^{a}	7.8±0.18^{b}

[1] *Presented values are the means (±SD) of two replicates*
$^{a\text{-}b\text{-}c}$ *Different letters in the same line indicate significantly different means at P<0.01*
A: *Control,* ***B:*** *Pasteurized milk was incubated with TGase at 50°C for 1 h,* ***C:*** *Prepared from milk added 1.5% of Dairy Lo before homogenization*

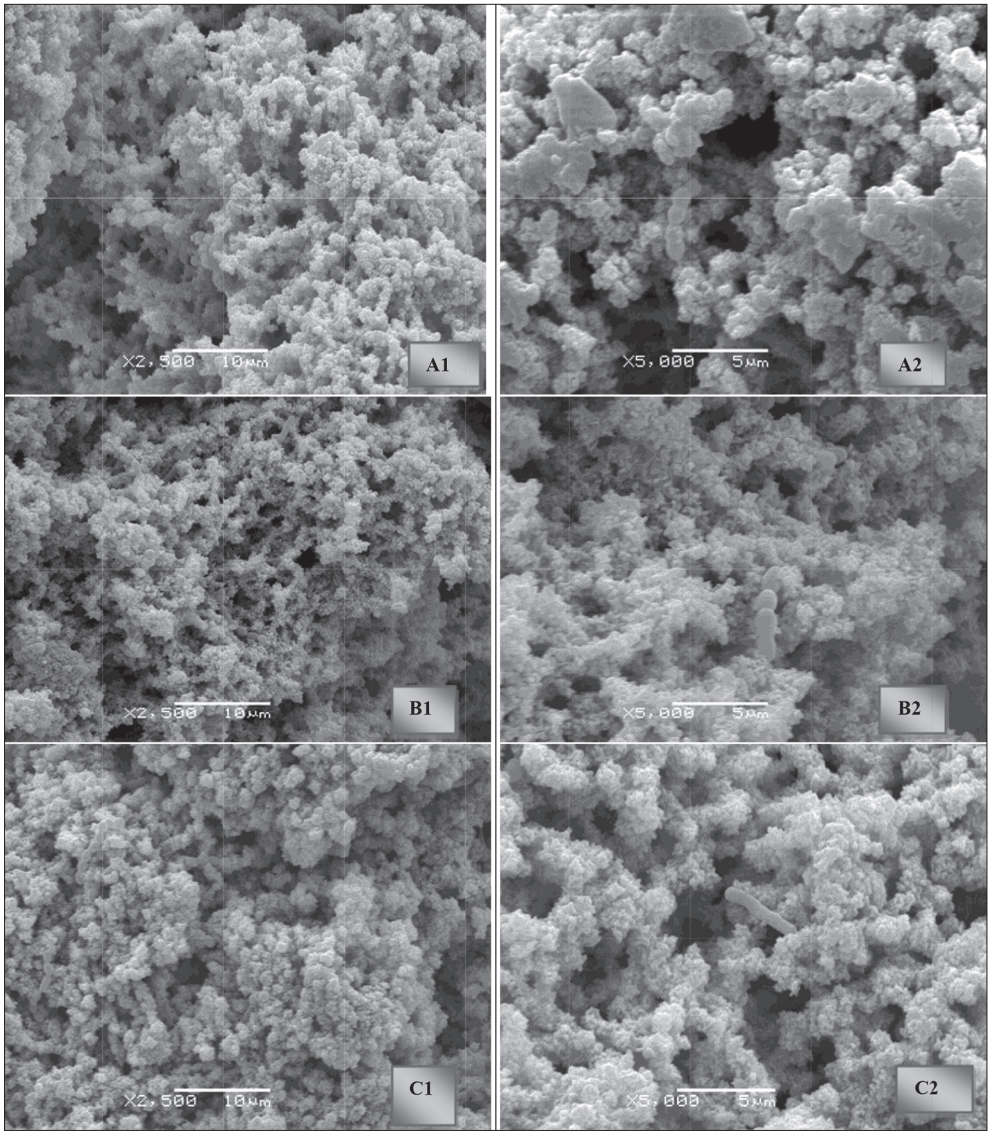

Fig 2. SEM micrographs of yoghurts. A1-B1-C1: Magnification is x2500, scale bar 10 µm. A2-B2-C2: Magnification is x5000, scale bar 5 µm. **A-** Control, **B-** Pasteurized milk was incubated with TGase at 50°C for 1 h, **C-** Prepared from milk added 1.5% of Dairy Lo before homogenization

Şekil 2. Yoğurtların SEM mikrofotoğrafları. A1-B1-C1: Büyütme x2500, ölçek çubuğu 10 µm. A2-B2-C2: Büyütme x5000, ölçek çubuğu 5 µm. **A-** Kontrol, **B-** Pastörize süt TGase ile 50°C'de 1 saat inkübe edilmiştir, **C-** Homojenizasyondan önce süte %1.5 Dairy Lo ilave edilmiştir

Table 2. *Sensory properties of non-fat yoghurt samples during storage period*[1]
Tablo 2. *Depolama sürecinde yağsız yogurt örneklerinin duyusal özellikleri*[1]

Variables (Scores)	Days	A	B	C
Appearance	1	4.50±0.14[a]	4.50±0.14[a]	2.80±0.14[b]
	10	4.10±0.14[a]	4.15±0.07[a]	2.55±0.07[b]
	20	3.70±0.00[b]	4.10±0.00[a]	3.00±0.00[c]
Consistency	1	3.05±0.07[b]	4.15±0.07[a]	2.85±0.07[c]
	10	2.90±0.00[b]	4.45±0.63[a]	2.40±0.14[b]
	20	2.70±0.00[b]	4.35±0.07[a]	2.60±0.00[b]
Odour	1	3.85±0.07	4.10±0.00	4.05±0.07
	10	3.95±0.07[a]	3.75±0.07[a]	2.95±0.07[b]
	20	4.05±0.00[a]	4.00±0.07[a]	3.60±0.00[b]
Taste	1	265±0.07[a]	2.80±0.00[a]	2.00±0.00[b]
	10	2.55±0.07[a]	2.20±0.00[b]	1.75±0.07[c]
	20	2.35±0.07[a]	2.40±0.00[a]	1.85±0.00[b]

[1] *Presented values are the means (±SD) of two replicates*
[a-b-c] *Different letters in the same line indicate significantly different means at P<0.01*
A: *Control,* ***B:*** *Pasteurized milk was incubated with TGase at 50°C for 1 h,* ***C:*** *Prepared from milk added 1.5% of Dairy Lo before homogenization*

sample B (P<0.01), while the samples A (control) and C received similar scores for consistency. These results were consistent with the instrumental analysis results. Panelists could not detect any difference in odors of the samples at the beginning of storage time; however, the sample C was found different from the other samples in terms of odors at days 10 and 20 (P<0.01).

The variations in the acetaldehyde, acetone and diacetyl levels of the yoghurt samples during storage period are presented in *Table 1*. The acetaldehyde levels of the yoghurt samples at 10 d of storage were found significantly different (P<0.01). The highest acetaldehyde level was detected at the sample C added with Dairy-Lo. This may be associated with the increase in protein content of yoghurt which acts as a precursor compound for the formation of acetaldehyde in yoghurt [8]. However, there was no significant difference between the sample B and the control sample regarding the level of acetaldehyde.

DISCUSSION

The acidity of the yoghurt increased with addition of Dairy-Lo; however, the presence of mTGase in yoghurt did not affect the acidity. Also, Lorenzen and Schlimme [22] did not detect any significant difference among the yoghurts with and without mTGase in regard to acidity during the storage period of 14 days. Moreover, the titratable acidity and pH values of the sample treated with mTGase was higher than the control sample (P<0.01) at 1st day of storage. On the contrary, some authors reported that the treatment with mTGase caused slower production of acidity in yoghurt [17,26]. In addition, the mTGase application did not cause any delay in fermentation period of non-fat yoghurt production. The incubation of the yoghurt samples was terminated when the pH reached to 4.6. Fermentation time were 225 and 215 min in the samples A (control) and sample B (containing mTGase), respectively. Similar results were reported by Schey [35] that there is no interaction between mTGase and starter bacteria through the fermentation of yoghurt. The acidities of all samples increased throughout storage.

Tyrosine is an indicator of the level of proteolysis. Tyrosine value of the sample treated with mTGase was found to be the lowest during storage. These findings indicate that cross-linking of proteins catalyzed by the enzyme results in proteins become more stable against to proteolysis. The level of tyrosine increased during storage as a consequence of the proteolytic activity of yoghurt starter culture. However, the increment in the tyrosine content of the sample treated with TGase was slightly slower than the other samples. Yüksel and Erdem [36] reported that mTGase active yoghurt samples had lower peptide content and tyrosine values than those without mTGase and mTGase inactive samples.

The viscosity and consistency values of all samples increased during the storage time and the highest levels were observed at 20 day of storage. These increases during storage period could be as a result of protein rearrangement and protein-protein interactions [26]. However, the remarkable increase was determined in the sample treated with mTGase. This result could indicate that activity of enzyme continued after fermentation. Similar findings were reported by Özer et al.[26].

Scanning electron microscopy images confirmed that gel strength of yoghurt made from milk treated with mTGase was higher due to a more regular distribution of protein network with smaller pores, leading to less serum separation during storage [18]. Also, some researchers reported that decrease in gel porosity resulted in the decrease in yoghurt whey expulsion because of the fact that the cross-linking of protein chains can stabilize the three dimensional network of yoghurt gel [18,26]. Scanning electron micrographs of yoghurt made with Dairy-Lo showed that the addition of protein based-fat replacer caused to differences in arrangement of the gel network. It can be explained that protein based-fat replacer integrated into the aggregates and as a result of these interactions between denatured whey proteins and the surface casein micelles were prevented in milk. Thus, the microstructure of the yoghurt including Dairy-Lo was coarser and fluffier *(Fig. 2 C1-C2)*.

The mTGase treatment did not have a negative effect on aroma and flavour. Panelists reported that the flavour of the yoghurt treated with mTGase (sample B) was the same as the flavour of the control sample. However, Dairy-Lo had a negative impact on the taste. Sample C added with Dairy-Lo was perceived lower taste scores by the panelists than the other samples (A and B) (P<0.01).The difference in the appearance of the yoghurt samples was found to be significant (P<0.01). Dairy-Lo added sample had the lowest appearance score than the other samples.

There is a general agreement in literature that the aroma and flavour of yoghurt consist mainly of non-volatile and volatile acids and carbonyl compounds. One of the major flavour carbonyl compounds in yoghurt is acetaldehyde [37]. Acetaldehyde concentration in all yoghurt samples were declined with storage time. This decrease in the level of acetaldehyde could arise from the alcohol dehydrogenize activity of yoghurt starters. This enzyme transforms acetaldehyde to ethyl alcohol during storage [37]. No significant difference was observed among the yoghurt samples regarding diacetyl contents *(Table 1)*. In addition, the presence of mTGase in the yoghurt did not have any effect on the acetone levels. However, the acetone level of the sample C was higher than the other samples (sample A and sample B).

The results indicated that mTGase enzyme may be useful for production of non-fat yoghurt without adversely

affecting the sensory properties of the end product. The rheological data and SEM indicate that mTGase had significant effects on the protein microstructure of non-fat yoghurt. This study also showed that the use of Dairy-Lo could negatively affect texture development and sensory properties of non-fat set yoghurts.

REFERENCES

1. Sandoval-Castilla O, Lobato-Calleros C, Aguirre-Mandujano E, Vernon-Carter RJ: Microstructure and texture of yogurt as influence by fat replacers. *Int Dairy J*, 14, 51-159, 2004. DOI: 10.1016/S0958-6946(03)00166-3

2. Folkenberg DM, Martens M: Sensory properties of low fat yogurts. Part A: Effect of fat content, fermentation culture and addition of non-fat dry milk on the sensory properties of plain yogurts. *Milchwissenschaft*, 58, 48-51, 2003.

3. Duboc P, Mollet B: Applications of exopolysaccharides in the dairy industry. *Int Dairy J*, 11, 759-768, 2001. DOI: 10.1016/S0958-6946(01)00119-4

4. Cayot P, Schenker F, Houze G, Sulmont-Rosse C, Colas B: Creaminess in relation to consistency and particle size in stirred fat-free yogurt. *Int Dairy J*, 18, 303-311, 2008. DOI: 10.1016/j.idairyj.2007.06.009

5. Barrantes E, Tammime AY, Davies G, Barclay MN: Production of low-calorie yogurt using skim milk powder and fat-substitutes 2: Compositional quality. *Milchwissenchaft*, 49 (3):135-139, 1994.

6. Barrantes E, Tamime AY, Sword AM: Production of low-calorie yogurt using skim milk powder and fat-substitutes 4: Rheological properties. *Milchwissenchaft*, 49 (5): 263-265, 1994.

7. Huygbaert A, Dewettinck K, Greyt W: Fat replacers. *IDF*, 317, 10-15, 1994.

8. Kök-Taş T, Güzel-Seydim Z: Determination of effects of using fat replacers andprobiotic on Ayran quality. *J Food*, 35 (2):105-11, 2010.

9. Anema SG, Lauber S, Lee SK, Henle T, Klostermeyer H: Rheological properties of acid gels prepared from pressure and transglutaminase-treated skim milk. *Food Hydrocolloids*, 19, 879-887, 2005. DOI: 10.1016/j.foodhyd.2004.12.001

10. Gauche C, Tomazi T, Barreto PLM, Ogliari PJ, Bordignon-Luiz MT: Physical properties of yoghurt manufactured with milk whey and transglutaminase. *LWT-Food Sci Technol*, 42, 239-243, 2009. DOI: 10.1016/j.lwt.2008.05.023

11. Jaros D, Jacob M, Otto C, Rohm H: Excessive cross-linking of caseins by microbial transglutaminase and its impact on physical properties of acidified milk gels. *Int Dairy J*, 20, 321-327, 2010. DOI: 10.1016/j.idairyj.2009.11.021

12. Dickinson E, Yamamoto Y: Rheology of milk protein gels and protein-stabilized emulsion gels cross-linked with transglutaminase. *J Agric Food Chem*, 44, 1371-1377, 1996. DOI: 10.1021/jf950705y

13. Jong GAH, Koppelman SJ: Tranglutaminase catalyzed reactions: Impact on food applications. *J Food Sci*, 67, 279-280, 2002. DOI: 10.1111/j.1365-2621.2002.tb08819.x

14. Bonisch MP, Huss M, Weitl K, Kulozik U: Transglutaminase cross-linking of milk proteins and impact on yoghurt gel properties. *Int Dairy J*, 17, 1360-1371, 2007. DOI: 10.1016/j.idairyj.2007.01.019

15. Motoki M, Seguro K: Transglutaminase and its use for food processing. *Food Sci Technol*, 9, 204-210, 1998. DOI: 10.1016/S0924-2244(98)00038-7

16. O'Sullivan MM, Kelly AL, Fox PF: Influence of transglutaminase treatment on some physicochemical properties of milk. *J Dairy Res*, 69, 433-442, 2002. DOI: 10.1017/S0022029902005617

17. Lorenzen PC, Neve H, Mautner A, Schlimme E: Effect of enzymatic cross-linking of milk proteins on functional properties of set-style yoghurt. *Int J Dairy Technol*, 55, 152-157, 2002. DOI: 10.1046/j.1471-0307.2002.00065.x

18. Faergemand M, Qvist KB: Transglutaminase: Effect on rheological properties, microstructure and permeability of set style acid skim milk gel. *Food Hydrocolloids*, 11, 287-292, 1997. DOI: 10.1016/S0268-005X(97)80058-6

19. Lauber S, Henle T, Klostermeyer H: Relationship between the crosslinking of caseins by transglutaminase and the gel strength of yoghurt. *Eur Food Res Technol*, 210, 305-309, 2000. DOI: 10.1007/s002170050554

20. Lorenzen PC: Techno-functional properties of transglutaminase-treated milk proteins. *Milchwissenschaft*, 55, 667-670, 2000.

21. Kuraishi C, Katsutoshi Y, Susa Y: Transglutaminase: Its utilization in the food industry. *Food Rev Int*, 17, 221-246, 2001. DOI: 10.1081/FRI-100001258

22. Lorenzen PC, Schlimme E: Properties and potential fields of application of transglutaminase preparations in dairying, Bulletin 332, 47-53, International Dairy Federation, Brussels, 1998.

23. Faergamand M, Sorensen M, Jorgensen U, Budolfsen G, Qvist KB: Transglutaminase: effect on instrumental and sensory texture of set style yoghurt. *Milchwissenschaft*, 54 (10): 563-566, 1999.

24. Öner Z, Karahan AG, Aydemir S, Sanlıdere-Aloglu H: Effect of transglutaminase on physicochemical properties set-style yogurt. *Int J Food Prop*, 11, 196-205, 2008. DOI: 10.1080/10942910701286346

25. Şanlı T, Sezgin E, Deveci O, Şenel E, Benli M: Effect of using transglutaminase on physical, chemical and sensory properties of set-type yoghurt. *Food Hydrocolloids*, 25, 1477-1481, 2011. DOI: 10.1016/j.foodhyd.2010.09.028

26. Ozer B, Kirmaci HA, Oztekin S, Hayaloglu A, Atamer M: Incorporation of microbial transglutaminase into non-fat yogurt production. *Int Dairy J*, 17, 199-207, 2007. DOI: 10.1016/j.idairyj.2006.02.007

27. Hooi R, Barbano DM, Bradley RL, Budde D, Bulthaus M, Chettiar M: Chemical and physical methods. **In,** Wehr HM, Frank JF (Eds): Standard Methods for the Examination of Dairy Products. 17th ed., 363-532, DC American Public Health Association, Washington, 2004.

28. Hull ME: Studies on milk proteins - II. Colorimetric determination of the partial hydrolysis of the proteins in milk. *J Dairy Sci*, 30, 881-884, 1947. DOI: 10.3168/jds.S0022-0302(47)92412-0

29. Atamer M, Sezgin E: The effect of increasing dry matter content on physical properties of yoghurts. *J Food*, 11, 327-331, 1986.

30. Ulbert F: Headspace gas chromatographic estimation of some yoghurt volatiles. *J AOAC Int*, 74, 630-634, 1991.

31. Bodyfelt FW: The Sensory Evulation of Dairy Products. 2nd ed., 227-299, An AVI Book, New York, 1988.

32. Skriver A, Buchheim W, Qvist KB: Electron microscopy of stirred yoghurt: Ability of three techniques to visualize exopolysaccharides prom ropy strains. *Milchwissenschaft*, 50, 683-686, 1995.

33. Hayat MA: Rinsing, dehydration and embedding. **In,** Hayat MA (Ed): Principles and Techniques of Electron Microscopy: Biological Applications. 4th ed., 85-93, Cambridge University Press, Cambridge, 2000.

34. Rosner B: Fundamentals of Biostatistics. 6th ed., Thomson Higher Education, USA, 2006.

35. Schey A: Texture improvement of fermented dairy products by enzymatic crosslinking with transglutaminase, Bulletin 301, 370-375, International Dairy Federation, Brussels, 2003.

36. Yüksel Z, Erdem YK: The influence of transglutaminase treatment on functional properties of set yoghurt. *Int J Dairy Technol*, 63, 86-97, 2010. DOI: 10.1111/j.1471-0307.2009.00539.x

37. Tamime AY, Robinson RK: Background to manufacturing practice. **In,** Tamime AY, Robinson RK (Eds): Yoghurt Science and Technology. 11-108, Woodhead Publishing Limited, Cambridge, 1999.

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Short Communication

Detection of Metals in Different Honey Brands

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Abstract

In this study, cadmium (Cd), lead (Pb), iron (Fe), zinc (Zn), aluminium (Al), mercury (Hg) and copper (Cu) levels determined in a total of 100 honey samples belonging to 15 different companies, by atomic absorption spectrometry. The mean values were found Cd 0.343, Pb 1.101, Fe 41.13, Zn 6.76, Al 1.490, Hg 0.618, Cu 0.06 mg/kg. These results showed that the metal levels were known to be within the acceptable limits so honey is safe for metals which are harmful for human health. This data also indicates that, honey production is made in accordance with the regulations and it's under control.

Keywords: *Honey, Metal, Atomic absorption spectrometry, Environmental contamination*

Farklı Marka Ballarda Metal Seviyelerinin Tespiti

Özet

Bu çalışmada, 15 farklı firmaya ait 100 adet bal numunesindeki kadmiyum (Cd), kurşun (Pb), demir (Fe), çinko (Zn), alüminyum (Al), civa (Hg) ve bakır (Cu) seviyeleri atomik absorbsiyon spekrometresi ile araştırılmış ve metallerin ortalama değerleri Cd 0.343, Pb 1.101, Fe 41.13, Zn 6.76, Al 1.490, Hg 0.618, Cu 0.06 mg/kg olacak şekilde saptanmıştır. Bu sonuçlar ballardaki metal miktarların kabul edilebilir seviyelerde olduğunu ve ülkemizde bal üretiminin yasal düzenlemelere uygun yapıldığını ve kontrol altında olduğunu da göstermektedir.

Anahtar sözcükler: *Bal, Metal, Atomik absorbsiyon spektrometresi, Çevresel kontaminasyon*

INTRODUCTION

Honey is a sweet, viscous substance mainly produced by honey bees *(Apis mellifera)* from nectar of different plant flowers and secretion of plants or plant-sucking insects [1]. In honey the main nutrition component is the carbohydrates, fructose and glucose which make it, an excellent energy source. Honey contains also a number of other constituents in small and trace amounts, producing numerous nutritional and biological effects: antimicrobial, antioxidant, antiviral, antiparasitic, antiinflammatory, antimutagenic, anticancer and immunosuppressive activities [2]. An important aspect of honey quality is the presence of contaminants due to environmental contamination or pharmacological (antiparasitical or acaricidal) residues [3]. One of the most concerned contaminant in honey is metals. Major metals are primarily derived from soil and nectar-producing plants [4]. Environmental pollution or other anthropogenic sources of metals in honey are also important for accumulation. Honey can also be contaminated with some transition metals during its processing through the equipment and tools used by the beekeepers long with the processing environment [5]. After prolonged evaluation studies on food additives and their toxicity, the World Health Organization (WHO) has concluded that even low levels of some metals, such as Pb and Cd, can cause several diseases in humans [6]. The objective of this study was to determine the levels of some metals, Cd, Pb, Fe, Zn, Al, Hg and Cu, which are considered among the most dangerous to human, in honeys from Turkey.

MATERIAL and METHODS

Samples

A total of 100 honey samples belonging to 15 different

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companies were analyzed as an indicator of environmental contamination. 250 g of each sample was placed in a plastic bottle and kept in a dark, dry and clean place until the analysis.

Sample Preparation

Approximately 0.5 g honey was placed in a polytetrafluoroethylene (PTFE) vessel and 10 ml of concentrated nitric acid (HNO_3) was added. The digestion was carried out with the following digestion program: 500 W 15 min up to 210°C; then 1050 W 15 min at 210°C. After cooling, the vessels were opened and 2 mL H_2O_2 was added to the digest and the same temperature program was repeated. The digests were made up to 25 mL using ultrapure water. Process blanks were also prepared in a similar manner.

Apparatus

Analytical grade reagents were used throughout the analysis. Metal concentrations in the honey were determined by using a continuum source graphite furnace atomic absorption spectrometer (CS-GFAAS) Model Contra AA 700 equipped with a transversely heated graphite tube atomizer and a MPE 60 autosampler (Analytik Jena AG, Jena, Germany). A xenon short arc lamp in hot-spot mode operated at 300 W was used as a continuum radiation source. A high-resolution double monochromator, consisting of a prism as pre-monochromator and an echelle grating monochromator, providing a spectral bandwidth *per* pixel of *ca.* 2 pm at 200 nm, was used to promote spectral dispersion of the continuum radiation and a linear charge coupled device array detector with 588 pixels for the detection of the radiation. Argon with a purity of 99.99% was used as the purge gas with a flow rate of 2 L min^{-1} in all stages, except during atomization step. The samples were digested in high pressure 1500 Teflon vessels using a MARS (CEM, Matthews, NC, USA) microwave digester equipped with temperature and pressure sensors. In order to obtain maximum absorbance and minimum background values, the operational parameters were optimized [7,8].

RESULTS

Calibration curves showed good linearity over the entire range of concentrations with acceptable coefficients (r^2 >0.9). Validation data (n ≥5) obtained for the selected metals in honey were shown in *Table 1*. The attained LODs are sufficiently low for honey safety monitoring purposes considering the maximum metal levels established in honey by the European Commission Regulation. The accuracy of the method was checked by spike recovery experiments. All measurements were performed, at least, in triplicate. The recovery values obtained by spiking the honey samples with Cd, Pb, Cu, Fe, Zn, Al and Hg standards, ranged between 92% and 101%. Analytical blanks and standards were analyzed daily and regularly along with samples to check instrument performance.

Table 1. *Validation data (n≥ 5) obtained for the select/ed metals in honey*
Tablo 1. *Ballarda, belirlenen metallerin validasyon verileri (n≥ 5)*

Metal	Linear Range (µg/L)	Correlation Coefficient (r^2)	Limit of Detection (LOD) (µg/kg)	Limit of Quantification (LOQ) (µg/kg)	Recovery (%)	Relative Standard Deviation (RSD)%, n=3
Cd	0.25-1.25	0.9975	0.03	0.09	94	4
Pb	3.0-30.0	0.9878	1.3	3.67	99	3
Fe	0.2-06	0.9992	0.9	2.3	98	5
Zn	0.10-0.50	0.9583	0.05	0.17	101	8
Al	15-30	0.9694	7.0	22.1	97	9
Hg	1.0-5.0	0.9999	0.4	1.3	92	5
Cu	0.1-0.5	0.9957	1.2	3.36	98	6

Table 2. *Metal concentrations (mean and range; (mg/kg, wet weight) in honey*
Tablo 2. *Ballardaki metal konsantrasyonları sonuçları (ortalama, maks. ve min.,mg/kg, yaş ağırlık)*

Metal	Metal Concentration			SD (Standard Deviation)	Standard Error Mean (SEM)
	Mean	Minimum	Maximum		
Cd	0.343	0.216	1.553	0.205	0.021
Pb	1.101	0.699	12.300	1.277	0.128
Fe	41.13	13.45	97.30	22.87	2.29
Zn	6.76	3.82	17.96	3.88	0.39
Al	1.490	0.038	4.570	0.648	0.065
Hg	0.618	0.250	0.852	0.288	0.029
Cu	0.06	0.011	0.098	0.028	0.003

Elemental concentrations were determined on wet weight bases. Mean, minimum and maximum value of each heavy metal for the honey samples are reported in *Table 2*. As can be seen from these data most abundant elements in our honey samples are Fe and Zn with the mean values 41.13 mg kg and 6.79 mg kg, respectively. Since the average honey consumption in Turkey is 1.1 kg per year; consumed heavy metal contaminants were still found to be below the risk.

DISCUSSION

In Turkey there is no specific legislation on honey's metal contents. In Turkish Food Codex Regulation on Contaminants in Foodstuffs, only maximum limits for Pb, Cd and Hg exists for some foods including milk, meat, fish and seafoods, cereals, vegetables, fruit and fruit juices and food supplements. Maximum Pb, Cd and Hg levels are identified between 0.020 and 3 mg kg^{-1}, 0.050 and 3 mg kg^{-1} and 0.10 and 0.50 mg kg^{-1} respectively, for different foods. In the current study, the levels were found to be below the limits set for other foodstuff as mentioned [9]. The Joint FAO-World Health Organization Expert Committee on Food Additives (JECFA) established a provisional tolerable weekly intake (PTWI) for Pb as 0.025 mg kg^{-1} body weight (bw), as 0.007 mg kg^{-1} bw for Cd and for Hg as 1.6 µg kg^{-1} body weight week. When the mean consumption of honey in a diet was taken in account, our results showed that the level of these metals are tolerable [10]. Generally our results were found to be compatible with those reported from Turkey. Yılmaz and Yavuz [11] detected the contents of Na, K, Ca, Mg, Cu, Fe, Mn, Zn and Co in 30 samples of honey collected from different parts of south-eastern Anatolia, Turkey by atomic absorption spectrometer. The mean values for Cu, Fe and Zn were 1.8, 6.6, and 2.7 mg kg^{-1}, respectively. Tuzen et al.[12] reported that it was noticable that the honeys from the Marmara region (West Anatolia) showed high levels of Cu, Mn, Zn, Ni, Se and Fe contamination. The reason might be that the industry has been well developed in this area and possibly apiaries are located at a distance not far from the polluted habitat. In contrast, honeys from East Anatolia showed lower contents of Cu, Mn, Zn, Fe and Pb than the other honeys, due to the fact that this region does not have industrially polluted apiaries. Bilandzic et al.[13] reported that the levels of Pb determined in multifloral honey in Croatia were generally higher than concentrations obtained from other geographical origins in Europe. It was found that Pb levels in multifloral honeys in 2010 and 2011 were higher (189 µg kg^{-1} and 360 µg kg^{-1}) than previously determined values in the same region of Croatia. These high concentrations of Pb in multifloral honey samples in the central region may be related to the fact that this region has become more urban and that the network of motorways is growing every year. Perna et al.[14] determined the Cd, Pb, Cr, and As levels in nine areas of southern Italy. Pb (0.289 mg kg^{-1}), Cd (0.013 mg kg^{-1}) and Cr (0.707 mg kg^{-1}) levels in honeys from studied areas were influenced by local environmental conditions and results were known to be within the acceptable limits and lower than our findings. Only in one area Pb level was detected highly (1.390 mg kg), this linked to the petroleum extraction-related and touristic activities, high movement of vehicles, especially in the spring-summer period. In Saghaei et al.[15] study, metal levels detected from the honey collected from four different areas of Orumieh City, in Iran. The results indicated that Zn had highest concentration followed by Cr, Fe, Mn, Pb, Ni, Co and As.

In this study, Cd, Pb, Fe, Zn, Al, Hg and Cu levels were determined in a total of 100 honey samples collected from 15 different companies. Results showed that the metal levels were known to be within the acceptable limits; however there is no specific legislation on honey's heavy metal contents. To define the acceptable levels or criteria related to chemicals, potential enhanced exposures like honey must be take into consideration. These results indicate that, honey production is made in accordance with the regulations and it's under control. In Turkey, honey production areas are generally far from the industrial areas and the flora and soil are suitable for high quality honey production so as seen in our results, honey is safe for metals which are harmful for human health.

REFERENCES

1. Belitz HD, Grosch W, Schieberle P: Food Chemistry. Springer, Berlin, 2009.

2. Bogdanov S, Jurendic T, Sieber R, Gallmann P: Honey for nutrition and health: A review. *J Am Coll Nutr,* 27, 677-689, 2008. DOI: 10.1080/07315724.2008.10719745

3. Przybylowski P, Wilczyn´ska A: Honey as an environmental marker. *Food Chem,* 74, 289-291, 2001. DOI: 10.1016/S0308-8146 (01)00153-4

4. Pohl P: Determination of metal content in honey by atomic absorption and emission spectrometries. *Tren Anal Chem,* 28, 117-128, 2009. DOI: 10.1016/j.trac.2008.09.015

5. Lambert O, Piroux M, Puyo S, Thorin C, Larhantec M, Delbacc F, Pouliquen H: Bees, honey and pollen as sentinels for lead environmental contamination. *Environ Pollut,* 170, 254-259, 2012. DOI: 10.1016/j.envpol.2012.07.012

6. Kurnaz E, Filazi A: Determination of metal levels in the muscle tissue and livers of chickens. *Fresen Environ Bull,* 20 (11): 2896-2901, 2011.

7. Meeravali NN, Madhavi K, Manjusha R, Kumar SJ: Sequential extraction of platinum, cisplatin and carboplatin from environmental samples and pre-concentration/separation using vesicular coacervative extraction and determination by continuum source ETAAS. *Talanta,* 118, 37-44, 2014. DOI: 10.1016/j.talanta.2013.09.045

8. Filazi A, Baskaya R, Kum C, Hismiogullari SE: Metal concentrations in tissues of the Black Sea fish Mugil auratus from Sinop-Icliman, Turkey. *Hum Exp Toxicol,* 22, 85-87, 2003. DOI: 10.1191/0960327103ht323oa

9. Anonymous: Turkish Food Codex Regulation on Contaminants in Foodstuffs. 29.12.2011-28157, 2011.

10. Morais S, Garcia C, Lourdes F, Pereira M: Heavy Metals and Human Health. **In,** Oosthuizen (Ed): Environmental Health-Emerging Issues and Practice. 227-246, Intech, Croatia, 2012.

11. Yılmaz H, Yavuz O: Content of some trace metals in honey from South-Eastern Anatolia. *Food Chem,* 65, 475-476, 1999. DOI: 10.1016/S0308-8146(98)00205-2

12. Tuzen M, Silici S, Mendil D, Soylak M: Trace element levels in honeys from different regions of Turkey. *Food Chem,* 103, 325-333, 2007. DOI: 10.1016/j.foodchem.2006.07.053

13. Bilandzic N, Sedak M, Đokic M, Kolanovic BS, Varenina I, Bozic D, Simic B, Konc˘urat A, Brstilo M: Lead content in multifloral honey from central croatia over a three-year period. *Bull Environ Contam Toxicol,* 88:985-989, 2012. DOI: 10.1007/s00128-012-0585-z

14. Perna A, Intaglietta I, Simonetti A, Gambacorta E: Metals in honeys from different areas of Southern Italy. *Bull Environ Contam Toxicol,* 92, 253-258, 2014. DOI: 10.1007/s00128-013-1177-2

15. Saghaei S, Ekici H, Demirbas M, Yarsan E, Tumer I: Determination of the metal contents of honey samples from Orumieh in Iran. *Kafkas Univ Vet Fak Derg*, 18, 281-284, 2012. DOI: 10.9775/kvfd.2011.5426

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Short Communication

Evaluation of Motility Hormones in Dairy Cattle with Omasal Impaction and Caecal Dilatation

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Abstract

The aim of this study was to evaluate the motility hormones levels in cattle with caecal dilatation (CD) and omasal impaction (OI). In this study, four cows with OI, four cows with CD (without volvulus) and ten healthy controls were used. Serum ghrelin, motilin, gastrin and leptin concentrations were determined using ELISA. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT) activities and sodium (Na), potassium (K) and chloride (Cl) concentrations were measured using a spectrophotometer. Serum ALT, AST and GGT activities were higher in OI cows. Serum Cl concentrations were lower in OI cows than in CD cows. Higher gastrin and motilin levels in OI and lower leptin levels in CD cows were found. In conclusion, this study dose not supports the use of GI motility hormones agonists in cows suffering from omasal impaction and caecal dilatation. Leptin might be indicated in negative energy balance.

Keywords: *Motility, Omasal impaction, Caecal dilatation, Leptin*

Sekum Dilatasyonlu ve Omazum Konstipasyonlu Sütçü Sığırlarda Motilite Hormonlarının Değerlendirilmesi

Özet

Bu çalışmanın amacı, omazum konstipasyonlarında (OK) ve sekum dilatasyonunda (SD) sığırlarda motilite hormon düzeylerini değerlendirmektir. Çalışmada 4 OK, 4 SD (volvulus olmaksızın) ve 10 sağlıklı kontrol sığır kullanıldı. Serum ghrelin, motilin, gastrin ve leptin konsantrasyonları ELISA kullanılarak belirlendi. Serum alanine aminotrasferaz (ALT), aspartate aminotrasferaz (AST), gama glutamil transferaz (GGT) aktiviteleri ve sodyum (Na), potasyum (K) ve klor (Cl) konsantrasyonları spektrofotometre kullanılarak ölçüldü. Serum ALT, AST ve GGT aktiviteleri OK'lı sığırlarda yüksekti. Serum Cl konsantrasyonu ise OK'lu sığırlarda CD'lu sığırlardan düşüktü. OK'lı sığırlarda yüksek gastrin ve motilin seviyesi ve SD'lı sığırlarda düşük leptin seviyesi tespit edildi. Sonuç olarak, bu çalışma omazum konstipasyonu ve sekum dilatasyonlu sığırlarda GI motilite hormone agonistlerinin kullanımını desteklememektedir. Leptin negative enerji dengesini belirleyebilir.

Anahtar sözcükler: *Motilite, Omazum konstipasyonu, Sekum dilatasyonu, Leptin*

INTRODUCTION

The motility hormones include ghrelin, motilin and gastrin. Ghrelin was discovered in 1999 in rat stomach and was observed to stimulate gastrointestinal motility in a manner similar to that of gastrointestinal peptides, such as motilin, cholesystokinin and gastrin. Leptin is a major regulator of hormones for food intake and energy homeostasis and acts in opposition to ghrelin [1]. Omasum constipation/impaction and caecal dilatation, which occurs in high producing dairy cows, are gastrointestinal tract diseases. Omasum is an important organ for water resorption. When the transition in the omasum is blocked, water resorption can be hindered resulting in omasal content drying out. Drying of the content of the omasum generates mechanical pressure in the omasal mucosa and causes ischemia, necrosis and, eventually, omasitis in the lamina of the omasum [2]. The large intestine is made up of the caecum and colon, which are sacculated organs; they do not have villi or papillae, and they store waste materials. The sac contents can accumulate and slow the passage of digesta. Microorganisms present in the large intestine ferment any remaining available nutrients in the digesta, including cellulose and hemicellulose. They produce volatile fatty acids (acetate, propionate and butyrate) and microbial proteins. The volatile fatty acids produced in the

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large intestine can be absorbed and used by the animal [3]. CD without volvulus is a much less common disease, but it is a common cause of gastrointestinal dysfunction [4].

The aim of this study was to evaluate changes in motility hormones levels in cows with caecal dilatation and omasal impaction compared with healthy cows.

MATERIAL and METHODS

A total of 18 Holstein-Friesian cows, 4 with omasal impaction, 4 with caecal dilatation without torsion and 10 clinically healthy adults, with no previous history of gastrointestinal disease (control group) were included in this study. The ages of the affected cows varied from 3 to 8 years (mean 5.5 years). They had been ill for an average of three days (2-10 days). The control animals were 2 to 5 years old dairy cows living in the same environmental conditions. The cows with CD were two months postpartum and had clinical signs of CD, including anorexia, decreased milk yield, scanty, pasty faeces and slight pain. The cows with OI were in their fourth pregnancy and had clinical signs of OI, including anorexia, scanty faeces or cessation of defecation, dullness, ruminal distension, moderate to severe dehydration in spite of polydipsia, and excess fluid in the forestomachs.

Clinical examinations were performed. The respiratory and pulse rates and body temperatures were recorded and were normal in all groups. Abdominal percussion and auscultation at the right rib cage, rectal examination and abdominal ultrasonography were conducted and used for the diagnosis of CD. OI was confirmed by clinical symptoms and feeding with dusty hay. In addition, the OI cattle were in their fourth pregnancy. The diagnosis of OI was confirmed by a response to medical therapy.

Blood samples were obtained from jugular venipuncture. The serum samples were separated by centrifugation at 1550 *g* for 10 min. The serum samples were measured by spectrophotometry according to the standard procedures using commercially available diagnostic kits (DiaSys Diagnostic Systems) for ALT, AST, GGT, Na, K and Cl analyses. Serum ghrelin (Phoenix-EK, 031/30), motilin (USCN-E, 90575), gastrin (Raybiotech-EIA-GAS-I) and leptin (DRG-EIA, 2395) concentrations were analysed using commercial ELISA test kits according to the manufacturer's instructions.

All data are presented as means ± SEM, and SPSS software (version 18) for Windows was used for the statistical analyses. The Shapiro-Wilk test was used for evaluating the normal distribution of the variables. All data were normally distributed. One-way analysis of variance (ANOVA) was performed in a completely randomised design for the normal distributed parameters (motilin, gastrin, ghrelin, leptin, AST, ALT, GGT, Na, Cl, and K). A Bonferroni test was used to separate these differences as well.

RESULTS

Rectal palpation revealed that the rectum was empty or contained some constipated faeces in all cows with OI. CD was diagnosed by rectal touching, ultrasonography *(Fig. 1)*, and clinical signs. In the simultaneous auscultation and percussion of the right paralumbar fossa, high-pitched resonant pings and splashing sounds were noticed over the last ribs. However, this sign is not specific for CD. All the OI and CD cows were afebrile with a normal pulse and respiratory rates; however, they did have a poor appetite, a sudden drop in milk yield, slight pain, little defecation and scanty, pasty faeces. The cattle with OI and CD recovered via medical treatment.

The serum biochemical and motility hormones levels are presented *Table 1*. The serum electrolyte analysis demonstrated that potassium and sodium were normal, and chloride were significantly ($P<0.05$) lower in cases of OI compared with the control and CD animals *(Table 1)*. The biochemical analysis demonstrated a significant ($P<0.001$ and $P<0.05$) increase in the level of AST, ALT, and GGT in cows with OI. The CD cows showed differences ($P<0.05$) in ALT levels compared to other groups and GGT levels compared to control group. The serum motilin and gastrin concentrations were significantly higher ($P<0.001$) in the OI group compared with other groups. However, ghrelin concentrations were not significantly different between the groups. Leptin concentration was significantly lower ($P<0.05$) in the CD group, but fairly similar between the OI and control groups.

DISCUSSION

Omasal impaction can be caused by poor-quality feed, excessive consumption of poorly digestible and small particle wheat straw, perforated phytobezoars, the restriction of animals to small barns without exercise, and the feeding of dry concentrate daily with limited access to water; it is also affected by other gastrointestinal

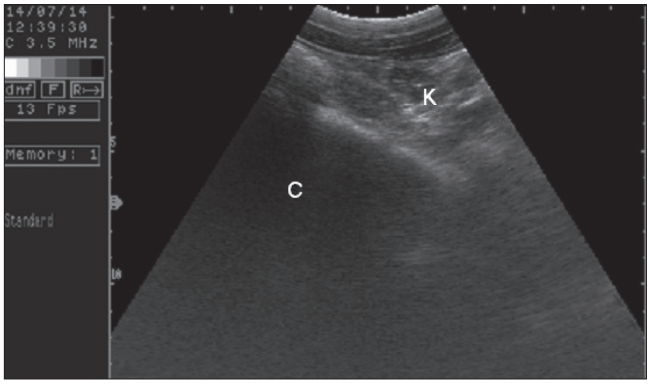

Fig 1. Ultrasonogram of caecal dilatation obtained at the right rib cage space, **K:** Kidney, **C:** Caecal gas

Şekil 1. Sağ kaburga alanında sekum dilatasyonunun ultrasonografik görüntüsü, **K:** Böbrek, **C:** Sekal gaz

Table 1. *Concentrations of serum ghrelin, motilin, gastrin, leptin and other biochemical parameters in omasal impaction, caecal dilatation and control groups (Means±SEM)*

Tablo 1. *Omazum konstipasyonu, sekum dilatasyonu ve kontrol gruplarında serum ghrelin, motilin, gastrin, leptin ve diğer biyokimyasal parametrelerin konsantrasyonları (Means±SEM)*

Parameters	Control (n:10)	Omasal Impaction (n: 4)	Caecum Dilatation (n: 4)	*P*-value
Gastrin (pg/ml)	139.33±8.58^{b}	228.50±22.01^{a}	124.9±9.49^{b}	0.000
Motilin (pg/ml)	226.70±6.56^{b}	328.75±23.89^{a}	240.50±19.79^{b}	0.000
Ghrelin (ng/ml)	113.30±4.99	129.50±21.15	120.25±14.77	0.581
Leptin (ng/ml)	3.66±0.07^{a}	3.45±0.09^{ab}	3.29±0.06^{b}	0.012
ALT (IU/l)	21.74±1.63^{c}	53.13±3.27^{a}	34.65±3.48^{b}	0.000
AST (IU/l)	99.82±6.32^{b}	134.40±14.98^{a}	86.53±4.42^{b}	0.013
GGT (IU/l)	28.13±2.24^{b}	51.73±2.56^{a}	40.83±3.92^{a}	0.000
Na (mmol/l)	140.24±2.34	140.40±4.43	125.75±9.00	0.086
Cl (mmol/l)	102.4±2.40^{a}	86.50±3.12^{b}	100.00±4.92^{ab}	0.012
K (mmol/l)	5.39±0.24	4.43±0.39	5.65±0.28	0.063

^{a,b,c} mean values marked with different superscripts in the same line are significantly different from each other (P<0.05)

tract disorders [5]. The symptoms of OI include anorexia, scanty faeces or cessation of defecation, dullness, ruminal distension, moderate to severe dehydration, congested mucous membranes and reduced milk yield [6]. In this study, we observed similar symptoms. Cows with anamnesis were fed a diet that included in dusty hay and were in their fourth pregnancy; they did not have severe disease and responded to medical treatment.

El-Attar et al.[7] analysed some biochemical parameters in cows with OI. Their results showed a significant decrease in glucose level and an increase in AST, ALT, LDH, CPK, urea and creatinine levels. Serum Cl levels were lower in cows with OI. Hypochloraemia could be attributed to fasting and long-standing anorectic status of the animals [2]. Chloride retention in rumen contents may caused low chloride level in omasal impaction [8]. In our study, serum Cl levels were lower because of fasting and retention in rumen. Serum ALT, AST, and GGT levels were higher in cows with OI but did not change in the control group. Non-lactating cows had mildly increased AST activity due to fatty liver, which resulted from dietary restriction and lipid infiltration in the muscle as well as the liver [9]. The elevation of GGT is associated with liver damage causing fatty liver syndrome in dairy cows [10].

The symptoms of simple CD without volvulus are not specific and include a drop in milk yield, reduced appetite and amount of faeces, and, occasionally, discrete signs of colic. The distended right paralumbar fossa, percussion (ping) and succession auscultation in the right flank are positive, extending from the tuber coxae to the last rib [11]. Our CD cases were diagnosed by clinical examination and ultrasonography *(Fig. 1)*. In anamnesis, the cattle were fed a high concentrate diet.

The haematological and blood biochemical analyses in dairy cattle with CD, associated with partial or complete interruption of the passage of intestinal contents, were not diagnostic [11]. Radostitis et al.[2] observed generalised fat mobilisation syndrome in late pregnancy and early lactation, particularly in high-yielding dairy cows. The laboratory findings of our study were similar to those of previous studies. However, serum ALT and GGT activities were slightly higher than control in the CD group but were not higher than those of the OI group. This increase was probably due to the periparturient period and metabolic changes in the cows. Other routine biochemical parameters were not changed.

Gastric motility hormones genes are differentially expressed in gastrointestinal tissues in humans and animals. Motilin and gastrin are synthesised in the upper gastrointestinal tract and have prokinetic activity on gastrointestinal motility [12]. Koenig et al.[13] reported a maximal concentration of motilin receptors in the duodenum, with decreasing numbers in the equine jejunum, large colon (i.e., pelvic flexure) and caecum [14]. Bunnett [15] found that cells containing gastrin immunofluorescence were confined to the mucosa of the abomasal antrum and the proximal small intestine and were not observed in the oesophagus, rumen, reticulum, omasum, abomasal fundus, ileum, large intestine, pancreas or gall-bladder. The ovine ghrelin gene was shown to be expressed in various tissues of the gastrointestinal tract, including the rumen, reticulum, omasum, duodenum, jejunum and ileum, although it was expressed primarily in the abomasums [16]. Another study found that ghrelin mRNA was not detectable in the reticulum, omasum, or rumen [14].

In our study, we observed that gastrin and motilin levels were higher in OI cows than in CD cows. Similar studies have shown that serum gastrin [17], ghrelin and motilin levels elevate the displacement of the abomasum in cattle [18]. This could be caused by omasal transport insufficiency. In addition, contents of the pre-stomach did

not pass the abomasum and produced gastrin and motilin. In contrast, caecal dilatation was not affected by motility hormones levels because motility hormones affect upper gastrointestinal tract diseases.

Serum ghrelin concentrations fluctates relative to nutritional status, but prolonged fasting for three days has not changed ghrelin concentrations, suggesting that the eating-related changes are rather decreases after food intake than increases due to fasting [19]. It was recently reported that experimentally induced gastric outlet obstruction and abomasal displacement increased gastric ghrelin production and plasma ghrelin concentrations [18,20]. However, in our study, ghrelin level was not different between groups. The content of the pre-stomach was not passed to the abomasum because OI was occurring. As a result, gastric obstruction was not complete and the abomasum could continue to empty.

Leptin in CD was significantly ($P<0.05$) lower than in the control group, but leptin was similar between the OI and control groups. Block et al.[21] stated that leptin is negatively correlated with the amount of non-esterified fatty acids, which reflects the amount of fat mobilisation. Leptin can decrease during abomasal displacement [18], fasting or complete food deprivation, and a period of negative energy balance and can increase in cases of obesity [21]. The large intestine includes a population of microorganisms which produce volatile fatty acids. If this product is not absorbed and used by the animal, it can produce a large amount of substrate for volatile fatty acid production. High gas production is common in CD cases [11]. Similarly, in this study, increasing volatile fatty acids in cecum might have caused CD in postpartum cattle. In addition leptin levels might be decreased due to negative energy balance in the postpartum period.

The levels of the motility hormones motilin and gastrin were increased in cows with OI but were unchanged in cows with CD. It was explained that gastric motility hormones activated the upper gastrointestinal tract. In addition, the use of gastric motility hormones should be reconsidered for gastrointestinal diseases. Leptin level was decreased in cows with CD. According to the results of this study, leptin alone might be related with negative energy balance which has a role in the pathogenesis of gastrointestinal diseases in the postpartum period. In conclusion, further studies are needed to clarify whether the use of gastroprokinetic agents and motility hormones in different gastrointestinal diseases in dairy cattle are beneficial and what effects they have.

REFERENCES

1. Inui A, Asakawa A, Bowers CY, Mantovani G, Laviano A, Meguid MM, Fujmiya M: Ghrelin, appetite, and gastric motility: The emerging role of the stomach as an endocrine organ. *FASEB J,*18, 439-456, 2004. DOI: 10.1096/fj.03-0641rev

2. Radostits OM, Gay CC, Hinchcliff KW, Constable PD: Impaction of the omasum. Diseases of the intestines of ruminants. **In,** Rodastits OM, Gay CC, Hinchcliff KW, Constable PD (Eds): Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats. 10th ed., 352-353, 375-376, Elsevier Health Sciences, Philadelphia, 2007.

3. Knowlton KF, Nelson JM: World of Dairy Cattle Nutrition. Holstein Foundation. pp.7-8, 1989.

4. Divers TJ, Peek SF: Rebhun's Diseases of Dairy Cattle. 2nd ed., p.184, Elsevier Health Sciences, ISBN-13: 978-1-4160-3137-62008.

5. Turkar S, Uppal SK: Blood biochemical and ruminal liquor profile in buffaloes *(Bubalus bubalis)* showing omasal impaction. *Vet Res Commun,* 31, 967-975, 2007. *DOI*: 10.1007/s11259-007-0027-2

6. Umakanthan T: Native drug therapy for omasal impaction in cattle. *Indian Vet J,* 79 (3): 295-296, 2002.

7. El-Attar HM, Ghanem MM, Abd El-Raof YM: Clinical, biochemical and haematological studies on omasal impaction in cows: The 2nd Scientific Conference, Faculty of Veterinary Medicine, Benha University, Egypt, January, 2007.

8. Hussain SA, Uppal SK, Randhawa C, Sood NK, Mahajan SK: Clinical characteristics, hematology, and biochemical analytes of primary omasal impaction in bovines. *Turk J Vet Anim Sci,* 37, 329-336, 2013. DOI: 10.3906/vet-1205-31

9. Gerloff BJ, Herdt TH, Emery RS: Relationship of hepatic lipidosis to health and performance in dairy cattle. *J Am Vet Med Assoc,* 188(8): 845–850, 1986.

10. Smith BP (Ed): Large Animal Internal Medicine. Fifth ed., Chapter 22: Gamma Glutamyltransferase (GGT). p.367, Elsevier Publishing, USA. 2015.

11. Braun U, Beckmann C, Gerspach C, Hässig M, Muggli E, Knubben-Schweizer G, Nuss K: Clinical findings and treatment in cattle with caecal dilatation. *BMC Vet Res*, 8, 1-9, 2012. DOI: 10.1186/1746-6148-8-75

12. Ohno T, Mochıkı E, Kuwano H: The roles of motilin and ghrelin in gastrointestinal motility. *Int J Pept,* Article ID 820794, 6 pages. 2010. DOI: 10.1155/2010/820794

13. Koenig JB, Cote N, LaMarre J, Harris WH, Trout DR, Kenney DG, Monteith G: Binding of radiolabeled porcine motilin and erythromycin lactobionate to smooth muscle membranes in various segments of the equine gastrointestinal tract. *Am J Vet Res*, 63, 1545-1550, 2002. DOI: 10.2460/ajvr.2002.63.1545

14. Wertz A, Knight T, Kreuder A, Bohan M, Beitz D, Trenkle AH: Effect of feed intake on plasma ghrelin concentration in beef cattle. *Animal Industry Report:* AS 650, ASL R1881. http://lib.dr.iastate.edu/ans_air/vol650/iss1/40, 2004. *Accessed:* 02.12.2014.

15. Bunnett NW: The localization of gastrin-like immunoreactivity (GIRL) in the alimentary tract of the sheep. *Q J Exp Physiol,* 69, 521-529, 1984.

16. Wang W, Cheng L, Guo J, Ma Y, Li F: Expression of Ghrelin in gastrointestinal tract and the effect of early weaning on Ghrelin expression in lambs. *Mol Biol Rep,* 41, 909-914, 2014. DOI: 10.1007/s11033-013-2935-2

17. Sen I, Ok M, Turgut K, Birdane FM, Guzelbektas H: Role of gastrin in the aetiology of abomasal displacement in dairy cows. *Vet Rec,* 151, 26-27, 2002. DOI: 10.1136/vr.151.1.26

18. Ozturk AS, Guzel M, Askar TK, Aytekin I: Evaluation of the hormones responsible for the gastro-intestinal motility in cattle with displacement of the abomasum: Ghrelin, motilin and gastrin. *Vet Rec,* 172, 636, 2013. DOI: 10.1136/vr.101322

19. Chan JL, Bullen J, Lee JH, Yiannakouris N, Mantzoros CS: Ghrelin levels are not regulated by recombinant leptin administration and/or three days of fasting in healthy subjects. *J Clin Endocrinol Metab,* 89, 335-343, 2004. DOI: 10.1210/jc.2003-031412

20. Iwasaki E, Suzuki H, Masaoka T, Nishizawa T, Hosoda H, Kangawa K, Hibi T: Enhanced gastric ghrelin production and secretion in rats with gastric outlet obstruction. *Digest Dis Sci,* 57, 858-864, 2012. DOI: 10.1007/s10620-011-1953-y

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

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Short Communication

Genomic Characterization of Goose Parvovirus and Muscovy Duck Parvovirus Co-infection in Fujian, China

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Abstract

In the present study, we sequenced and analyzed the complete genomes of the goose parvovirus (GPV) and the duck parvovirus (MDPV) isolates derived from a co-infected dead Muscovy duckling without any symptoms of other emerging Muscovy duck diseases. The genomic organization and sequences analysis indicates that the size of the full-length inverted terminal repeats regions determines the genomic length of GPV and MDPV, with which the lengths of the non-structural protein and capsid proteins coding region were highly conserved. According to the phylogenetic analysis, there are two genotypes of duck parvoviruses co-circulating in Muscovy ducks in China

Keywords: *Goose parvovirus; Muscovy duck parvovirus; Genome; Analysis*

Çin'in Fujian Eyaletinde Kaz Parvovirus ve Muscovy Ördek Parvovirus Koenfeksiyonunun Genomik Karakterizasyonu

Özet

Bu çalışmada, herhangi bir semptom göstermeyen bir Muscovy ördek palazından izole edilen kaz parvovirus (GPV) ve ördek parvovirus (MDPV) koenfeksiyon izolatlarının tüm genom sekansı ve analizi gerçekleştirilmiştir. Tüm uzunluk ters terminal tekrar bölgelerinin büyüklükleri GPV ve MDPV genomik boyutu belirlemektedir, ve yapısal olmayan protein ve kapsid proteinleri kodlayan bölgelerin uzunluklarının oldukça yüksek düzeyde korunduğu genomik organizasyon ve sekans analizleri ile gösterilmiştir. Filogenetik analizlere göre ördek parvoviruslarının iki genotipi Çin'de Muscovy ördeklerinde birlikte bulunmaktadır.

Anahtar sözcükler: *Kaz parvovirus, Muscovy ördek parvovirus, Genom, Analiz*

INTRODUTION

Anseriform dependoparvovirus 1 belonging to the family *Parvoviridae* that classified into two subfamilies (*Densovirinae* and *Parvovirinae*), which are further classified in thirteen generas (5 within *Densovirinae* and 8 within *Parvovirinae*). Anseriform dependoparvovirus 1 now have two groups that include goose parvovirus (GPV) and the duck parvovirus (MDPV). The MDPV is closely related to the GPV based on the southern hybridization assays and shared more than 80.0% sequence identity with GPV. GPV can cause highly contagious and fatal disease in goslings and Muscovy ducklings; whereas MDPV only cause disease with Muscovy ducklings [1,2].

The MDPV and GPV contain single-stranded DNA genome of approximately 5.1-kb in length, sharing closely related in genome and neutralization. The genome contains two major open reading frames (ORFs). The left ORF1 encodes the non-structural protein (NS) involved in viral replication, and the right ORF2 encodes the viral capsid proteins VP1, VP2 and VP3. The VP2 and VP3 shared the same C-terminal of the VP1, which were produced by alternate splicing [3,4].

The MDPV and GPV were reported once epidemic in many Muscovy duck breeding regions, such as Hungary, China, Japan, Thailand, France, and the USA. Though the attenuated vaccine against MDPV and GPV were used

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for more than twenty years in China, the MDPV and GPV are still mainly diseases in China waterfowl (especially for Muscovy ducks) industry. Many small Muscovy ducks farms, with poor breeding conditions, often sporadic outbreak with MDPV or GPV infection.

In this study, we demonstrated the occurrence of MDPV and GPV co-infection in a Muscovy duckling of age 15-day without any other waterfowl common diseases by sequencing the complete genomes of the two viruses. Derivation of the genomic sequences of the MDPV and GPV provides useful tools for studying viral infections in Muscovy ducklings as well as facilitates a better understanding of the phylogenetic relationship between MDPV and GPV.

MATERIAL and METHODS

Case History

In January 2013, a dead Muscovy duckling at the age of 15-day was collected from a farm that the breed regions located in Fujian, Southeast China. The Muscovy ducks shown mass symptoms including watery diarrhea, wheezing and locomotory dysfunction, with typical GPV-related and MDPV-related infection clinical signs. Twenty-five out of forty (62.5%) were dead at the age of 15-day. All the Muscovy duckling were purchased from commercial Muscovy duck farms, which had no previous history of MDPV or GPV exposure, and no attenuated vaccines against MDPV or GPV were used.

To determine the pathogens which were responsible for the disease, tissues were sampled from the dead ducklings for GPV or MDPV tests. The PCR results of ethe sample testing revealed that the succumbed ducking were co-infected with GPV and MDPV. All classical endemic and emerging viruses outbreaks in Muscovy duck flocks, such as avian influenza virus, Tembusu virus, duck hepatitis virus, Muscovy duck reovirus and novel duck reovirus, could be excluded as the causative agent by PCR (RT-PCR).

Sample Collection and DNA Extraction

The liver, spleen and intestinal cavity from the dead Muscovy duck aged 15 days were collected and homogenized in sterile phosphate-buffered saline (PBS, pH7.2) and centrifuged at 8.000 rpm for 30 min at 4°C. Supernatants were filtered through 0.45 μm and 0.22 μm filters (Merck KGaA, Darmstadt, Germany) and stored at -80°C prior for DNA extortion. DNA was extracted using the Viral DNA Kit (Omega Bio-Tek, GA, USA) according to the manufacture's instructions.

Genome Sequencing

The GPV and MDPV genomes were amplified by polymerase chain reaction (PCR) method using the corresponding primers according to the similar methodology by Shien [5] *(Table 1)*, which overlapped fragments encompassing the entire GPV and MDPV genome, designated as GPV strain G7 and MDPV strain FJM5, respectively. All PCR products were purified using the Gel Extraction Kit (Omega Bio-Tek, GA, USA) and ligated into the pBackZero8-T vector cloning kit (Takara, Dalian, China). In each case, five positive clones were selected at random and sequenced (Sangon Biotech, Shanghai, China) in both directions using an ABI model 3730 automatic DNA sequencer (ABI, CA, USA). The obtained sequences were used to compile the complete genome sequences assembly with Lasergene package (DNAStar, v7.1, Madison, WI, USA).

Genomic Characterization and Phylogenetic Analysis

Sequence comparison and percent identity was calculated using the Megalign program in the same package by CLUSTAL-W method. Phylogenetic analysis was performed with MEGA 6.0 using the neighbor-joining method with the maximum-likelihood model. Bootstrap scores were generated from 1.000 replicates.

Considering the VP1 coding region contains the immuno-domiant region that can induce neutralizing antibodies and the VP3 is the most abundant region with high genetic stability, we aligned the full-genome sequences, the VP1 genes and the VP3 genes in this study. Two Muscovy duck-origin GPV virus (PT strain, GenBank No: JF926695 and DY strain, GenBank No: EF515837) and the attenuated vaccine MDPV virus (P1 strain, GenBank No: JF926698) were added when constructing the VP1 gene and VP3 gene phylogenetic tree for evolution analysis.

RESULTS

Genomic Organization and Sequence Analysis

The genome of G7 was found to be 5106 nucleotides in length. The genome contained two major ORFs. The left ORF encodes the non-structural protein (NS) with 627 amino acids (aa) (nt 537-2420), and the right ORF encodes the viral capsid proteins VP1 with 732 aa (nt2439-4637) with the VP3 began at position nt 3008, respectively. However, the G7 genome shared the highest identity (98.9%) with the SYG61v strain, which was licensed as a vaccine virus used in goslings for preventing Dersy's disease in China over three decades [6]. When compared with other reported GPV genomes, the G7 genome shared 94.1% to 98.8% nucleotides sequence identities, respectively. Compared with other reported MDPV genomes, the G7 genome shared 79.4%-83.2% nucleotides sequence identities, respectively.

The genome of FJM5 was found to be 5017 nucleotides in length. The non-structural protein (NS) encodes 627 aa (nt 490-2373), the VP1 encodes 732 aa (nt2392-4590), the VP3 encodes 534 aa (nt 2986-4590), respectively. The inverted terminal repeats (ITRs) was found to be 359 bp

Table 1. *Primers used to amplify the complete genome of GPV and MDPV*
Table 1. *GPV ve MDPV tam genomlarını amplifiye etmekte kullanılan primerler*

Type	Primers	Position	Sequences	Product Length
GPV	G-F1	1-22	CTCATTGGAGGGTTCGTTCGT	241
	G-R1	218-241	GCATGCGCGTGGTCAACCTAACAGCCGGAAA	
	G-F2	166-185	TGACGTGTTTCCGGCTGTTA	617
	G-R2	762-782	TTCGTCCTGGTTGAACTGATT	
	G-F3	528-548	GTCGGAGAGATGGCACTTTCT	1028
	G-R3	1535-1555	GTGGTGGCAGGTCCGTAGAGC	
	G-F4	1309-1331	CTTCTTCAAATAATAGACAAGTG	1137
	G-R4	2425-2445	TAGACATATCTGCTTTGAGTC	
	G-F5	2317-2337	ATGAACATGGGTGGTATGATT	1299
	G-R5	3595-3615	ACTAGAATGCACTCCGGTCAT	
	G-F6	3330-3349	AACCATTGGGGAATCAGACC	1410
	G-R6	4687-4709	TCCAATGAGACTCAAGGACAAGA	
MDPV	M-F1	1-21	CTCATTGCAGGGTTCGTTCGT	238
	M-R1	214-238	ACGCACTTCCTTTGATGACGTATTT	
	M-F2	165-188	CTTTGATGACGTATTTCCGGTTGT	669
	M-R2	812-833	TGTACTCACTGCCTTCTTCCAA	
	M-F3	500-521	TTCATTCGTTGCTCTGCTCTCA	1228
	M-R3	1705-1727	CTTTTGCGGATTCCACTACTTTA	
	M-F4	1542-1562	TATGGCTCTACGGACCTGCGA	1182
	M-R4	2701-2723	TGAGCTGCTGGTCGTACGCTTTA	
	M-F5	2573-2596	AGAAAACCCCAACGAAAAGACAAT	1173
	M-R5	3721-3745	TAGCCTGTCTAAATCCTGTGAATGA	
	M-F6	3595-3616	AGCGAGATTCAATGACAGAAGT	1323
	M-R6	4898-4917	TCAAGGCTGATCGGGCGCAT	

GPV and MDPV amplification primers according the position in the GPV B strain genome (GenBank No. U25479, 5106 bp) and MDPV FM strain genome (GenBank No. U22967, 5132 bp), respectively. F and R denote the forward and reverse primers, respectively

in length, which was present at the 5′ and 3′ terminal ends of the genome. The FJM5 genome shared the highest identity (98.6%) with the FM strain, which was isolated in Hungary. When compared with other reported MDPV isolates SAAS-SHNN and MDPV-GX5 genome, the FJM5 genome shared 90.3% and 92.7% nucleotide sequence identities, respectively. Compared with other reported GPV genomes, the FJM5 genome shared 77.7%-79.6% nucleotides sequence identities, respectively. Nucleotide identities of the G7 VP1 gene with other GPV and MDPV isolates varied between 88.7%-98.6% and 80.0%-88.4%, respectively. Whereas, nucleotide identities of the FJM5 VP1 gene with other MDPV and GPV isolates varied between 85.2%-99.1% and 80.0%-85.4%, respectively.

The inverted terminal repeats (ITRs) of G7 and FJM5 was found to be 444 bp and 359 bp in length, respectively., which was presented at the 5′ and 3′ terminal ends of the genome structure. The ITRs region sequences of the GPV and MDPV isolates download from the GenBank were summarized in *Table 2, which can* conclude that the size of the full-length ITRs determine the genomic length of GPV and MDPV.

The complete genome of GPV strain G7 and MDPV strain FJM5 had been submitted to GenBank under accession No. KR029617 and KR075689, respectively.

Phylogenetic Analysis

Phylogenetic tree based on the GPV and MDPV isolates revealed that the GPV strain G7 clustered together with other GPV strains, and also the MDPV strain FJM5 clustered together with other MDPV strains *(Fig. 1-1)*.

The phylogenetic tree based on the VP1 *(Fig. 1-2)* and VP3 *(Fig. 1-3)*, we can found that the G7 belonged to the GPV clusters; also the FJM5 belonged to the MDPV clusters. However, four Muscovy duck-origin parvovirus (GPV PT strain, GPV DY strain, MDPV SAAS-SHNN strain and MDPV MDPV-GX5 strain) shared more closely than other GPV and MDPV isolates, the four isolates clustered in the subgroup, with specific lineage. From the phylogenetic tree based on the VP1, the four Muscovy duck-origin parvovirus VP1 genes belonged to the MDPV clusters *(Fig. 1-2)*. Whereas, from the phylogenetic tree based on the VP3, the four Muscovy duck-origin parvovirus VP3 genes belonged to

Table 2. *Comparison the ITRs sizes of the genome from GPV and MDPV*
Table 2. *GPV ve MDPV genomlarında ITR boyutlarının karşılaştırılması*

Type	GenBank No.	Strain	Genome	ITR	Host
GPV	U25749	B	5106	444	goose
	EU583392	VG32/1	5104	443	vaccine
	EU583391	06-0329	5054	418	goose
	EU583390	82-0231	5050	416	goose
	EU583389	82-0231V	4980	381	vaccine
	HQ891825	GDaGPV	5106	444	goose
	JF333590	SH	5106	444	goose
	KC178571	Y	5106	444	Muscovy duck
	KC184133	E	5125	443	goose
	KC478066	SHFX1201	5050	416	swan
	KC996729	SYG61V	5102	442	vaccine
	KC996730	YZ99-6	5046	414	goose
	KM272560	LH	5047	414	goose
	KR029617	G7	5106	444	Muscovy duck
MDPV	U22967	FM	5132	457	Muscovy duck
	KC171936	SAAS-SHNN	5061	381	Muscovy duck
	KM093740	MDPV-GX5	5132	384	Muscovy duck
	KR075689	FJM5	5017	359	Muscovy duck

the GPV clusters *(Fig. 1-3)*. The results indicated that more genotype duck parvovirus circulating in Muscovy ducks, leading more diverse evolutionary directions of MDPV.

DISCUSSION

When compared with the GPV reference isolates, the GPV attenuation strains and their parental strains or field viruses showed that only 2.9% different between 82-0321 and 82-0321V (exclude the ITRs region sequences), the MDPV attenuation strains and their parental strains or field viruses showed that only 1.0 % different between P (GenBank No. JF926697) and P1 (GenBank No. JF926698). The results indicated that the genomic sequences of the GPV and MDPV had highly stable in the field for more than three decades, which also verified the GPV G7 strain and MDPV FJM5 strain may the pathogen for the dead Muscovy duck.

Comparing the GPV and MDPV genome structure retrieved from GenBank, the lengths of the NS and VP1 coding region were highly conserved; all references isolates had a NS coding region of 1884 nucleotides and a VP1 coding region of 2199 nucleotides. The length of the ITRs region determines the genomic length of GPV and MDPV, with different nucleotides deletions. Though deletions can be observed compared with the virulent B and FM strain, but no deletion affected the formation of the hairpin structure, no matter the deletions occurs in the "stem" or "bubble" region of ITRs, because of all substitutions occurred in pairs, and the sequences in each pair were complementary to each other so that these substitutions did not affect the formation of the hairpin structure.

Genetic natural recombination has been reported among parvoviruses, especially with the persistent immunological pressure, which was thought to play an important role for parvoviruses evolution [7]. Poonia B reported new duck parvovirus from Muscovy duck, only shared 84.6% sequence identity with GPV and 84.5% identity with MDPV [8]. Wang reported a part of VP gene sequence of the GPV PT strain isolated from Muscovy ducks had the characteristics of MDPV sequence [9]. Zhu recently reported a recombinant Muscovy duck parvovirus with two putative genetic recombination regions between GPVs and MDPVs [10]. The phylogenetic tree showed that the GPV DY strain and MDPV MDPV-GX5 strain also had genetic natural recombination between GPVs and MDPVs. Meanwhile, the GPV DY strain and PT strain (though no ITRs regions were sequenced) may belong to the new-genotype MDPV.

In summary, although the co-infection with GPV and MDPV shared similar symptoms with GPV or MDPV infection as previously reported, the co-infection of GPV and MDPV in the pathogenicity and virulence of certain isolates still needs further investigation.

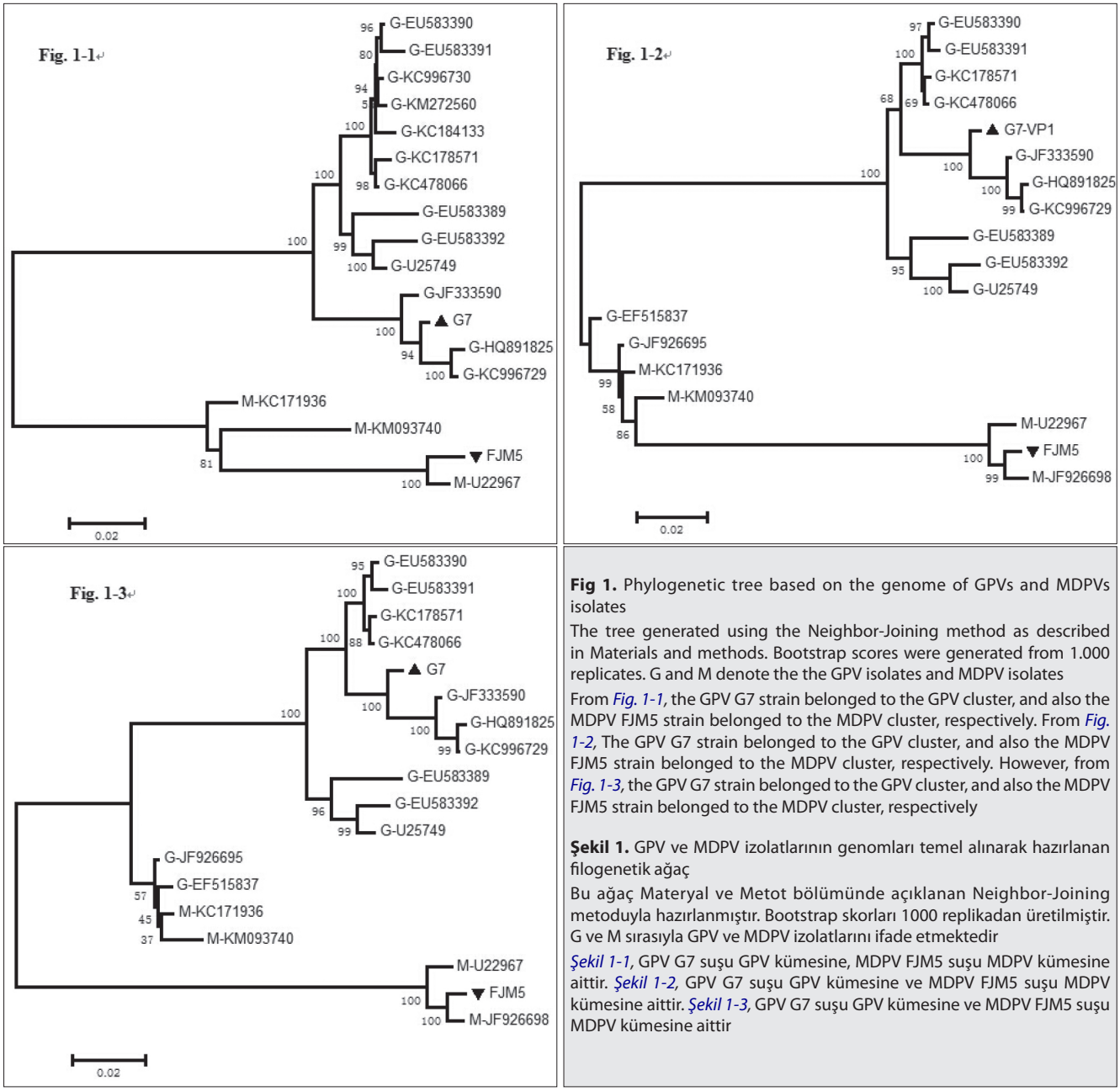

Fig 1. Phylogenetic tree based on the genome of GPVs and MDPVs isolates

The tree generated using the Neighbor-Joining method as described in Materials and methods. Bootstrap scores were generated from 1.000 replicates. G and M denote the the GPV isolates and MDPV isolates

From *Fig. 1-1*, the GPV G7 strain belonged to the GPV cluster, and also the MDPV FJM5 strain belonged to the MDPV cluster, respectively. From *Fig. 1-2*, The GPV G7 strain belonged to the GPV cluster, and also the MDPV FJM5 strain belonged to the MDPV cluster, respectively. However, from *Fig. 1-3*, the GPV G7 strain belonged to the GPV cluster, and also the MDPV FJM5 strain belonged to the MDPV cluster, respectively

Şekil 1. GPV ve MDPV izolatlarının genomları temel alınarak hazırlanan filogenetik ağaç

Bu ağaç Materyal ve Metot bölümünde açıklanan Neighbor-Joining metoduyla hazırlanmıştır. Bootstrap skorları 1000 replikadan üretilmiştir. G ve M sırasıyla GPV ve MDPV izolatlarını ifade etmektedir

Şekil 1-1, GPV G7 suşu GPV kümesine, MDPV FJM5 suşu MDPV kümesine aittir. *Şekil 1-2*, GPV G7 suşu GPV kümesine ve MDPV FJM5 suşu MDPV kümesine aittir. *Şekil 1-3*, GPV G7 suşu GPV kümesine ve MDPV FJM5 suşu MDPV kümesine aittir

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REFERENCES

1. Chang P, Shien J, Wang M, Shieh H: Phylogenetic analysis of parvoviruses isolated in Taiwan from ducks and geese. *Avian Pathol,* 29, 45-49, 2000. DOI: 10.1080/03079450094270

2. Woźniakowski G1, Samorek-Salamonowicz E, Kozdruń W: Quantitative analysis of waterfowl parvoviruses in geese and Muscovy ducks by real-time polymerase chain reaction: Correlation between age, clinical symptoms and DNA copy number of waterfowl parvoviruses. *BMC Vet Res,* 8, 29, 2012. DOI: 10.1186/1746-6148-8-29

3. Zádori Z, Stefancsik R, Rauch T, Kisary J: Analysis of the complete nucleotide sequences of goose and muscovy duck parvoviruses indicates common ancestral origin with adeno-associated virus 2. *Virology,* 212, 562-573, 1995.

4. Liu H, Wang H, Tian X, Zhang S, Zhou X, Qi K, Pan L: Complete genome sequence of goose parvovirus Y strain isolated from Muscovy ducks in China. *Virus Genes,* 48, 199-202, 2014. DOI: 10.1007/s11262-013-1001-4

5. Shien J, Wang Y, Chen C, Shieh H, Hu C, Chang P: Identification of sequence changes in live attenuated goose parvovirus vaccine strains developed in Asia and Europe. *Avian Pathol,* 37, 499-505, 2008. DOI: 10.1080/03079450802356979

6. Wang J, Duan J, Meng X, Gong J, Jiang Z, Zhu G: Cloning of the genome of a goose parvovirus vaccine strain SYG61v and rescue of infectious virions from recombinant plasmid in embryonated goose eggs. *J Virol Methods,* 200, 41-46, 2014. DOI: 10.1016/j.jviromet.2014.02.014

7. Ren X, Tao Y, Cui J, Suo S, Cong Y, Tijssen P: Phylogeny and evolution of porcine parvovirus. *Virus Res,* 178, 392-397, 2013. DOI: 10.1016/j.virusres.2013.09.014

8. Poonia B, Dunn PA, Lu H, Jarosinski KW, Schat KA: Isolation and molecular characterization of a new Muscovy duck parvovirus from Muscovy ducks in the USA. *Avian Pathol,* 35, 435-441, 2006. DOI: 10.1080/03079450601009563

9. Wang S, Cheng X, Chen S, Zhu X, Chen S, Lin F, Li Z: Genetic characterization of a potentially novel goose parvovirus circulating in Muscovy duck flocks in Fujian Province, China. *J Vet Med Sci,* 75, 1127-1130, 2013.

10. Zhu Y, Zhou Z, Huang Y, Yu R, Dong S, Li Z, Zhang Y: Identification of a recombinant Muscovy Duck parvovirus (MDPV) in Shanghai, China. *Vet Microbiol,* 174, 560-564, 2014. DOI: 10.1016/j.vetmic.2014.10.032

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Short Communication

A New Louse Species: *Aegypoecus guralpi* sp. n. (Phthiraptera: Ischnocera) from a Long-legged Buzzard *(Buteo rufinus)* [1]

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Abstract

A total of ten louse samples were collected from a long-legged buzzard *(Buteo rufinus)* in Hatay province in Mediterranean Region of Turkey. All of the louse samples were identified as a new species morphologically and named as *Aegypoecus guralpi*.

Keywords: *Aegypoecus guralpi, Louse, Long-legged buzzard*

Kızıl Şahin *(Buteo rufinus)*'de Yeni Bir Bit Türü: *Aegypoecus guralpi* sp. n. (Phthiraptera: Ischnocera)

Özet

Türkiye'nin Akdeniz Bölgesi'nde yer alan Hatay ilinde bir kızıl şahinden *(Buteo rufinus)* toplanan 10 adet bit örneği morfolojik olarak incelenmiş, örneklerin tamamı yeni bit türü olarak teşhis edilmiş ve *Aegypoecus guralpi* adıyla isimlendirilmiştir.

Anahtar sözcükler: *Aegypoecus guralpi, Bit, Kızıl şahin*

INTRODUCTION

The *Aegypoecus* genus was generated by Clay and Meinertzhagen [1] as *Helluo* from Aeygpiinae. Round-bodied louse species of vultures have adapted to live on head and neck niches [2,3]. There are a few lice species reported in the genus *Aegypoecus.* In 1838, Burmeister described *Docophorus brevicollis*, Kellogg [4] identified *Aegypoecus (Docophorus) perspicuous* from *Corvus* spp.. *Aegypoecus neophron (Helluo neophron)* was reported from *Neophron percnopterus* by Clay and Meinertzhagen [1]. Ansari [5] reported *A. brevicollis* from *Aegypius monachus* and *Aegypoecus perspicuus* (Kellogg, 1914) (as *Aegypoecus griffoneae)* from *Gyps himalayenesis* Hume in Punjab, India. Dhanda [6] recorded a new species, as *Aegypoecus hopkinsi* from *Necrosyrtes monachus.* Pérez-Jiménez et al.[2] later renamed it as *A. brevicollis.* In the current study, a new louse species for this genus has been morphologically identified in all collected samples from a long-legged buzzard *(Buteo rufinus)* in Hatay province in Mediterranean Region of Turkey.

MATERIAL and METHODS

A total of ten louse samples were collected from a long-legged buzzard *(Buteo rufinus)* in Hatay (Batiayaz district, 36° 16′ N, 35° 99′ E) in Mediterranean Region of Turkey along the Syria border in 2012. The lice were stored in ethanol 70% and cleared in KOH 10% for 24 h. The lice were slide-mounted in Canada balsam and kept in an incubator for 20 days to dry. The louse samples were individually examined using phase contrast microscope (Leica DM 750) morphologically and identified at a species level. The new louse species described here was deposited in the collection at the Department of Parasitology, Faculty of Veterinary Medicine, University of Selcuk, Konya, Turkey.

RESULTS

All of the ten louse samples were identified as a new species *Aegypoecus* sp. and named as *Aegypoecus guralpi*

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in memory of Prof. Dr. Nevzat Guralp (deceased) who was well respected by the first author.

Aegypoecus guralpi sp.n.

Type Host: *Buteo rufinus*

Male: (Holotype) Rather small, but head is very large, whereas thorax and abdomen are short *(Fig. 1A, 1B; Fig. 2A)*. The triangular head is narrowed anteriorly. The length of the head is equal to the width. The anterior margin is slightly rounded and a marginal carina is interrupted by a hyaline membrane at the median. The clypeal plate is relatively broad, short and weakly developed, and also narrowed as a small prolongation to the posterior end. The ventral anterior plate is narrowed as a strip. The mandibles are large and very stout, and dark coloured in apical ends. The preantennal region is well developed in the lateral aspect. The length of the preantennal head region is subequal to the postantennal head region. The trabeculae are small and hypopharyngeal sclerite is well developed. The antenna have five segments, the first and second segments are wide and long, respectively. The preantennal and ocular setae are short. There are also five long marginal temporal setae in *Fig. 1A, 1B*.

The thorax is small, prothorax and pterothorax are quadrangular and rounded laterally, and the posterior margin is slightly convex. The pronotum has two long setae on each side of lateromarginal angles. The prosternal plate is small and pentagonal shaped, pointed at the anterior, without setae. It is pteronotum in almost all parts, except a little anterior part that is fully divided as a narrow strip at the median. The anterior part of this line is of a width wider than the posterior part with 11-12 long setae on the each side of the posterior margin.

The abdomen is very short, relatively broad and rounded. Pleural plates are present, narrowed and well developed. Paratergal plates are slightly well-developed, paratergal plates of segment II-IV are dark and distinct in paratype, narrowed and rounded at the median, and also equal in segments II-VII (well developed in paratype). There are two irregular rows of tergocental setae on the posterior margin of the abdominal segments.

Genitalia is displayed in *Fig. 1C; Fig. 2C*. Male genitalia was drawn from the paratype male due to it was not clear in holotype. It was also take the photos of paratype of male due to it was more clear than holotype male. The basal plate

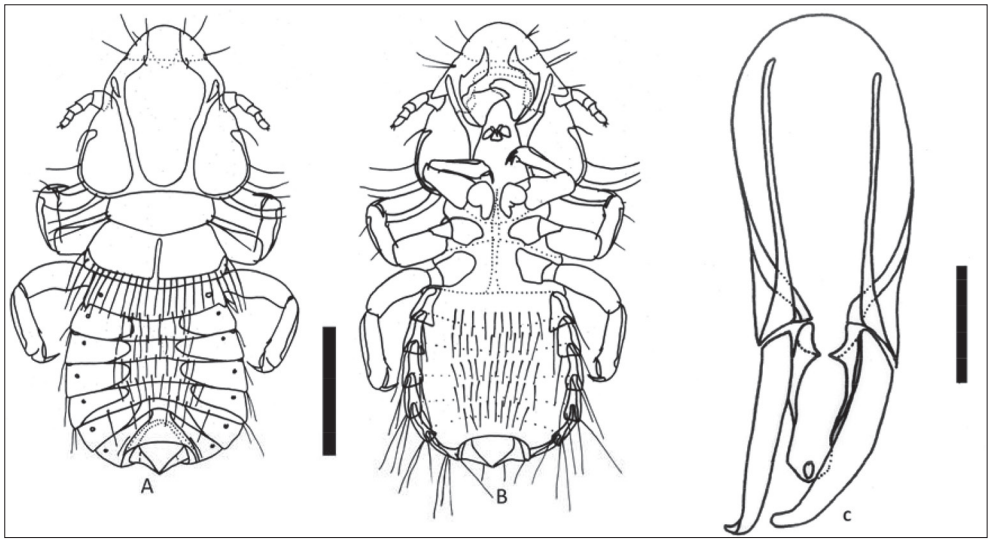

Fig 1. *Aegypoecus guralpi*, sp.n; holotype male; A- Dorsal; B- Ventral (scale bar: 0.5 mm), C- Paratype male genitalia (scale bar: 0.25 mm), Original

Şekil 1. *Aegypoecus guralpi*, sp.n; holotip erkek, A- Dorsal; B- Ventral (cetvel: 0.5 mm), C- Paratip erkek genital (cetvel: 0.25 mm), Orjinal

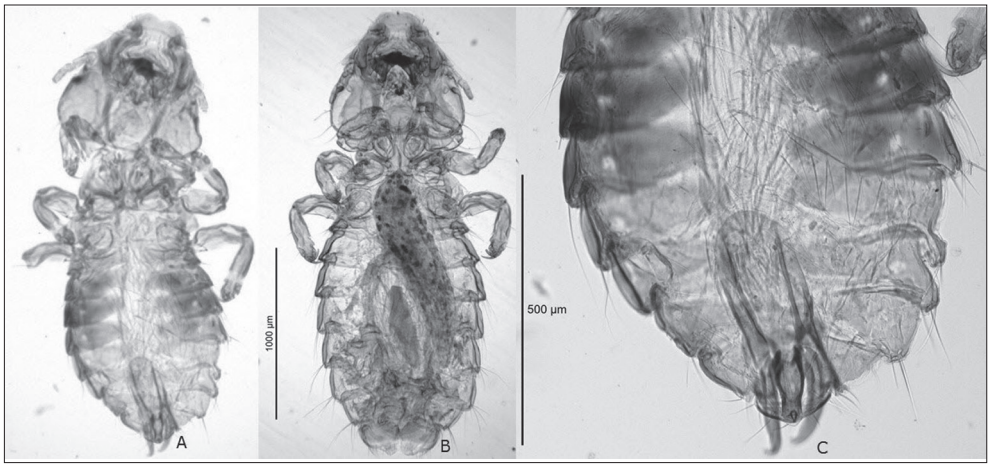

Fig 2. *Aegypoecus guralpi*, sp.n; paratype male; A- Ventral; Allotype Female; B- Ventral; Paratype male genitalia; C- Original

Şekil 2. *Aegypoecus guralpi*, sp.n; paratip erkek; A- Ventral; Allotip Dişi; B- Ventral; Paratip, Erkek genital; C- Orjinal

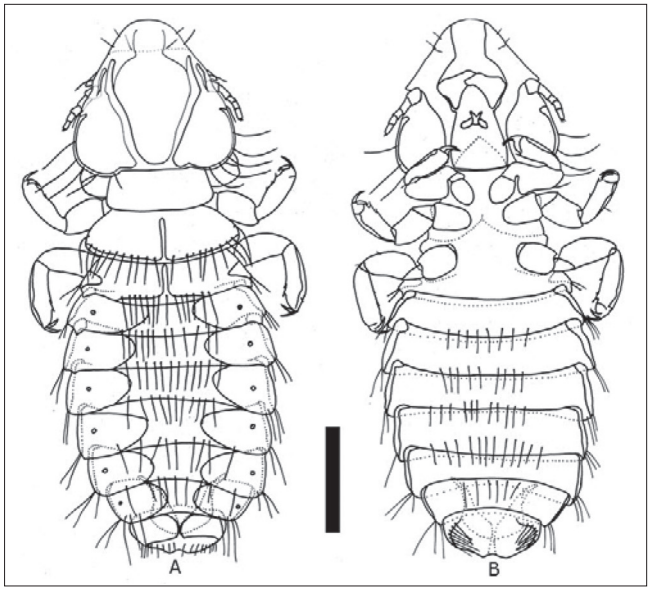

Fig 3. *Aegypoecus guralpi*, sp.n; Allotype female; A- Dorsal; B- Ventral (scale bar: 0.5 mm)

Şekil 3. *Aegypoecus guralpi*, sp.n; Allotip Dişi; A- Dorsal; B- Ventral (cetvel: 0.5 mm)

Table 1. *Measurements of Aegypoecus guralpi sp. n.*
Tablo 1. *Aegypoecus guralpi sp. n. ölçümleri*

Body Measurements	Female (n: 3)	Male (n: 2)
Head length	0. 76 mm	0.66 mm
Head width	0. 77 mm	0.67 mm
Head index	1. 00	0.97
Thorax length	0.48 mm	0.42 mm
Thorax width	0.72 mm	0.53 mm
Abdomen length	1.45 mm	0.76 mm
Abdomen width	1.06 mm	0.64 mm
Total length	2.64 mm	1.82 mm

is relatively short, rounded anteriorly, and reaches to the posterior part of segment V. Parameres are well developed.

Female: Similar to the male, but longer *(Table 1)*. The abdomen is short, relatively broad, rounded and slightly longer than the total length of the head and thorax. Segment IX is bilobed. The genital plate is large and distinct. There is no lanceolate hair on abdominal segments *(Fig. 2B; Fig. 3)*.

Abdominal chaetotaxy: Tergocentral setae numbers in posterior margin: II, 15; III, 15; IV, 15; V, 13; VI, 10; VII, 8; VIII, 6. Sternit IX has a cluster with 16 setae on the lateral margins. Short post-spiracular setae do not reach to the anterior part of next segment.

DISCUSSION

Natural louse infestations of vultures caused by the genus *Aegypoecus* are rarely reported. Only seven species of *Aegypoecus*; *A. brevicollis* (Burmeister, 1838); *A. trigonoceps* (Giebel, 1874); *A. perspicuus* (Kellogg, 1914); *A. hopkinsi* (Dhanda, 1959); *A. clayae* (Dhanda, 1960); *A. jordani* (Dhanda, 1960); and *A. africanus* Dhanda, 1960, have been described until now.

The specimens in this report were similar with Clay's description [1]. Clay described large heads, short broad abdomens, short ventral trabeculae protruding beyond the lateral margin of the head, widely-separated tergal plates, and numerous dorsal and ventral lanceolate-like hairs for the *Aegypoecus (Helluo)* species. Clay also emphasized that the abdomen of females had three, small, irregular shaped dark chitinious plates in the centre of segment VII; one was flanked median by a lateral plate on each side, and the terminal segment was bilobed and rounded posteriorly in the male. This study identified two clear descriptive differences: (i) no lanceolate-like hairs were found on the abdominal segments of the louse specimens and (ii) two dark and large chitinous plates were found at the terminal abdominal segments of the female.

Dhanda [6] stated that the *Aegypoecus* species parasitized on vultures had four, long marginal temporal setae, and even if a fifth one was present, it was short and spine-like. Dhanda [6] also reported that a long seta was found on each side of the prothorax in the genus *Aegypoecus*. Our specimens have two relatively short setae at the posterolateral margins of the prothorax. The specimens have five long marginal temporal setae, two prothorasic setae and no lanceolate hairs on the abdominal segments. The results of morphological evidence from the lice collected on a non-vulture host indicate that these specimens are different from the other species in the genus *Aegypoecus*. This species may be considered as a "straggle" for *B. rufinus*. Because all lice species were described under the genus *Aegypoecus* found on Vulturidae, *Buteo rufinus* may be accepted as a natural host for this new louse species. Indeed there are few studies about louse species of the wild birds in Turkey although on intercontinental route of migratory birds [7-10].

REFERENCES

1. Clay T, Meinertzhagen R: New genera and species of Mallophaga. *Entomol,* 71, 275-279, 1938.

2. Dhanda V: A new species of *Aegypoecus* Clay and Meinertzhagen, 1939 (Mallophaga). *Ann Mag Nat Hist*, 2, 725-730, 1960. DOI: 10.1080 /00222935908655759

3. Pérez-Jiménez JM, Soler-Cruz MD, Ruiz-Martínez I, Díaz-Lopez M, Granados-Torres JE: A redescription of *Aegypoecus brevicollis* (Burmeister, 1838) (Ischnocera: Philopteridae). *Sys Parasitol,* 22, 215-219, 1992. DOI: 10.1007/BF00009668

4. Kellogg VL: Mallophaga from birds of the South Atlantic. *Sci Bull Mus Brook Inst Art Sci,* 2, 80-89, 1914.

5. Ansari AR: Mallophaga (Ischnocera) infesting birds in the Punjab (India). *Proc Nat Inst Sci India*, 13, 253-303, 1947.

6. Dhanda V: A revision of the Mallophagan genus *Aegypoecus* Clay

and Meinertzhagen, 1939 (Ischnocera: Philopteridae), with descriptions of three new species. *Ann Mag Nat Hist,* 13, 657-683, 1961. DOI: 10.1080/00222936008651072

7. Dik B, Yamaç E, Uslu U: Chewing lice (Phthiraptera) found on wild birds in Turkey. *Kafkas Univ Vet Fak Derg,* 17, 787-794, 2011. DOI: 10.9775/kvfd.2011.4469

8. Goz Y, Dik B, Kılınç Ö, Yılmaz A, Aslan L: Chewing lice (Phthiraptera: Amblycera, Ischnocera) on several species of wild Birds around the Lake Van basin, Van, Eastern Turkey. *Kafkas Univ Vet Fak Derg*, 21, 333-338, 2015. DOI: 10.9775/kvfd.2014.12484

9. Dik B, Yamaç E, Uslu U: Studies on Chewing Lice (Phthiraptera: Amblycera, Ischnocera) Species from Domestic and Wild Birds in Turkey. *Kafkas Univ Vet Fak Derg,* 19, 553-560, 2013. DOI: 10.9775/kvfd.2012.8207

10. Girisgin AO, Dik B, Girisgin O: Chewing lice (Phthiraptera) species of wild birds in northwestern Turkey with a new host record. *Int J Parasitol Parasites Wildl*, 2, 217-221, 2013. DOI: 10.1016/j.ijppaw.2013.07.001

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Case Report

First Reports of *Sarconema eurycerca* and *Trinoton anserinum* in The Whooper Swan *(Cygnus cygnus)* in Van, Turkey

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Abstract

Whooper swan *Cygnus cygnus* (Linnaeus, 1758) with wounded wing that found in the Ercis district of Van province was brought into Directorship of Wild Animal Protection of University of Yuzuncu Yıl. Despite the surgical interventions, the whooper swan could not be rescued. Five lice in the feather and three nematodes in the heart were found at examination of the whooper swan. These lice specimens were identified as *Trinoton anserinum* (Fabricius, 1805). After the necropsy, nematodes were found in the heart of the whooper swan. Nematodes were identified as *Sarconema eurycerca* according to their morphological pecularities. *Sarconema eurycerca* have been reported for the first time in Whooper swan in Van, Turkey.

Keywords: *Trinoton anserinum, Sarconema eurycerca, Cygnus cygnus, Whooper swan, Turkey*

Ötücü Kuğuda *(Cygnus cygnus) Sarconema eurycerca* (Filarioidea: Nematoda) ve *Trinoton anserinum* (Phthireptera: Amblycera)'un Van'da (Türkiye) İlk Bildirimi

Özet

Van'ın Erciş ilçesinden Yüzüncü Yıl Üniversitesi Yaban Hayvanlarını Koruma Müdürlüğüne kanadından yaralı getirilen Ötücü kuğu *(Cygnus cygnus* Linnaeus, 1758) cerrahi müdahalelere rağmen kurtarılamayarak ölmüştür. Kuğunun tüyleri arasında 5 adet bite, kalbinde ise 3 adet nematoda rastlanmıştır. Bitlerin *Trinoton anserinum* (Fabricius, 1805) türü oldukları tespit edilmiştir. Nekropsi sonrası kuğunun kalbinde nematodlar bulunmuş, nematodların morfolojik özellikleri göre *Sarconema eurycerca* olduğu tespit edilmiştir. Ötücü kuğuda tespit edilen *Sarconema eurycerca* Türkiye'de ilk kez bildirilmiştir.

Anahtar sözcükler: *Trinoton anserinum, Sarconema eurycerca, Cygnus cygnus, Ötücü kuğu, Türkiye*

INTRODUCTION

Sarconema eurycerca known as heartworm in swans and geese is a filarial nematode of the superfamily Filarioidea [1,2]. In the previous studies whistling, trumpeter, tundra, black, whooper and mute swan were found to infested with *S. eurycerca*. Moreover *S. eurycerca* was found in snow, white-fronted and bean geese. *Sarconema eurycerca* has been implicated as a cause of death among wild birds [2,3]. It was first described by Wehr [4] from a Whistling Swan, *Cygnus columbianus columbianus*, where it was found to be parasitic in the heart muscle [5]. *Sarconema eurycerca* has an indirect life cycle. Female adult heartworms release microfilariae into the bloodstream of the definitive host bird [5].

Trinoton anserinum serves as natural cyclo-developmental vector for a *S. eurycerca* [6] within the sub-order Amblycera. This family of lice was classified by Clay [7] and the genus *Trinoton* is distinguished by the presence of two large sternal plates bearing many setae [8]. Among the largest lice are those of the genus *Trinoton*, which can reach 5-6 mm in length. These lice feeds with feathers, also they can feed on blood [9]. One is *Trinoton querquedulae* which is found on Anas and is related with genera and the other is *T. anserinum* on Anser and is related with genera.

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In Turkey, there are limited studies [10-12] about *Trinoton* species but *T. anserinum* had been reported on a mute swan before *(Cygnus olor)* [3]. This is the first record for *T. anserinum* and *S. eurycerca* in whooper swan in Turkey.

CASE HISTORY

In February 2014, wounded whooper swan was found in Ercis area of Van Lake and was brought into Directorship of Wild Animal Protection of University of Yüzüncü Yıl. Five lice were found after examination of the swan and the specimens were transferred into tube containing 70% ethyl alcohol. Then it was cleared in 10% KOH, the specimens were washed in distilled water for 24 h, and was passed through a graded series of 70%, 80%, 90% and 96% alcohol, and was mounted in Canada balsam on the slides. The slides were examined by light microscopy for the identification of species and measurements were taken [12]. Whooper swan was necropsied after death. Three nematodes were found in the myocardium of the heart and stored in 70% alcohol until identification. The nematodes were examined with a light microscope to take morphological measurements and to determine sex. *Trinoton anserinum* and *Sarconema eurycerca* were identified according to literatures [13-15].

Identification of Parasites

Trinoton anserinum (Fabricius, 1805): The body of *T. anserinum* is dorso-ventrally flattened *(Fig 1A, 1B)*. Head triangular in shape than long, greates width at temporal region rounded and broader, the laterodorsal margin of head with small protuberance bearing setae. Chaetotaxy consisting of long and short setae of normal appearance and stout, spine-like setae. Clay and Hopkins [13] indicated that there are two species in the group of quaerguedulae as *Trinoton anserinum* and *Trinoton querquedulae*, these species are on the 3rd femora's ventral part *(Fig. 1C)* and there are spine-like stae groups in 4-5 sternites. Measurements of this species are shown in *Table 1*.

Sarconema eurycerca (Wehr, 1939): The parasite both of eliptical ends are extremely visible and string *(Fig. 2A)*. The uteri occupies most of the body cavity, eggs are visible in them through the cuticle and different stages of development of microfilariae were visible in the eggs *(Fig. 2C)*. The vulva is nearly to the anus and uteri extends almost to the anus. Eggs are thin shelled and microfilariae of *S.eurycerca* consists of a long, narrow nucleated body surrounded by a sheath. The posterior edges of microfilariae are narrower *(Fig. 2B)*. Measurements of this species and eggs and microfilers of this species are shown in *Table 2*.

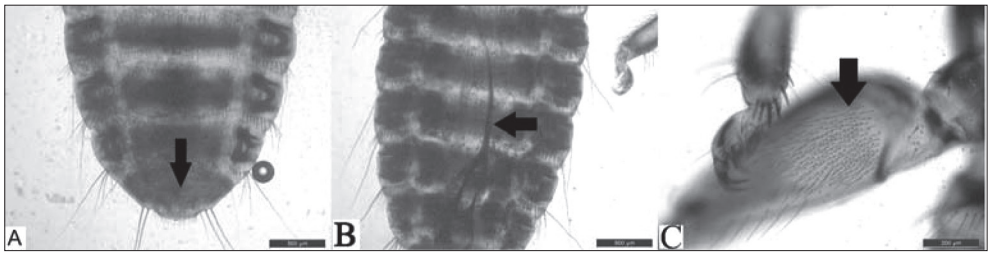

Fig 1. A- *Trinoton anserinum* (female), **B-** *Trinoton anserinum* (male), **C-** *Trinoton anserinum* brushes setae ventrale side of 3rd femur

Şekil 1. A- *Trinoton anserinum* (Dişi), **B-** *Trinoton anserinum* (erkek), **C-** *Trinoton anserinum* 3. femurun ventral tarafında fırça kıllar

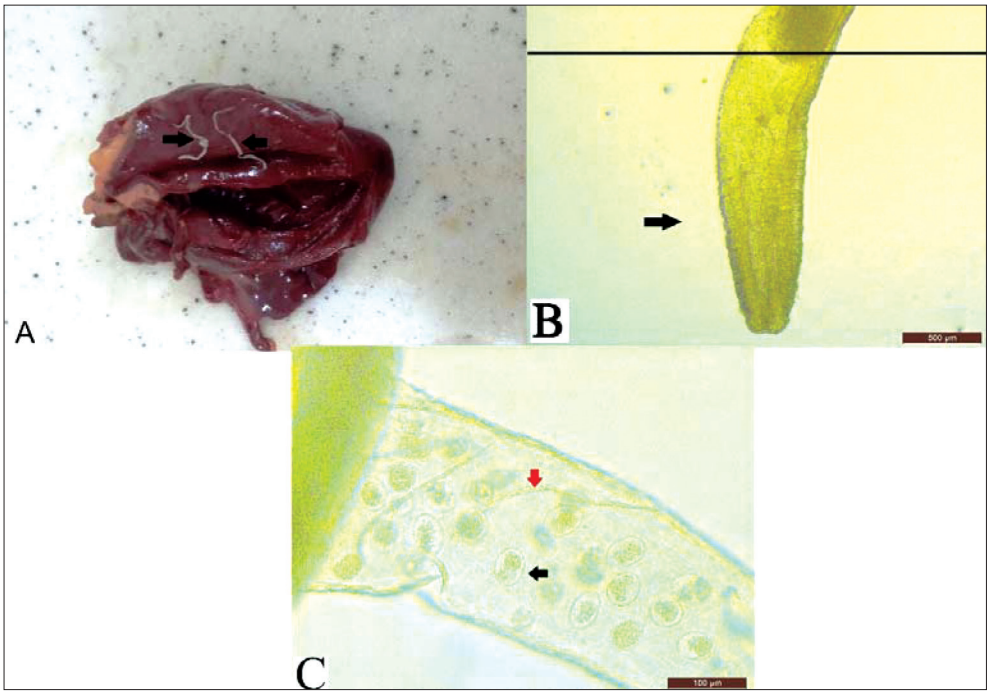

Fig 2. A- Heartworms on the myocardium of the heart of whooper swan, **B-** Anterior section of adult female nematode *(S. eurycerca)* under a light microscope, **C-** Fully developed microfilariae *(red arrow)* hatched from eggs *(black arrow)*, still within uteri

Şekil 2. A- Ötücü kuğunun kalbinde (miyokardium) *Sarconema eurycerca* (kalp kurdu), **B-** Erişkin dişi nematodun *(S. eurycerca)* ön ucu, **C-** *Sarconema eurycerca*'nın uterusunda yumurta *(siyah ok)* ve gelişmiş mikrofilarya *(kırmızı ok)*

Table 1. *Body size of Trinoton anserinum (mm) on various hosts according to different authors*
Tablo 1. *Farklı araştırmacılara göre çeşitli konaklar üzerinde Trinoton anserinum'un (mm) vücut uzunlukları*

Male n=2	References		Present Data n=2	Female n=3	References		Present Data n=3
	Cohen [15]	Castresana et al.[16]			Clay and Hopkins [13]	Castresana et al.[16]	
Length of head	0.91	-	0.80	Length of head	0.93	-	0.90
Width of head	1.54	-	1.38	Width of head	1.59	-	1.53
Width of thorax	-	-	0.60	Width of thorax	-	-	0.64
Length of thorax	-	-	2.9	Length of thorax	-	-	2.4
Length of abdomen	-	-	3.26	Length of abdomen	-	-	3.5
Width of abdomen	-	1.38	1.63	Width of abdomen	-	1.60	2.0
Total length of body	5.67	6.17	5.76	Total length of body	6.05	5.31-6.28	6.38

Table 2. *Body size of females of Sarconema eurycerca (µm) on various hosts according to different authors*
Tablo 2. *Farklı araştırmacılara göre çeşitli konaklar üzerinde dişi Sarconema eurycerca'nın (µm) vücut uzunlukları*

Character	References		Present Data
	Cohen [15]	Seegar [14]	
Length of microfilariae	263-382	270-340	278-290
Width of microfilariae	-	4.5-6.5	6-7
Length of eggs	-	-	54-55
Width of eggs	-	-	33-34

DISCUSSION

Approximately 4.000 valid lice species have been reported on the birds worldwide [17]. In the studies done relevant to the chewing lice found on wild birds, approximately 100 lice species have been detected in the birds in Turkey, until today. This species have been reported from Greylag Goose *(Anser anser)* in Van [18] and Wild Swans in Samsun, Turkey [10]. *T. anserinum* was recorded from Whooper Swan *(Cygnus cygnus)* for the first time in this study, in Turkey. In this study, one lice species, *Trinoton anserinum* was found on the whooper swan *(Cygnus cygnus)*.

Heartworm in swans and geese (birds of the order Anseriformes) is caused by *Sarconema eurycerca*, a filarial nematode of the superfamily filarioidea. *S. eurycerca* was recorded from whooper swan *(Cygnus cygnus)* for he first time in this study, in Turkey. This species has been previously reported from Mute Swan *(Cygnus olor)* in England [14], Mute Swan *(Cygnus olor)* in Netherlands [19], Whistling Swan *(Cygnus columbianus)* in Maryland [20] and Whooper Swan *(Cygnus cygnus)* in Korea [2].

Castresana et al.[16] compared species *T. querguedulae* and *T. anserinum* as morphologically and reported that while in the *T. anserinum*'s quetotaxia spines are shorter and mostly silky-like setae, the *T. querquedulae* species are longer and mostly spine-like setae. Additionally, they reported that 3rd femura has fewer and smaller sized brunch setae in *T. anserinum* compared to *T. querguedulae* [16]. Therefore, these specimens were identified as *T. anserinum*.

Previous researchers reported that *T. anserinum* which was found in the host is seen in all species of the genus Anser [6,16,21,22]. It has also been cited that *S. eurycerca* whether causes to or being the primary cause of death of whistling swan and mute swan [3,20]. Three avian filarioids *(Pelecitus*, *Splendidofilaria* and *Sarconema)* may infect swan and geese of the Anatidae family. *Splendidofilaria* species induce lesions in various organs and tissues, such as the heart, aorta, pulmonary arteries, abdominal cavity, kidney, trachea, esophagus, eyes and skin. Various species of wild birds may be infected. *S. eurycerca* cause lesions only in the heart and only swans and geese were affected. Seegar [14] reported that the microfilariae of *S. eurycerca* was sheathed and measured 270-340 µ in length and 4.5-6.5 µ in width, and Cohen et al.[23] also reported the measurement 263-382 µ. In this study the microfilariaes were measured between 278-290 µ x 6-7 µ.

In conclusion, *T. anserinum* and *S. eurycerca* are reported in whooper swan in Turkey for the first time.

REFERENCES

1. Cole RA: Heartworm of swans and geese. **In,** Friend M, Franson JC (Eds): Field Manual of Wildlife Diseases: General Field Procedures and Diseases of Birds Biological Resources Division, U.S. Geological Survey, Washington, DC, 233-234, 1999.

2. Woo GH, Jean YH, Bak EJ, Kang S, Roh IS, Lee KH, Hwang EK, Lee OS: Myocarditis by Nematodes ınfection, presumably *Sarconema eurycerca*, in

a wild whooper swan *(Cygnus cygnus)* in Korea. *J Vet Med Sci*, 72, 1233-1235, 2010. DOI: 10.1292/JVMS.10-0075

3. Irwin JC: Mortality factors in whistling swans at Lake St. Clair, Ontario. *J Wild Dis*, 11, 8-12, 1975.

4. Wehr E: New genera and species of Filaroidea. III. *Sarconema eurycerca* n. gen. n. sp. *Proc Helminthol Soc Wash*, 6, 95-97, 1999.

5. Seegar WS: Prevalence of the heartworm, *Sarconema eurycerca*, Wehr. 1939 (Nematoda), in whistling swan, *Cygnus columbianus columbianus*. *Can J Zool*, 57, 1500-1502, 1979. DOI: 10.1139/z79-194

6. Seegar WS, Schiller EL, Sladen WJ, Trpis M: A mallophaga, *Trinoton anserinum*, as a cyclodevelopmental vector for a heartworm parasite of waterfowl. *Science*, 194, 739-741, 1976.

7. Clay T: Some problems in the evolution of a group of ectoparasites. *Evolution*, 3, 279-299, 1949. DOI: 10.2307/2405715

8. Keirans JE: The Mallophaga of New England birds. Agricultural Experiment Station University of New Hampshire Durham, New Hampshire. Station Bulletin, 492, 1967.

9. Fryderyk S: *Trinoton querquedulae* (Linnaeus,1758) (Phthiraptera: Amblycera, Menoponidae) a rare parasite of the Eider duck *Somateria mollissima* (Linnaeus, 1758). *Ann Parasitol*, 59, 21-23, 2013.

10. Açıcı M, Umur Ş, Gürsoy A: Yabanıl bir kuğuda *Trinoton anserinum* olgusu. *XIV. Ulusal Parazitoloji Kongresi,* 18-25 Eylül, İzmir, pp.225-226, 2005.

11. Aksin N: Chewing lice (Insecta: Phthiraptera) on Mallards *(Anas platyrynchos)* in Turkey. *J Anim Vet Adv*, 10, 1656-1659, 2011. DOI: 10.3923/javaa.2011.1656.1659

12. Dik B, Uslu U: Studies on chewing lice species found on some duck (Anseriformes: Anatidae) species at Lake Akşehir, Turkey. *Kafkas Univ Vet Fak Derg*, 18, 1055-1060, 2012. DOI: 10.9775/kvfd.2012.7020

13. Clay T, Hopkins GHE: The early literature on Mallophaga (Part IV, 1787-1818). *Bull Br Mus (Nat Hist) Entomol,* 9, 1-61, 1960.

14. Seegar WS: Prevalence of parasitic heartworms in swans in England. *Wildfowl,* 30, 147-150, 1979.

15. Cohen S: *Sarconema eurycerca* (Wehr): The heartworm of swans and the role of *Trinoton anserinum* (F) as an intermediate host. *A Doctoral Thesis.* Submitted in partial fulfillment of the requirements for the award of Doctor of Philosophy of Loughborough University. 1988.

16. Castresana L, Notarıo A, Mateo PM: Study of the ectoparasitic mallophaga of Anatidae (Insecta, Mallophaga) in the Iberian Peninsula. Identification, biometric characteristics and biological aspects. *Zool Baetica*, 10, 63-86, 1999.

17. Price RD, Hellenthal RA, Palma RL, Johnson KP, Clayton DH. The Chewing Lice: World Checklist and Biological Overview. Illinois Natural History Survey Special Publication, 24, 2003.

18. Göz Y, Dik B, Kılınç ÖO, Aslan L: Chewing lice (Phthiraptera: *Amblycera*, *Ischnocera*) on several species of wild birds around the Lake Van basin, Van, Eastern Turkey. *Kafkas Univ Vet Fak Derg,* 21, 333-338, 2015. DOI: 10.9775/kvfd.2014.12484

19. De Bruijn ND, Velkers FC, Gröne A: Heartworm in a mute swan *(Cygnus olor). Tijdschr Diergeneeskd*, 134, 882-884, 2009.

20. Holden BL, Sladen, WJL: Heartworm, *Sarconema eurycerca*, infection in whistling swans, *Cygnus columbianus*, in the Chesapeake Bay. *Bull Wildl Dis Assoc,* 4, 126-8,1968. DOI: 10.7589/0090-3558-4.4.126

21. Pennycott TW: Lead poisoning and parasitism in a flock of mute swans *(Cygnus olor)* in Scotland. *Vet Rec Jan*, 142, 13-17, 1998.

22. Acuna-Gonzales D, Lara J, Cicchino A: New records of lice (Insecta: Phthiraptera) from domestic and ornamental birds from Chile. *Arc Med Vet*, 41, 181-184, 2009. DOI: 10.4067/S0301-732X2009000200013

23.Cohen S, Green Wood MT, Fowler JA: The louse *Trinoton anserinum* (Amblycera: Phthiraptera), an intermediate host of *Sarconema eurycerca* (Filarioidea: Nematoda), a heartworm of swans. *Med Vet Entomol*, 5, 101-110, 1991.

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Case Report

Belçika Malinois Köpeğe Ait 12 Adet Fötusta Schistosoma Reflexum Olgusu

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Özet

Dört yaşlı Belçika Malinois ırkı köpeğin gebeliğinin 60. gününde USG muayenesinde yavruların kalp atımları alınmamıştır. Sezeryan operasyonuyla çıkarılan ve Erciyes Üniversitesi Veteriner Fakültesi Patoloji Anabilim Dalı'na nekropsi isteği ile getirilen 12 adet ölü yavrusu bu olgu sunumunun materyalini oluşturmaktadır. Yapılan nekropsi sırasında ağırlıkları 220-440 g arasında değişen yavruların tamamında, karın bölgesinde şekillenen 0.4-1.7 cm uzunluğundaki yarıklardan bağırsakların bir kısmının dışarıya çıktığı tespit edildi. Oluşan şiddetli otolitik değişiklikler nedeniyle mikroskobik değerlendirme yapılamadı. Yavruların lateral ve ventro-dorsal çekilen columna vertebralis radyografilerinde patolojik bir bulguya rastlanmadı. Bu rapora, nekropsi bulguları ve radyografik incelemeler sonucunda Schistosoma reflexum tanısı konmuş ve köpeklerde ender görülmesi nedeniyle yayın haline getirilmiştir.

Anahtar sözcükler: *Schistosoma reflexum, Köpek, Radyografi, Konjenital anomali*

A Case of Report Schistosoma Reflexum in the 12 Fetuses of Belgian Malinois Dog

Abstract

The USG examinations 4-year-old female Belgian Malinois dogs on the 60th day of pregnancy, the heartbeat of puppies were not measured. The material of case was 12 puppies were referred to the Erciyes University, Faculty of Veterinary Medicine and Department of Pathology to necropsy introduced were formed by cesarean. At necropsy all of the puppies weights' ranging between 220-440 g, shaped region of 0.4-1.7 cm in height rupture was determined that out of part intestines. Microscopic evaluation was not possible due to severe autolysis changes are occurring. Ventro-dorsal lateral radiographs taken vertebral column did not show any pathological findings. This report with necropsy findings and radiographic examinations were diagnosed with Schistosoma reflexum which was became into publication because it is rare in dogs.

Keywords: *Schistosoma Reflexum, Dog, Radiography, Congenital Anomaly*

GİRİŞ

Konjenital anomaliler; evcil hayvanlarda, eksojen ve endojen (kalıtsal) faktörlere bağlı olarak ontogenez sırasında normalden ayrı bir yapılışta olmasıdır [1]. Gözlenen bu anomalilerin çoğunun etiyopatolojisi hakkında kesin bir bilgi bulunmamasının yanında, multifaktöriyel sebepler; genetik faktörler, mutasyonlar, infeksiyon ajanlar, çevresel faktörler veya bu faktörlerin kombinasyonu söz konusudur [1,2].

Amnion zarının hatalı şekillenmesi sonucu gövde yarıklarının oluştuğu düşünülmekle birlikte, cyclin kinase inhibitör proteinleri ile ilgili metabolizma bozukluklarına bağlı olabileceği de bildirilmiştir [1,3]. Schistosoma reflexum, fissura abdominalisin özel bir formu olup, göğüs ve karın bölgesindeki organların, ince amniojen bir kese ile örtülü olarak karın boşluğundan dışarı çıkması şeklinde görülmektedir [1]. Çiftlik hayvanlarından en çok sığırlarda karşılaşılan bir konjenital anomali [4] olup görülme sıklığı %1.3 olarak bildirilmektedir [5]. Fakat deve [6], Köpek [7], kedi [8] ve insanlarda [1] nadirde olsa görülmektedir. Evcil hayvanlar dışında, bu anomalinin görülebildiği ve bir hayvanat bahçesinde beyaz gergedanın *(Ceratotherium simum simum)* yavrusunda rastlanıldığı rapor edilmiştir [9].

Türkiye'de bugüne kadar schistosoma reflexum olarak tanımlanan anomali, sığırlarda bildirilirken, köpek ve kedide birer tane olarak literatüre geçmiştir [5,7,8].

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Schistosoma reflexum olgularında güç doğum sebebiyle, sezeryan ya da fötotomi uygulamalarına zorunlu olarak başvurulmaktadır [1,4].

Bu raporda, aynı batına ait sezeryan operasyonuyla alınan 12 adet ölü köpek yavrusuna nekropsi bulguları ve radyolojik incelemeler sonucunda Schistosoma reflexum tanısı konulan olgular sunulmuştur. Uluslararası ve ülkemiz literatür verilerinde köpeklerde Schistosoma reflexum'un ender görülmesi ve olgumuzdaki gibi bir yayının olmaması nedeniyle bu konuda daha sonra bildirilecek vakalara ışık tutması amaçlanmıştır.

OLGUNUN TANIMI

Dört yaşlı Belçika Malinois ırkı köpeğin gebeliğinin 60. gününde USG muayenesinde yavruların kalp atımları alınmamıştır. Sezeryan operasyonuyla çıkarılan ve Erciyes Üniversitesi Veteriner Fakültesi Patoloji Anabilim Dalı'na nekropsi isteği ile getirilen 12 adet ölü yavrusu bu çalışmanın materyalini oluşturmaktadır *(Tablo 1)*. Anne daha önce 2012 ve 2013 yıllarında gebe bırakılarak normal görünümlü ve sağlıklı, sırasıyla 5 ve 12 adet yavru dünyaya getirmiştir. Bu gebeliklerde aynı ırktan farklı erkek köpekler kullanılmıştır. Vakamızda da aynı ırktan farklı bir köpek ile çiftleştirilmiş ve gebe kalan dişi köpeğin, 12 adet yavrusu ölü olup, sezeryanla alınmıştır. Yapılan sistemik nekropsi sonucunda hazırlanan doku kesitleri, %10'luk nötral formalin solüsyonunda tespit edildikten sonra rutin prosedür izlendi ve parafine gömüldü. Doku kesitleri 5-7 mikron kalınlığında kesildi ve hematoksilen-eozin ile boyanarak ışık mikroskobunda değerlendirildi.

Sezaryen operasyonuyla çıkarılan 12 adet yavrunun tamamında karın bölgesindeki yırtıklardan barsakların bir kısmının dışarıya çıktığı tespit edildi *(Şekil 1)*.

Mikroskobik olarak; şiddetli otolitik değişiklikler nedeniyle 12 adet yavrudan alınan doku örneklerinden hazırlanan preparatların histolojik değerlendirilmesi yapılamadı.

Yavruların lateral ve ventro-dorsal yönlerde alınan columna vertebralis radyografilerinde patolojik bir bulguya rastlanılmadı *(Şekil 2, Şekil 3)*.

Tablo 1. *Yavruların ağırlıkları ve karın bölgesindeki yırtıkların boyutları*
Table 1. *The weight of the puppies and rupture in the abdominal region*

Olgu No	Yavrunun Ağırlığı	Karın Bölgesindeki Yırtığın Boyutu
1	350 g	1.7 cm
2	300 g	1.3 cm
3	290 g	1 cm
4	310 g	0.7 cm
5	330 g	0.5 cm
6	330 g	1 cm
7	320 g	0.5 cm
8	335 g	1 cm
9	440 g	0.8 cm
10	320 g	0.5 cm
11	280 g	0.8 cm
12	400 g	0.4 cm

TARTIŞMA ve SONUÇ

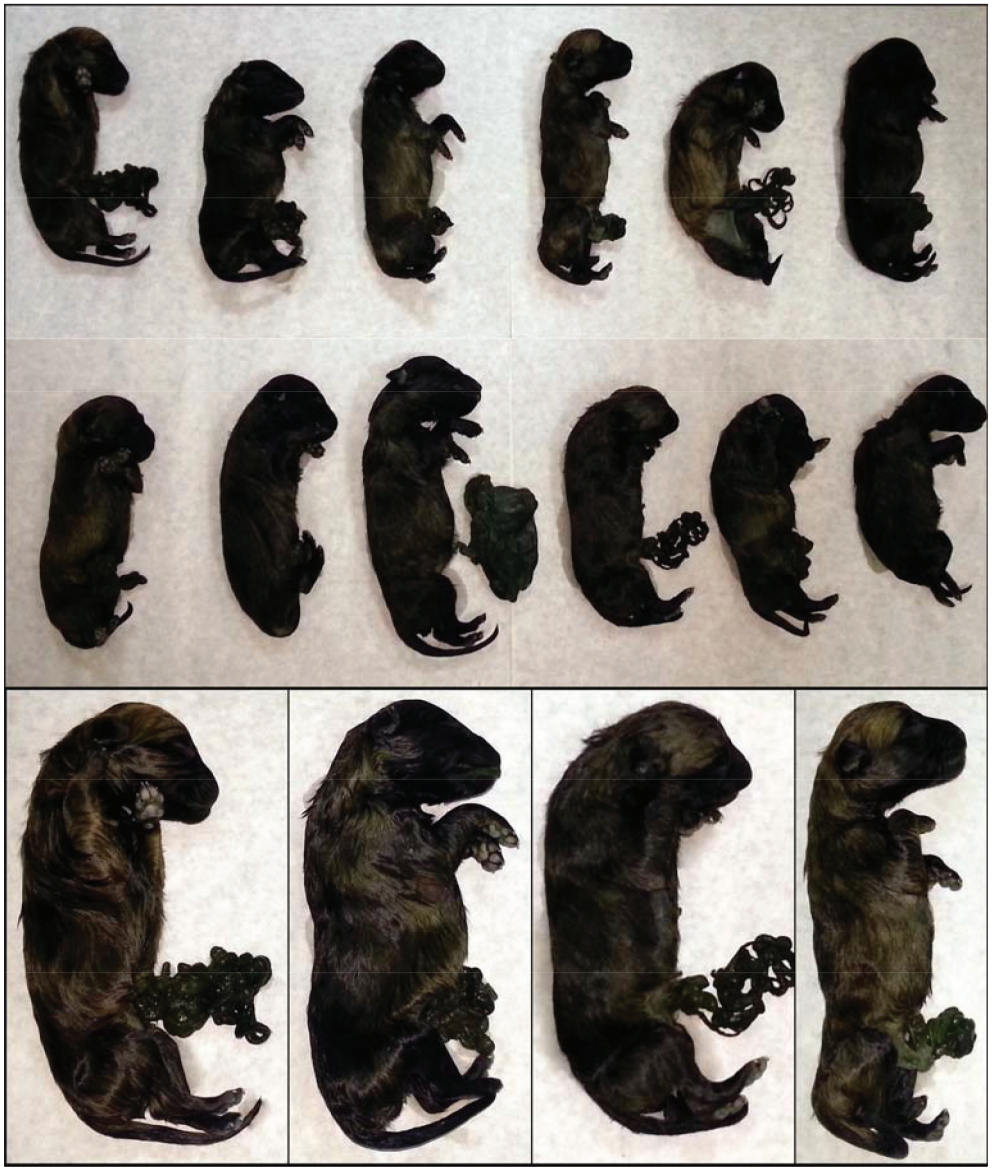
Schistosoma reflexum anomalisinin oluşumuyla ilgili olarak literatürlerde teratojenik etkilerden çok mutant genler veya kromozomal anomaliler sonucu oluşabileceği düşünülmektedir [8]. Özalp ve ark.[8] schistosoma reflexum olgularında, yavrudan alınan lenfosit kültürlerinde, kromozom ve kromatidlerin uç bölgelerinin, homolog olmayan eşleşmeler yaptığını ve bu uçların ezilmiş ve disentrik fragmentler halinde bulunduğunu saptamış, bu kromozomal bozukluğun annede herhangi bir değişikliğe sebep olmadığını bildirmişlerdir. Sunulan olguda anne ve yavrulardan kültür alınmadığı için, kromozomal bir deformitenin değerlendirmesi yapılamamıştır.

Schistosoma reflexum olgularında göğüs ve karın organlarının dışarı çıkması ile birlikte omurgada lordozis [1,8] ve bacaklarda ankiloz [5] durumları da gözlenebilmektedir. Özellikle olgumuzda karın boşluğu organlarından barsakların dışarı çıkması literatür [1,8] verileriyle uyumluluk göstermekle birlikte, omurgada lordozis [1,8] ve bacaklarda ankiloz [5] durumunu bildiren vakalardan farklı olarak yavruların Lateral (lordozis tespiti için) ve ventro-dorsal (skoliozis tespiti için) çekilen radyografilerinde bu durumlar tespit edilememiştir.

Fissura abdominalis American Staffordshire Terrier, Yorkshire Terrier, Chihuahua, English Bulldog ırkı köpeklerde gözlenirken [7], German Shepherd ırkı köpek yavrusunda [8] ve iki yaşlı Persian ırkı bir kedinin üç yavrusunun, ikisinde [10] schistosoma reflexum olgusunun tanımlandığı bildirilmiş olup, bu vakadaki (Belçika Malinois) ırk ile bir benzerlik göstermemesi ve farklı ırklarda görülmesi bir ırk predispozisyonun olmadığını düşündürmektedir.

Organların, özellikle akciğer, karaciğer ve kalbin hipoplazik olarak tanımlandığı schistosoma reflexum vakalarında, mikroskobik olarak, otolitik dokuların yanısıra nekrotik ve dejeneratif karaciğer lezyonları bildirilmiştir [7,8]. Olgumuzda incelenen yavruların organlarında makroskobik olarak bir lezyon gözlenmezken, alınan doku örneklerinin histopatolojik incelemelerinde otolitik değişikliklerin görülmesi daha önceki bildirilen olgularla [7,8], benzerlik gösterirken, oluşan şiddetli otoliz nedeniyle nekrotik ve dejeneratif karaciğer değişiklikleri hakkında yorum yapılamamıştır.

Anomali oluşumuna sebep olan mekanizmaların karmaşıklığından dolayı pek çok konjenital anomalide etiyoloji tam olarak belirlenememektedir. Genetik veya çevresel faktörlerin etiyolojide rol aldığı düşünülmekte-



Şekil 1. Schistosoma reflexum'lu köpek yavrularının görünümü
Fig 1. View of the Schistosoma reflexum puppies

dir [11]. Schistosoma reflexum olgularının tümü, tam anlamıyla kalıtsal olmasa da, babanın genetik durumu söz konusu olarak kalıtsal bir hastalık olduğu düşünülmektedir [4]. X'e bağlı otozomal resesif kalıtım ile ilişkili olup, atalarını oluşturan erkek ya da dişide dominant olmadığı savunulmaktadır [12]. Vakada anne daha önceki iki gebeliğinde, farklı erkek köpeklerde çiftleşmiş, sağlıklı yavrular dünyaya getirmiştir. Son gebeliğinde ise farklı bir köpek ırkı ile çiftleşmesi, doğuma yakın yavrularda USG muayenesi'nde kalp atımları alınamayınca sezaryen operasyonu ile çıkarılan yavrularda schistosoma reflexum görülmesi, genetik bir anomali olabileceğini düşündürmüştür.

Sonuç olarak, uluslararası ve ülkemiz literatür verilerinde köpeklerde Schistosoma reflexum'un ender görülmesi ve olgumuzdaki gibi bir yayının olmaması nedeniyle bu konuda daha sonraki bildirimlere ışık tutması amacıyla vaka rapor haline getirilmiştir.

Teşekkür

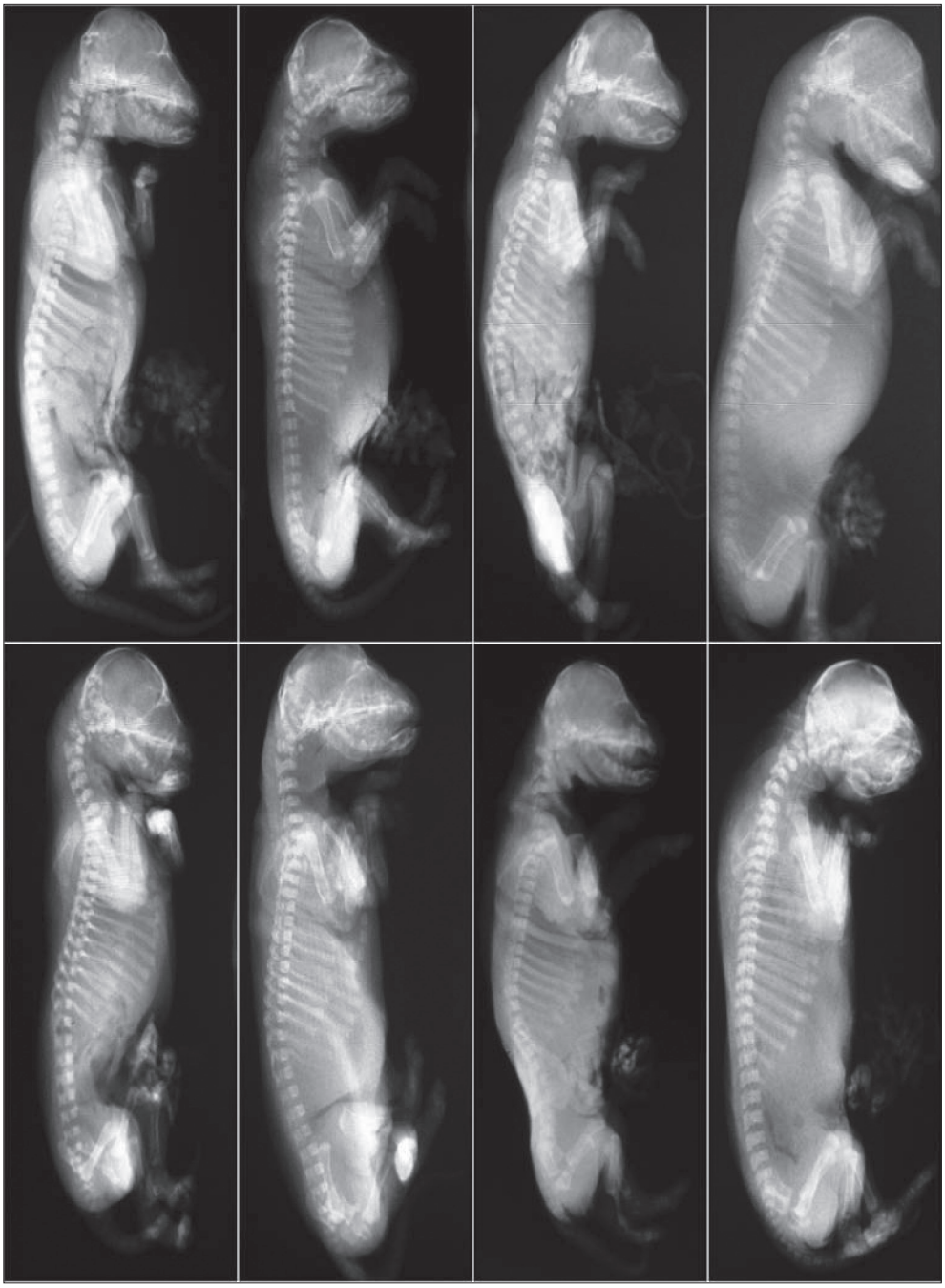
Bu olguda materyali sağlayan Nevşehir Jandarma At ve Köpek Eğitim Merkezi Komutanlığı, Veteriner Kısım Amiri Yüzbaşı Ünal YAVUZ'A teşekkür ederiz.

KAYNAKLAR

1. Kıran MM: Anomaliler. **In,** Erer H (Ed): Veteriner Genel Patoloji. İkinci Baskı. 184-224, Bahçıvanlar Basım Sanayi A.Ş, Konya, 2007.

2. Mazzulo G, Germana A, De Vico G, Germana G: Diprosopiasis in a lamb. *Anat Histol Embryol*, 32, 60-62, 2003. DOI: 10.1046/j.1439-0264.2003.00438.x

3. Sarpestein G: Congenital abnormalities of internal organs and body cavities. *Vet Clin North Am: Food Anim Pract,* 9, 114-125, 1993.



Şekil 2. Köpek yavrularının lateral radyografilerinde karın duvarındaki yırtıktan, bir kısmı dışarı çıkmış bağırsakların görünümü

Fig 2. View of the intestine gone out abdominal wall which Puppies of a rupture in the lateral radiograph

4. Citek J: Pedigree analysis of Czech Holstein calves with schistosoma reflexum. *Acta Vet Scand*, 54, 22-26, 2012. DOI: 10.1186/1751-0147-54-22

5. Aydın M, Karan M, Yüksel M: Buzağılarda rastlanılan farklı schistosoma reflexum olgularının anatomik olarak karşılaştırılması. *F.Ü. Sağlık Bil. Dergisi*, 20 (3): 249-252, 2006.

6. Elias E: Left ventrolateral cesarean section in three dromedary camels *(Camelus dromedarius). Vet Surg*, 20, 323-325, 1991. DOI: 10.1111/j.1532-950X.1991.tb01277.x

7. Özsoy SY, Oto C, Haziroğlu R: Schistosoma reflexum in a dog. *Ankara Üniv Vet Fak Derg*, 56, 225-6, 2009.

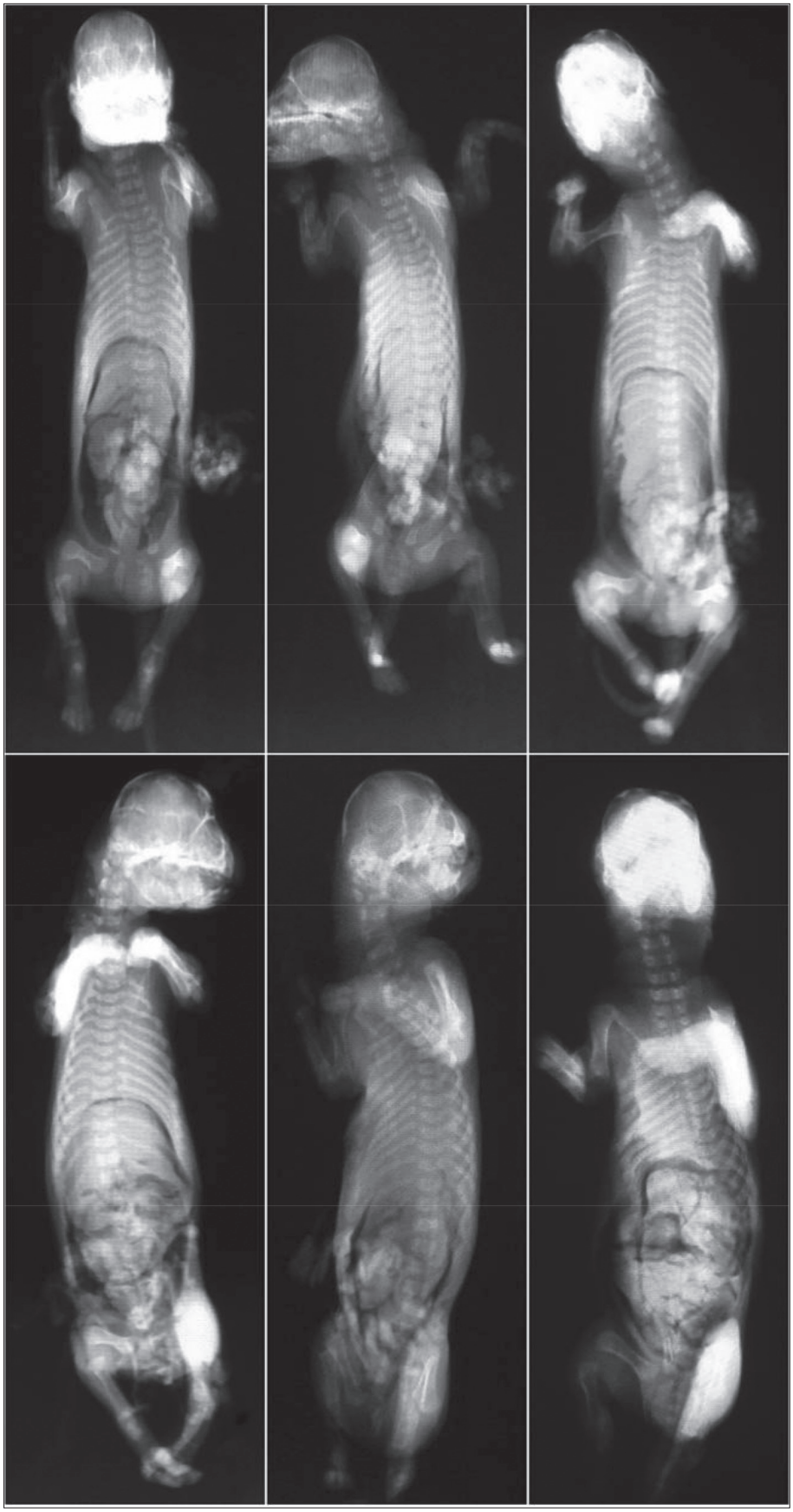
8. Ozalp G R, Celikler S, Simsek G, Ozyigit M O, Inan S: A case of Schistosoma reflexum in a cat with chromosomal aberrations. *Reprod Domest Anim*, 46, 373-376, 2011. DOI: 10.1111/j.1439-0531.2010.01649.x

9. Lankton JS, VanderHart DJ, Terrell SP: Schistosomus reflexus-like malformatıon in a southern white rhinoceros *(Ceratotherıum simum simum). J Zoo Wildlife Med*, 45 (3): 708-711, 2014.

10. Buck BC, Ulrich R, Wohlsein P, Distl O: A case of schistosoma reflexum in a black and white German Holstein calf. *Dtsch Tierarztl Wchschr*, 10, 363-368, 2009.

11. Max A: Congenital fissure of the abdominal wall in puppies. *Med Weter*, 66 (2): 131-133, 2010.

12. Dennis SM, Leipold HW: Ovine congenital defects. *Vet Bull*, 49, 233-239, 1979.



Şekil 3. Köpek yavrularının ventro-dorsal radyografilerinden columna vertabralisin görünümü
Fig 3. View of the columna vertabralis ventro-dorsal radiography of puppy

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Letter to the Editor

Sino-nasal Aspergillosis in a Dog (Bir Köpekte Sino-nazal Aspergilloz)

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Dear Editor,

We would like to report an interesting case of sino-nasal aspergillosis in a dog with the rhinoscopic results.

Chronic nasal discharge is a common clinical sign of respiratory tract disease in dogs [1]. It is resulted from fungal infection, neoplasia, foreign body or some oral disease [2,3]. Fungal infections of the nasal cavity are relatively uncommon in dogs [3-6]. In a study evaluated 80 dogs with nasal disease [6], the reported rates of the diagnosis include non-specific rhinitis (23.7%), neoplasia (15%), fungal infection (nasal aspergillus) (8.7%), cleft palate (8.7%), periodontal disease (4%), parasites (1.3%), foreign body (1.3%), primary bacterial disease (1.3%) and idiopathic (36%). In practice, most of the clinician at the first step of diagnostic work-up on nasal discharge in dogs with aspegillosis has been focusing on bacterial etiology, thereby resulting in increasing disease severity and time-consuming effects of antibacterial administrations and its unfavorable results. As most of the available diagnostic tests have limitations, a combination of tests is often necessary to confirm a diagnosis [4,5]. Thus, the aim of this case was to highlight the importance of rhinoscopic examination and fungal culture to diagnose of sino-nasal aspergillosis in a dog with chronic nasal discharge.

A dog (5 years old, Setter, male) was referred to Small Animal Clinic (Faculty of Veterinary Medicine, Uludag University, Bursa) with an anamnesis of chronic intermittent muco-hemorrhagic discharge from the right nasal passage *(Fig. 1)*. Also, dog was un-responsive to empirical antibiotic use (Enrofloxacin, 5 mg/kg, SC, once a day, for 5 days; Baytril®, Bayer, Italy) by a private vet. Clinically, sneezing and snoring were observed. Routine hematological (HM5, automatic blood analyzer, Abaxis) and biochemistry panels (Comprehensive profile, VetScan, Abaxis) were within reference ranges (data not shown). Rhinoscopic examination was applied to evaluate the nasal cavity and to help the exact diagnosis.

General anesthesia was acquired with 10 mg/kg/i.m., ketamine HCl (Alfamine®, Egevet, Turkey) injection after 2 mg/kg/i.m., xylazine HCl (Alfazyn®, Egevet, Turkey) sedation. The dog was laid down sternoabdominal and rhinoscopic examination was performed using 2.7 mm diameter rigid telescope and additional equipment (Karl Storz®, Germany). During examination, cold lactate ringer solution was insufflated locally to provide the clear endoscopic view and to prevent the mucosal hemorrhage from the inflammative spots on mucosal layer. Lesion was determined over the right ostium nose *(Fig. 1)*. Rhinoscopy revealed local hemorrhagic spots, congestion, mucosal polyp-like structures and erosions in the nasal cavity,

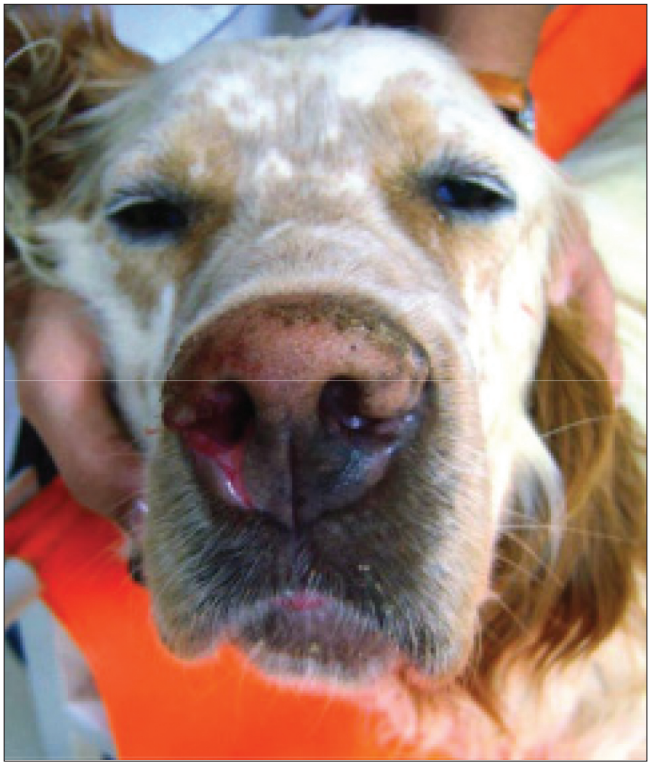

Fig 1. Lesion over the right ostium nose due to nasal discharge

Şekil 1. Sağ ostium nazi çevresinde nasal akıntı nedenli şekillenmiş lezyon

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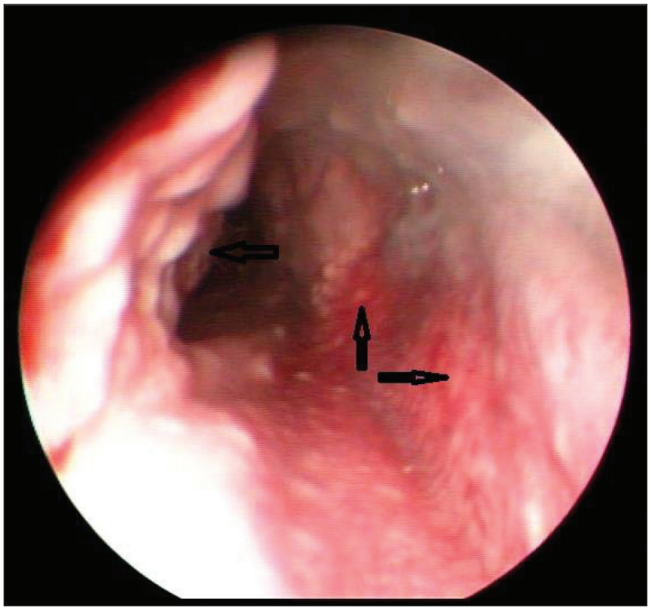

Fig 2. Congestion and mucosal protrusions *(arrows)* on nasal mucosa
Şekil 2. Nazal mukozadaki konjesyon ve mukozal çıkıntılar *(oklar)*

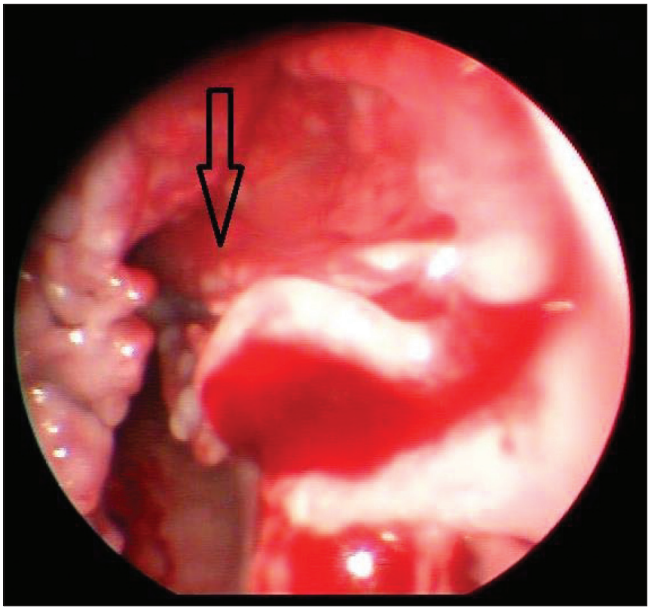

Fig 3. Hemorrhage and polyp-like proliferations on concha mucosa *(arrow)*
Şekil 3. Konha mukozasında kanama ve polip benzeri üremeler *(ok)*

particularly in the conchal part *(Fig. 2)*. Nasal sample was collected for microbiologic culture, and then the rhinoscopy of left nasal cavity was completed, which was clear from the pathology and considered as normal. Nasal sample was cultured with SDA, and incubated with 25°C for a week. Microbiological examination revealed the *Aspergillus spp*. Thus, sino-nasal aspergillosis was diagnosed, and then treated with oral antifungal agent, Ketocanozole 10 mg/kg (Ketoral tablet, Bilim Ltd, Turkey). Fourteen days later, therapy resulted with full recovery, based on the clinical and rhinoscopic examinations.

REFERENCES

1. Beniath N: Canine nasal aspergillosis. *Clin Tech Small Anim Pract*, 21, 82-88, 2006. DOI: 10.1053/j.ctsap.2005.12.015

2. Cooke K: Sneezing and nasal discharge. **In,** Ettinger SJ, Feldman EC (Eds): Textbook of Veterinary Internal Medicine. 207-210, Elsevier Saunders, Saint-Louis, USA, 2005.

3. Tasker S, Knottenbelt CM, Munro EA, Stonehewer J, Simpson JW, Mackin AJ: Etiology and diagnosis of persistent nasal disease in the dog: A retrospective study of 42 cases. *J Small Anim Prac*, 40, 473-478, 1999. DOI: 10.1111/j.1748-5827.1999.tb02998.x

4. Sharman MJ, Mansfield CS: Sinonasal aspergillosis in dogs: a review. *J Small Anim Pract,* 53, 434-444, 2012. DOI: 10.1111/j.1748-5827.2012.01245.x

5. Peeters D, Clercx C: Update on canine sinonasal aspergillosis. *Vet Clin Small Anim Pract*, 37, 901-916, 2007. DOI: 10.1016/j.csvm.2007.05.005

6. Meler E, Dunn M, Lecuyer M: A retrospective study of canine persistent nasal disease: 80 cases (1998-2003). *Can Vet J,* 49, 71-76, 2008.

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 şekil ve tablo gibi görsel öğelerin 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 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 oluşur ve toplam (metin, tablo, şekil vs dahil) 12 sayfayı geçemez. Yabancı dilde yazılmış makaleler yabancı dilde başlık, yabancı dilde özet ve anahtar sözcükler, Türkçe başlık, Türkçe özet ve anahtar sözcükler dışında Türkçe makale yazım kurallarında belirtilen diğer bölümlerden oluşur. Türkçe ve yabancı dilde özetlerin her biri yaklaşık 200±20 sözcükten oluşmalıdır.
Kısa Bildiri, konu ile ilgili yeni bilgi ve bulguların bildirildiği fakat orijinal araştırma olarak sunulamayacak kadar kısa olan yazılardır. Kısa bildiriler, orijinal araştırma makalesi formatında olmalı, fakat özetlerin her biri 100 sözcüğü aşmamalı, referans sayısı 15'in altında olmalı ve 6 sayfayı aşmamalıdır. Ayrıca, en fazla 4 şekil veya tablo içermelidir.
Ön Rapor, kısmen tamamlanmış, yorumlanabilecek aşamaya gelmiş orijinal bir araştırmanın kısa (en çok 4 sayfa) anlatımıdır. Bunlar orijinal araştırma makalesi formatında yazılmalıdır.
Gözlem (Olgu Sunumu), uygulama, klinik veya laboratuar alanlarında ender olarak rastlanılan olguların sunulduğu makalelerdir. Bu yazıların başlık ve özetleri orijinal makale formatında yazılmalı, bundan sonraki bölümleri Giriş, Olgunun Tanımı, Tartışma ve Sonuç ile Kaynaklar bölümlerinden oluşmalı ve 4 sayfayı geçmemelidir.
Editöre Mektup, bilimsel veya pratik yararı olan bir konunun veya ilginç bir olgunun resimli ve kısa sunumudur ve 2 sayfayı geçmemelidir.
Derleme, güncel ve önemli bir konuyu, yazarın kendi görüş ve araştırmalarından elde ettiği bulguların da değerlendirildiği özgün yazılardır. Bu yazıların başlık ve özet bölümleri orijinal araştırma makalesi formatında yazılmalı, bundan sonraki bölümleri Giriş, Metin, Sonuç ve Kaynaklar bölümlerinden oluşmalı ve 12 sayfayı geçmemelidir.
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.
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