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RESEARCH ARTICLE

Survey of Anaplasma Infections in Small Ruminants from East Part of Turkey

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Summary

This study was carried out to determine the presence and frequency of *Anaplasma ovis* and *Anaplasma phagocytophilum* in small ruminants from Bingol, Elazig, Malatya and Mus provinces. A total of 422 (291 sheep and 131 goats) blood samples were collected from apparently healthy animals. To determine of *A. ovis* and *A. phagocytophilum* in small ruminants, species-specific PCRs were set up using 60 kDA chaperonin gene (cpn60, also known as hsp60 or groEL) and 16S SSU rRNA gene primer sets, respectively. A total of 301 (71.32%) animals were found infected with *A. ovis* and/or *A. phagocytophilum*. The percentages of positive animals for *A. ovis* and *A. phagocytophilum* were 67.06% (283/422) and 19.66% (83/422), respectively. The rate of concurrent infections was 15.40% (65/422). Four PCR products from positive samples were purified from agarose gel and sequenced. These sequences were identical to the reported nucleotide sequences of *A. ovis* and *A. phagocytophilum*. This is the first molecular based study on the detection of *A. phagocytophylum* and *A. ovis* in small ruminants from East Anatolia Region. Further studies are needed on the determination of the genotypes and vectors of the species.

Keywords: *Anaplasma ovis, Anaplasma phagocytophilum, Sheep, Goat, East Anatolia Region*

Doğu Anadolu Bölgesinde Koyun ve Keçilerde *Anaplasma* Enfeksiyonlarının Araştırılması

Özet

Bu çalışma Bingöl, Elazığ, Malatya ve Muş yöresindeki koyun ve keçilerde *Anaplasma ovis* ve *Anaplasma phagocytophilum*'un araştırılması amacıyla yapılmıştır. Rastgele seçilen 422 sağlıklı (291 koyun 131 keçi) hayvandan kan örneği alınmıştır. *A. ovis* için 60 kDA chaperonin gen (cpn60, hsp60 veya gro EL olarak da bilinir) ve *Anaplasma phagocytophilum* için 16S SSU rRNA genlerine spesifik primerler kullanılarak tür spesifik PCR yapılmıştır. PCR sonucunda toplam 301 (%71.32) hayvan *A. ovis* ve/veya *A. phagocytophilum* ile enfekte bulunmuştur. Örneklerin %67.06 (283/422)'sı *A. ovis*, %19.66 (83/422)'sı *A. phagocytophilum* ve %15.40 (65/422)'ı her iki tür yönünden pozitif olarak tespit edilmiştir. *A. ovis* ve *A. phagocytophilum* yönünden pozitif olan PCR ürünleri agaroz jelden purifiye edilerek sekanslanmıştır. Elde edilen DNA dizilimlerin daha önce bildirilen *A. ovis* ve *A. phagocytophilum*'a ait dizilimlerle aynı olduğu görülmüştür. Bu çalışma Doğu Anadolu Bölgesinde koyun ve keçilerde *A. ovis* ve *A. phagocytophilum*'un moleküler yöntemlerle teşhisi üzerine yapılan ilk araştırmadır. Türlerin genotipleri ve vektörlerinin belirlenmesine yönelik çalışmaların gerekliği düşünülmektedir.

Anahtar sözcükler: *Anaplasma ovis, Anaplasma phagocytophilum, Koyun, Keçi, Doğu Anadolu Bölgesi*

INTRODUCTION

Anaplasma species are known to be important tick-borne pathogens of humans and animals. The genus *Anaplasma* comprises *A. phagocytophilum* (previously recognised as *Ehrlichia equi* and *E. phagocytophila*), *A. centrale, A. marginale, A. bovis, A. ovis* and *A. platys*. The species are mainly transmitted by ixodid ticks [1-4].

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Anaplasma phagocytophilum has a wide range hosts including domesticated ruminats, equids, cats, dogs, wild animals and humans. The pathogen causes tick-borne fever in ruminants and granulocytic anaplasmosis in humans, equines and canines [1]. *A. ovis* infects sheep, goats and wild ruminants [5]. It is known as a non-pathogenic species, but the agent has been detected to cause severe disease in bighorn sheep [6]. On the other hand, like with other *Anaplasma* infections, *A. ovis* may predispose hosts to other pathogens [4].

The main tick-borne diseases in Turkey are babesiosis and theileriosis. They are endemic in almost all regions of Turkey [7,8]. In small ruminants, clinical and subclinical *A. ovis* infections were detected in a few studies by microscopic examination [9,10]. Until 2005, in Turkey there was no report that shows the presence of *A. phagocytophilum* in sheep. In parallel with the emergence of tick-borne diseases in humans in recent years, this agent that is thought to be zoonotic was determined in domestic animals and ticks [11-14]. Further studies are needed for epidemiological information on the anaplasmosis.

There are a few molecular studies on *Anaplasma* sp. in the country. Recently, the presence of *A. phagocytophilum* was documented in cattle while *A. ovis* was reported in sheep and some tick species [11-14].

Molecular diagnostic methods as polymerase chain reaction (PCR) have become widely used as sensitive and specific tools for detection and discrimination of tick-borne parasites such as *Theileria* sp., *Babesia* sp. and *Anaplasma* sp. in their vectors and hosts [7,8]. This study was carried out to determine the presence and frequency of *A. ovis* and *A. phagocytophilum* in small ruminants using species-specific polymerase chain reaction (PCR) and sequence analyse methods in the eastern region of Turkey.

MATERIAL and METHODS

The blood samples from sheep and goats were collected from Bingol, Elazig, Malatya, and Mus located in East Anatolia Region of Turkey. A total of 422 (291 sheep and 131 goats) blood samples were collected from apparently healthy animals *(Table 1)*. All of the animals were older than one year.

Total genomic DNA extraction from the blood samples was performed according to previously described method [15]. Briefly, 125 µl of blood was added to 250 µl of lysis mixture (0.32 M sucrose, 0.01 M Tris, 0.005 M $MgCl_2$, 1% Triton X-100, pH 7.5) and the mixture was centrifuged at 11.600 x g for 1 min. The pellet was washed three times by centrifugation with 250 µl lysis buffer. The supernatants were discarded and the final pellets were resuspended in 100 µl of PCR buffer (50 mM KCl, 10 mM Tris-HCl (pH 8), 0.1% TritonX-100, pH 8.3). Proteinase K (50 µg/ml) was added to the pellet suspension and the mixture was then incubated at 56°C for one h. At last, the samples were boiled at 95°C for 10 min.

To determine the presence and frequency of *A. ovis* and *A. phagocytophilum* in small ruminants, species-specific PCRs were set up using 60 kDA chaperonin gene (cpn60, hsp60 or groEL) and 16S SSU rRNA gene primer sets, respectively. Primers SSAP2f (5'-GCTGAATGT GGGGATAATTTAT - 3') and SSAP2r (5'-ATGGCTGCTTCCTTT CGGTTA - 3') are specific for *A. phagocytophilum* [16]; JH0011 (5'- TAAAAGCCAAGGAGGCTGTG - 3') and JH0012 (5'- TTGCTCTCCTCGACCGTTAT - 3') are specific for *A. ovis* [17]. The PCRs were performed according to previously described methods [16,17].

Four positive PCR products, 2 for each species were purified from agarose gel using a commercial PCR purification kit (Wizard SV gel and PCR clean-up system, Promega, Madison, WI, USA). The partial sequences correponding to the 60 kDA chaperonin gene of *A. ovis* and 16S SSU rRNA gene of *A. phagocytophilum* were obtained. The sequences were compared to sequence databases using the BLAST algorithm. The new sequences were submitted to EMBL/GenBank database.

A chi-squared test was used to evaluate the differences between different parameters. $P<0.05$ was accepted to be statistically significant.

RESULTS

The infection prevalences were determined as the percentage of positive animals for the pathogen DNA detected by species-specific PCR. Four hundred and twenty two blood samples, 291 from sheep and 131 from goats were investigated for presence of *A. phagocytophilum* and *A. ovis* in four provinces (Bingol, Elazig, Malatya, Mus) of East Anatolia Region. As summarized in *Table 1*, 301 (representing 71.32% of analyzed small ruminats) animals were found infected with *A. ovis* and/or *A. phagocytophilum* (data not shown). The percentages of positive animals for *A. ovis* and *A. phagocytophilum* were 67.06% (283/422) and 19.66% (83/422), respectively *(Table 1)*. 65 (15.40%) of analyzed animals was concurrently infected with *A. ovis* and *A. phagocytophilum* (data not shown). The number of *A. ovis* infected sheep and goats were 196 (67.35%) and 87 (66.41%) respectively, whereas the number of *A. phagocytophilum* infected sheep and goats were 55 (18.90%) and 28 (21.37%), respectively *(Table 1)*.

There was not statistically significant diffrences in the prevalence of *Anaplasma* spp. infections between sheep and goats ($P>0.05$). On the other hand, *A. ovis* infection rate was higher than *A. phagocytophilum* in analyzed animals ($P<0.05$).

Table 1. *Anaplasma phagocytophilum and Anaplasma ovis species - specific PCR results by location and animal species in East Anatolia Region*
Tablo 1. *Doğu Anadolu Bölgesinde yerleşim yeri ve hayvan türlerine göre Anaplasma phagocytophilum ve Anaplasma ovis tür spesifik PCR sonuçları*

Locations	*Anaplasma ovis* Percentage (Number) of Positivity						*Anaplasma phagocytophilum* Percentage (Number) of Positivity					
	Sheep		Goats		Total		Sheep		Goats		Total	
	%	n	%	n	%	n	%	n	%	n	%	n
Bingöl	91.30	42/46	45.00	18/40	69.76	60/86	34.78	16/46	10.00	4/40	23.25	20/86
Elazığ	57.55	80/139	46.42	13/28	55.68	93/167	12.94	18/139	35.71	10/28	16.76	28/167
Malatya	62.71	37/59	95.23	20/21	71.25	57/80	16.94	10/59	14.28	3/21	16.25	13/80
Muş	78.72	37/47	85.71	36/42	82.02	73/89	23.40	11/47	26.19	11/42	24.71	22/89
Total	67.35	196/291	66.41	87/131	67.06	283/422	18.90	55/291	21.37	28/131	19.66	83/422

PCR products positive for *A. ovis* and *A. phagocytophilum* were purified and sequenced. The partial sequences of 16S SSU rRNA of *A. phagocytophilum* (GenBank accession nos. JF807995 and JF807994) and 60 kDA chaperonin gene of *A. ovis* (EMBL accession nos. HE580282 and HE580283) were identical to the reported nucleotide sequences of the *A. phagocytophilum* and *A. ovis*.

DISCUSSION

Anaplasmosis is a worldwide tick-borne disease caused by *Anaplasma* species [3]. A paucity of information exists concerning the current actual size of anaplasmosis in Turkey. Recently, *A. phagocytophilum* has been reported in cattle, sheep and ticks [12-14] and *A. ovis* in *R. bursa* [11]. this study was planned to investigate the presence and frequency of *A. phagocytophilum* and *A. ovis* in sheep and goats from the provinces of East Anatolia Region (Bingol, Elazig, Malatya, Mus). We used species-specific PCRs to amplify 60 kDA chaperonin gene (cpn60 or hsp60) [17] and 16S SSU rRNA gene [16] of *A. ovis* and *A. phagocytophilum*, respectively. We confirmed specificity of the PCRs with sequences analysis. The sequences of DNA fragments obtained in this study showed 100% identity to the recently reported sequences of *A. phagocytophilum* and *A. ovis*.

From the 422 animals, 301 (71.32%) were infected with *A. phagocytophilum* and/or *A. ovis* (data not show). The concurrent infection rate in analyzed animals was 15.40% (65/422) (data not show). Prevalence of *A. phagocytophilum* and *A. ovis* were 18.90% (55/291) and 67.35% (196/291) in sheep, 21.37% (28/131) and 66.41% (87/131) in goats, respectively. Prevalence of *A. ovis* was higher than *A. phagocytophilum* in both sheep and goats ($P<0.05$). There is not any report on ovine anaplasmosis based on molecular diagnostic tools in East Anatolia Region. This study is first molecular survey on the ovine anaplasmosis in the region.

The presence and frequence of *Anaplasma* infections might be associated with many factors including geographical and climatic diversity, tick species and reservoir hosts. *Ixodes ricinus* known to be main vector of *A. phagocytophilum* and the prevalence of the pathogen is closely related with the ratio of *Ixodes* spp. in the same enviroment [16,18]. *A. ovis* is transmitted by *Rhipicephalus. bursa* and other ticks [3]. Several Ixodid tick species are distributed in Turkey [19]. While *I. ricinus* is dominant tick species in the Black Sea region of Turkey, *Hyalomma* and *Rhipicephalus* species are dominant in the East of Turkey [10,18]. According to the literatures [9,10], *A. phagocytophilum* was detected in *I. ricinus* in the Black Sea region and *A. ovis* was detected in *R. bursa* in the East of Turkey. Tick infestation status of the examined animals was not investigated in this study. We reported that *A. ovis* and *A. phagocytophilum* infections are frequent in the East of Turkey *(Table 1)*. Further studies are needed to determine the tick vectors, reservoirs, hosts and genotypes of *Anaplasma* species.

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RESEARCH ARTICLE

A Comparison of Some Random Regression Models for First Lactation Test Day Milk Yields in Jersey Cows and Estimating of Genetic Parameters [1]

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Summary

This study was conducted to compare random regression models for third order Ali Schaeffer (AS), Wilmink (W) and Legendre polynomials (L) on estimation of genetic parameters for first lactation milk yield in Jersey cows. For this aim, data used in this study were 6387 official milk yield records from monthly recording of 686 first lactations between 1996 and 2011 in Karakoy Agricultural State Farm, Samsun (Turkey). In this study, (co)variance components, heritability for first lactation test day milk yields (TDMY) and genetic correlations among these TDMYs were estimated by using DFREML statistical package under DXMRR option. To compare the models, -2LogL, Akaike's information criterion (AIC), Bayesian information criterion (BIC), Residual variances (RV) and Log likelihood values were used. Heritabilities (0.08 to 0.28), additive genetic correlations (0.68 to 0.99) and phenotypic correlations (0.21 to 0.66) were estimated by AS(4,4) random regression model which had the lowest AIC and BIC values. As a result, it was decided that the AS(4,4) random regression model can be used for management decisions and genetic evaluation of Jersey cows for milk production.

Keywords: *Random regression, Test day milk yield, Jersey, Genetic parameters*

Jersey Sığırlarında İlk Laktasyon Test Günü Süt Verimleri için Bazı Şansa Bağlı Regresyon Modellerinin Karşılaştırılması ve Genetik Parametre Tahminleri

Özet

Bu çalışma Jersey sığırlarında ilk laktasyon süt verimleri için genetik parametrelerinin tahmini üzerine Ali Schaeffer, Wilmink ve Legendre polinomlarının 3 farklı uyum sırasında şansa bağlı regresyon modellerini karşılaştırmak için yürütülmüştür. Bu amaçla, çalışmada Samsun Karaköy Tarım İşletmesi'ndeki 1996-2011 yılları arasındaki 686 ilk laktasyonun 6387 adet aylık süt verim kaydı kullanılmıştır. Çalışmada ilk laktasyon test günü süt verimleri (TGSV) için kovaryans bileşenleri, kalıtım dereceleri ve TGSV arasındaki genetik korelasyonlar DFREML istatistik paket programı içerisindeki DXMRR opsiyonu kullanılarak tahmin edilmiştir. Modelleri karşılaştırmak için -2LogL, Akaike bilgi kriteri (AIC), Bayesian bilgi kriteri (BIC), Hata varyansı (RV) ve Log olabilirlik değerleri kullanılmıştır. En küçük AIC ve BIC değerlerine sahip AS(4,4) modeli ile kalıtım derecesi değerleri (0.08 - 0.28), eklemeli genetik korelasyonlar (0.68 - 0.99) ve fenotipik korelasyonlar (0.21 - 0.66) tahmin edilmiştir. Sonuç olarak, AS(4,4) modelinin Jersey sığırlarının genetik değerlendirmesi ve süt üretimi açısından işletme yönetim kararları için kullanılabilir olduğuna karar verildi.

Anahtar sözcükler: *Şansa bağlı regresyon, Test günü süt verimi, Jersey, Genetik parametreler*

INTRODUCTION

The objective in breeding is to improve the animal's genotype for the traits of interest (breeding goal). Breeding values are used as a tool for selecting the best animals. Animals with the most favorable genotype are

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selected to produce the next generation [1]. One of the main incomes is milk production for dairy cattle farms and therefore milk yield records have great deal of importance for dairy herds [2]. Milk yield is a trait where the phenotype of an animal can be represented by a continuous function of time. Thus, this trait is characterized by a trajectory with a theoretically infinite number of measurements [3]. Therefore, an appropriate model is one that considers a complex covariance structure. In the infinite-dimensional approach, the covariance structure is modeled as a Covariance Function (CF) [4]. A useful tool for the estimation of CF is the Random Regression Model (RRM) [5].

Recently, Test-Day Models (TDM) based on different random regression functions have been suggested for the genetic evaluation of dairy cows. TDMs analyzes individual test-day records instead of 305 day milk yields of cows, which are currently used by Ptak and Schaeffer [6], Swalve [7], Jamrozik and Schaeffer [8], Pool and Meuwissen [9], Schaeffer et al.[10], Takma and Akbas [11], Takma and Akbas [12], Bignardi et al.[13], Galic and Kumlu [14].

Moreover, Random Regression Models are able to predict covariance structures among the test day points along a continuous scale [15]. Thus, the (co)variances between records for additive genetic and permanent environmental effects can be described by using different covariance functions as Ali-Schaeffer [16], Wilmink [17] or Legendre polynomials [12].

Several Random Regression Models (e.g Wilmink, Ali-Schaeffer or Legendre polynomial models) were used to estimate the genetic parameters of milk yields and to compare the each other [18-22]. However, it cannot be founded a study on the applications of random regression models for estimating the genetic parameters of Jersey cows. Thus, the aims of this study are to compare of different order Ali-Schaeffer, Wilmink and Legendre polynomial random regression models and to find the best model that provided a good description of the genetic parameters for Jersey herds.

MATERIAL and METHODS

Data were 6387 first lactation milk yield official records from monthly recording of 686 lactations between 1996 and 2011 (over 15 years) of Jersey cattle herd under pasture-based dry seasonal production system in Karakoy Agricultural State Farm in Samsun (Turkey). Also, each data set was composed of the test days and the total amount of milk at the morning and evening milking of the test days. The lactation had variable length with a minimum of 150 and maximum of 305 days long. The average and standard deviation of test-day milk yields were 12.71 and 3.35 kg, respectively and a coefficient of variation of 26.38%.

In general, Random Regression Models (RRM) include lactational submodels, frequently using the lactation functions proposed by Ali and Schaeffer [16] and by Wilmink [17]. The first has shown better performance in adjusting observed daily phenotypes. The second, with a good adjustment performance, provides a more parsimonious model. Beside, Legendre polynomials (L(2,2), L(3,3) L(4,4)) were used to describe the (co)variance matrix within a Random Regression Test Day Model. Previous studies suggest that at least a three coefficient polynomial is needed to model the (co)variance structure of the random components of the data for RRM based on Legendre polynomials [21,23,24].

In this study, (co)variance components, heritability for first lactation test day milk yields (TDMY) and genetic correlations among these TDMYs were estimated by derivative-free REML (DFREML) statistical package using a RRM models Ali-Schaeffer, Wilmink functions and Legendre polynomials under DXMRR option [25] with different orders of fit for additive genetic (α_{jm}=2,3,4) and permenant environmental effects (p_{jm}=2,3,4) [12].

The equation for all the models analyzed can be written in scalar notation as:

$$Y_{ijk} = HTD_i + \sum_{m=1}^{K_B} \beta_m x_{(m)}(t_{ij}) + \sum_{m=1}^{K_A} \alpha_{jm}\varphi_m(t_{ij}) + \sum_{m=1}^{K_P} P_{jm}\varphi_m(t_{ij}) + e_{ijk}$$

Where;

Y_{ijk}= kth TDMY of the cow j obtained at ith herd-test day (month)

HTD_i: ith herd-test day (month)

β_m: mth fixed regression coefficients for cow j,

t_{ij}: ith test day of the cow j

$x_{(m)}$ (t_{ij}): mth covariates evaluated at and represented just by the Ali-Schaeffer and Wilmink functions, where C = 305, X_1 = 1, X_2 = DIM/C, X_3 = $(X_2)^2$, X_4 = ln(C/DIM), X_5 = $(X_4)^2$ for Ali-Schaeffer function and X_1 = 1, X_2 = t and X_3 = exp(-0.05t) for Wilmink function,

α_{jm}: mth additive genetic random regression coefficients for cow j,

P_{jm}: mth permanent environmental random regression coefficients for cow j,

φ_m: mth polynomial evaluated for the age t_{ij},

K_B, K_A and K_P are the order of fitted fixed, random additive and random permanent regression coefficients,

e_{ijk}: random residual effect for Y_{ijk}.

The RRMs were compared using the Akaike's (AIC) and Schwarz's Bayesian (BIC) information criteria [26], as well as by the exam of the variance components, the eigenvalues of the covariance functions and correlation estimates between milk yields on different test-days. The AIC and BIC

allow the comparison between non-hierarchical models and penalize those models that contain a larger number of parameters, with the BIC attributing a more rigorous penalty [27]. AIC was computed as:

$$AIC = -2\log L + 2k$$

where k is the number of free parameters in the model. The model with the minimum AIC is chosen as the best approximating model, i.e. the closest one to the real and unknown process that generated the observed data [24,28]. BIC was computed by the expression:

$$BIC = -2\log L + k\log(\lambda)$$

where k is as in AIC criteria, and, using REML, $\lambda = n - r(X)$, n being equal to the number of test day records and $r(X)$ equal to the rank of the systematic effects incidence matrix. The lowest BIC specifies the best fitting model [24]. Significant differences in the fit of Legendre polynomials with order from k=2 to k=4 were tested using a chi-square (χ^2) test of the likelihood [11].

RESULTS

In the present study we used Ali Schaeffer, Wilmink and Legendre polynomials of different orders to model genetic and permanent environmental variations during lactation. Estimated the logarithm of the likelihood function (-2LogL), Akaike's Information Criterion (AIC), Bayesian Information Criterion (BIC) and Residual Variance (RV) for used models were given in *Table 1*. In studied models, the number of parameters ranged from 7 to 21. Values of the AIC, BIC and -2LogL were changed between 14213.06 and 16694.53.

The maximum log likelihood values and changes in the log likelihoods from the models with different orders of fit were presented in *Table 2*. While -2LogL values were increased, AIC, BIC and RV values were decreased with increasing order of model. The most change in the estimated log likelihood values for order of fit estimated by Ali-Schaeffer, Wilmink and Legendre polynomials have been found to be significant (P<0.05).

Table 1. *Criteria used for comparison of the models*
Tablo 1. *Modellerin karşılaştırılmasında kullanılan kriterler*

Models	Number of Parameters	-2LogL	AIC	BIC	RV
AS (2,2)	7	15496.82	14559.28	14606.37	2.38
AS (3,3)	13	15686.70	14285.40	14372.75	2.03
AS (4,4)	21	16074.12	14213.06	14354.31	1.85
W (2,2)	7	16180.67	15243.14	15290.25	2.63
W (3,3)	13	16337.22	14935.92	15023.41	2.19
W (4,4)	21	16694.53	14833.56	14974.89	2.00
L (2,2)	7	15954.84	15017.31	15064.40	2.61
L (3,3)	13	15843.62	14442.32	14529.77	2.09
L (4,4)	21	16172.05	14310.98	14452.25	1.89

-2LogL: *logarithm of the likelihood function,* ***AIC:*** *Akaike's information criterion,* ***BIC:*** *Bayesian information criterion,* ***RV:*** *residual variance*

Table 2. *Maximum log likelihood values and changes in the log likelihoods from the models with different orders of fit*
Tablo 2. *Farklı uyum sıraları ile modellerden elde edilen log olabilirlikteki değişimler ve maksimum log olabilirlik değerleri*

Models	Number of Parameters	Log Likelihood	Changes in Log Likelihood	Changes in Log Likelihood (%)	X^2
AS (2. 2)	7	-7272.64	-	-	-
AS (3. 3)	13	-7129.70	142.94*	2.00	12.59
AS (4. 4)	21	-7085.53	44.17*	0.62	15.51
W (2. 2)	7	-7614.57	-	-	-
W (3. 3)	13	-7454.96	159.61*	2.14	12.59
W (4. 4)	21	-7395.78	59.18*	0.80	15.51
L (2. 2)	7	-7501.65	-	-	-
L (3. 3)	13	-7208.16	293.49*	4.07	12.59
L (4. 4)	21	-7134.49	73.67*	1.03	15.51

*** Significant change (P<0.05)*

In *Table 3*, first three eigenvalues of the additive genetic (co)variance matrix and their relative proportions (in parenthesis) estimated by Ali-Schaeffer, Wilmink and Legendre polynomial random regression models were given. The fourth eigenvalue had negligible proportions.

Eigenvalues of the estimated permanent environmental (co)variance matrix and their relative proportions (in parenthesis) were given in *Table 4* for different order of fit with Ali-Schaeffer, Wilmink and Legendre polynomial models.

Table 3. *Eigenvalues of the additive genetic (co)variance matrix and the proportion of total variance (%) estimated from Ali-Schaeffer, Wilmink functions and Legendre polynomials*
Tablo 3. *Ali-Schaeffer, Wilmink fonksiyonları ve Legendre polinomiyallerden tahmin edilen eklemeli genetik (ko)varyans matrislerinin özdeğerleri ve toplam varyanstaki payı (%)*

Eigenvalues			
Models	**First**	**Second**	**Third**
AS (2,2)	2.09786 (98.81)	0.02524 (1.19)	-
AS (3,3)	2.24101 (93.94)	0.04168 (1.75)	0.10286 (4.31)
AS (4,4)	2.36989 (94.00)	0.03752 (1.49)	0.11365 (4.51)
W (2,2)	2.75402 (99.99)	0.00011 (0.01)	-
W (3,3)	8.06202 (97.97)	0.00019 (0.01)	0.16641 (2.02)
W (4,4)	2.89087 (94.27)	0.01239 (0.40)	0.16331 (5.33)
L (2,2)	7.09811 (99.62)	0.02685 (0.38)	-
L (3,3)	2.23548 (93.06)	0.05556 (2.31)	0.11128 (4.63)
L (4,4)	2.34273 (93.76)	0.05293 (2.12)	0.10287 (4.12)

Table 4. *Eigenvalues of the permanent environmental (co)variance matrix and the proportion of total variance (%) estimated from Ali-Schaeffer, Wilmink functions and Legendre polynomials*
Tablo 4. *Ali-Schaeffer, Wilmink fonksiyonları ve Legendre polinomiyallerden tahmin edilen kalıcı çevre (ko)varyans matrislerinin özdeğerleri ve toplam varyanstaki payı (%)*

Eigenvalues				
Models	**First**	**Second**	**Third**	**Fourth**
AS (2,2)	3.53207 (77.87)	1.00352 (22.13)	-	-
AS (3,3)	3.57297 (73.27)	0.99176 (20.34)	0.31201 (6.39)	-
AS (4,4)	3.50227 (69.14)	1.11461 (22.00)	0.33763 (6.67)	0.11079 (2.19)
W (2,2)	3.84564 (78.21)	1.07157 (21.79)	-	-
W (3,3)	0.00002 (0.00)	1.87079 (84.42)	0.34528 (15.58)	-
W (4,4)	3.91735 (69.22)	1.26276 (22.31)	0.37336 (6.60)	0.10586 (1.87)
L (2,2)	0.00002 (0.01)	1.37299 (99.99)	-	-
L (3,3)	3.59741 (73.26)	1.00259 (20.42)	0.31061 (6.32)	-
L (4,4)	3.56877 (69.72)	1.10422 (21.58)	0.33405 (6.53)	0.11091 (2.17)

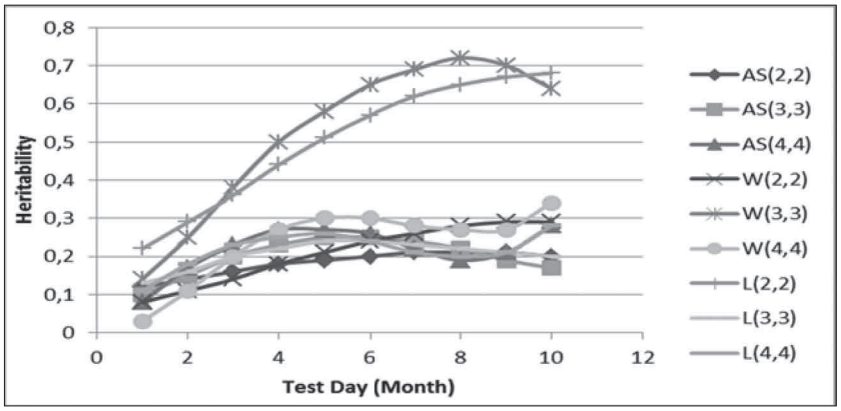

Fig 1. Changes of heritability for TDMYs during lactation estimated from Ali-Schaeffer, Wilmink and Legendre polynomial models

Şekil 1. Ali-Schaeffer, Wilmink Legendre polinomiyallerinden tahmin edilen test günü süt verimleri için kalıtım derecesi değişimleri

Table 5. *Heritability (diagonal), additive genetic (above diagnal) and phenotypic (below diagonal) correlations among test day milk yields estimated from AS (4,4) models*
Table 5. *AS (4,4) modelinden elde edilen test günü süt verimleri arasındaki kalıtım derecesi (köşegen), eklemeli genetic (köşegen üstü) ve fenotipik korelasyonlar (köşegen altı)*

Test Day (TD) (months)	TD1	TD2	TD3	TD4	TD5	TD6	TD7	TD8	TD9	TD10
TD1	0.08	0.94	0.87	0.81	0.76	0.73	0.73	0.77	0.82	0.78
TD2	0.60	0.17	0.98	0.95	0.93	0.91	0.91	0.94	0.93	0.82
TD3	0.46	0.61	0.23	0.99	0.98	0.97	0.97	0.98	0.93	0.78
TD4	0.37	0.55	0.63	0.26	0.99	0.99	0.99	0.98	0.91	0.74
TD5	0.32	0.49	0.59	0.64	0.27	0.99	0.99	0.98	0.89	0.70
TD6	0.29	0.43	0.53	0.59	0.65	0.26	0.99	0.98	0.88	0.68
TD7	0.27	0.37	0.45	0.53	0.61	0.66	0.22	0.98	0.90	0.72
TD8	0.26	0.33	0.39	0.47	0.55	0.62	0.66	0.19	0.96	0.82
TD9	0.25	0.29	0.34	0.40	0.48	0.55	0.61	0.66	0.21	0.95
TD10	0.21	0.27	0.30	0.33	0.37	0.41	0.46	0.54	0.64	0.28

Heritabilities for TDMYs during lactation estimated from Ali-Schaeffer, Wilmink and Legendre polynomials were given in *Fig. 1*. The values were changed from 0.03 to 0.68.

Heritability, additive genetic and phenotypic correlations between TDMYs estimated from AS(4,4) models were given in *Table 5*.

DISCUSSION

Choice of best model partly depends on the criteria's (-2LogL, AIC, BIC and RV values). The W(2,2) models had the highest values for AIC, BIC and RV (15243.14, 15290.25, 2.63 respectively), while the W(4,4) models had the highest values for -2LogL (16694.53) *(Table 1)*. According to estimated AIC and BIC by using the Ali-Schaeffer and Legendre polynomials were better than the finding of Wilmink function. Similar results have been reported by Takma and Akbas [12], Bignardi et al.[13] and Costa et al.[22]. While the AS(4,4) models had lowest AIC, BIC and RV values, the AS(2,2) models had lowest -2LogL values. Also, AIC, BIC and RV values were decreased while -2LogL values were increased with increasing order of model.

Although the L(3,3) model had the largest change (4.07%) of Log likelihood values, the Ali-Schaeffer and Legendre polynomial models which had the lowest AIC and BIC values was better than the Wilmink model in terms of Log likelihood values *(Table 2)*. So, the AS(4,4) model showed a good fit than other models (*Table 1* and *Table 2*). At the same time, the L(4,4) model have nearly similar values with AS(4,4) model.

For estimated values by Ali-Schaeffer, Wilmink and Legendre polynomial models, first eigenvalues belonging to additive genetic effect account for over 95% of total variation *(Table 3)*.

For estimated values by Ali-Schaeffer, Wilmink and Legendre polynomial models, first and second eigenvalues belonging to permanent environmental effect account for over 90% of total variation. But second and third eigen values of W(3,3) models for permanent environmental effect account for over 90% of total variation. Also, the second eigenvalues for the L(2,2) model was account for 99% of total variation *(Table 4)*.

The heritability estimates for TDMYs from W(3,3) model and L(2,2) model showed higher variability from other models *(Fig. 1)*. This figure showed that the estimates of heritability for W(4,4) model was similar at early part of lactation and was higher from other models in the rest of the lactation. Additionally, for estimates the Ali-Schaeffer and Legendre Polynomials models were determined to be better than Wilmink model. Also, the estimates of heritability with AS(4,4), W(2,2), W(4,4) and L(4,4) models showed higher in the middle and at the end of lactation. In there, the W(4,4) model and L(4,4) model were estimated nearly similar heritability values. While the estimates of heritability increased in the middle of lactation was similar to the study by Takma and Akbas [12], increased at the end of lactation was not similar to the studies by Takma and Akbas [11] and Cobuci et al.[29].

The additive genetic correlations were higher than the phenotypic correlations for AS(4,4) model *(Table 5)*. These findings were similar with other studies [11,14]. While additive genetic correlations were changed from 0.68 to 0.99, the phenotypic correlations for TDMYs estimated from AS(4,4) model varied from 0.21 to 0.66. Both additive genetic correlations and phenotypic correlations were a decline, due to the increased distance between the periods. Heritability estimates were altered from 0.08 to 0.28. In addition, heritability estimates from AS(4,4) model was reached the point of peak at the middle and last part of lactation *(Table 5)*.

In our study, the AS(4,4) models was a better performance than others for estimating the genetic parameters of Jersey cows under pasture-based dry seasonal production system in Karakoy Agricultural State Farm in Samsun (Turkey). Also, some studies have found the same performance with Ali-Schaeffer function for Holstein Friesian cows [12-14]. Due to the fact that there has no studies comparing different order of fit (L(2.3), L(2.4), …, L(5.6), L(6.6)) Ali-Schaeffer, Wilmink and Legendre polynomial random regression model for Jersey cows in first lactation, it can be apparently declared that the AS(4,4) models can be used for management decisions and genetic evaluation of Jersey cows for milk production.

It seems that there is no consensus in literature for Jersey cows about the best order of fit Ali-Schaeffer, Wilmink and Legendre polynomials models to be used to model of TDMY with RRM. So, several RRMs obtained with these models have been compared for fitting performance and estimated genetic parameters for TDMY with the AS(4,4) models that fits best. As a result, this study would be helpful to give the literature for estimating the genetic parameters of Jersey cows with RRMs.

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RESEARCH ARTICLE

Farelerde 3-Metilkolantrenle İndüklenen Fibrosarkoma Üzerine Sisteamin, Putresin ve Sisteamin-Putresin Kombinasyonunun Etkileri

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Özet

Bu çalışmada farelerde 3-metilkolantren (3-MC) ile indüklenen fibrosarkoma üzerine sisteamin, putresin ve sisteamin-putresin kombinasyonunun etkileri araştırıldı. Araştırmada *Mus musculus albino* ırkı, 2-3 aylık ve 20±2.0 g ağırlığında olan toplam 135 adet erkek fare kullanıldı. Fareler her grupta 15 adet olacak şekilde 9 gruba ayrıldı. Fareler standart diyet ve su ile *ad libitum* olarak beslendi. Birinci grup negatif kontrol grubu olarak tutuldu. İkinci gruba deri altı yolla 0.2 ml susam yağı, üçüncü gruba deri altı yolla 3-MC enjekte edildi. Dördüncü gruba içme suyuyla %0.1 oranında sisteamin, beşinci gruba %0.1 oranında putresin, altıncı gruba %0.1 oranında sisteamin ve %0.1 oranında putresin karışımı *ad libitum* olarak verildi. Yedinci gruba deri altı yolla 0.2 ml 3-MC çözeltisi (1 mg 3-MC/0.2 ml susam yağı) ve bir ay sonra içme suyuyla %0.1 oranında sisteamin, sekizinci gruba deri altı yolla 0.2 ml 3-MC çözeltisi ve bir ay sonra içme suyuyla %0.1 oranında putresin, dokuzuncu gruba deri altı yolla 0.2 ml 3-MC çözeltisi ve bir ay sonra içme suyuyla %0.1 oranında sisteamin + %0.1 oranında putresin karışımı *ad libitum* olarak verildi. Bir yılın sonunda ferelerin dokuları morfolojik ve histopatolojik olarak değerlendirildi. Araştırma sonucunda 3-MC ile indüklenen fibrosarkomaya karşı çoktan aza doğru putresin, sisteamin+putresin ve sisteaminin koruyucu etki gösterdiği belirlendi.

Anahtar sözcükler: *Fibrosarkoma, 3-Metilkolantren, Sisteamin, Putresin*

Effects of Cysteamine, Putrescine and Cysteamine-Putrescine Combination on 3-Methylcholanthrene-Induced Fibrosarcoma in Mice

Summary

In this study the effects of cysteamine, putrescine and the combination of cysteamine and putrescine were investigated in mice with 3-Methylcholanthrene (3-MC) induced on fibrosarcoma. A total of 135 adult male Mouse (*Mus musculus*) albino, 2-3 months old and weighting 20±2.0 g was used in this study. Mice in each group were divided 15 consisting of 9 individual. The first group was kept as a negative control group. The second group received subcutaneous injection of 0.2 ml sesame oil, and the third group was given subcutaneously 3-MC (1 mg/0.2 ml sesame oil). The 4th, 5th and 6th groups recived 0.1% cysteamine, 0.1% putrescine and 0.1% cysteamine + 0.1% putrescine mix with drinking water *ad libitum* respectively. The 7th, 8th and 9th groups were injected with 0.2 ml of 3-MC solution. After 1 month 0.1% cysteamine, 0.1% putrescine and 0.1% cysteamine + 0.1% putrescine combinations were administrated in drinking water to 7th, 8th and 9th groups respectively. After 1 year of all experiments mice tissues were evaluated morphologic and histopathologically. As a result were respectively demonstrated protective effect of putrescine, cysteamine+putrescine and cysteamin against 3-MC induced fibrosarcoma.

Keywords: *Fibrosarcoma, 3-Methylcholanthrene, Cysteamine, Putrescine*

GİRİŞ

Kanser tedavisinde toksisitesi düşük, ancak etkinliği yüksek ilaç geliştirme çalışmaları spesifik ve başarılı bir farmakoterapi için büyük önem arz etmektedir [1,2]. Aflatoksinler, nitrozo bileşikleri, aromatik aminler ve doyma-

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mış polisiklik aromatik hidrokarbonlar (PAH; benzantresen, fenantren vb) tümöre neden olan kimyasal maddeler arasında bulunmaktadır. PAH'lar kuvvetli karsinojenik etkileriyle tanınırlar. Deneysel çalışmalarda 3-metilkolantren (3-MC) tümör indüksiyonu amacıyla sıklıkla kullanılmaktadır [1-9]. Yapılan araştırmalarla 3-MC'nin immünotoksik, mutajenik, genotoksik ve karsinojenik etkileri ortaya konmuş ve farelerde 3-MC'nin mikronükleus testiyle mutajenik, deri testiyle de kanserojenik etkileri gösterilmiştir [9]. Deney hayvanlarına 3-MC haftada bir kez 200 mg/kg dozda oral yolla verildiğinde kansere ve genotoksik etkilere neden olmaktadır [10]. Organizmaya giren 3-MC'nin genetik yapılarla birleşmesi kansere neden olmaktadır [1,7,8,11]. Vücuda alınan 3-MC'nin epoksit, dihidroksile ve metile gibi metabolitleri kanserojenik etkiyi başlatırlar [1,3,12]. Zarar gören DNA tümör başlatıcısı olarak görev yapar. Oluşan tümör hücresi immun sistem tarafından kontrol altına alınamazsa gelişerek tümörü oluşturur. 3-MC'nin etkileri arilhidrokarbon reseptörlerini indüklemesiyle de yakından ilişkilidir [13-15]. Flavin taşıyan monoksijenazlarda ortaya çıkan bu tip indüksiyon ilaç metabolizmasını doğrudan etkilemektedir [13]. Karsinojen maddelerin etki şiddeti maddelerin fiziksel-kimyasal özellikleri, vücudun metabolizma kapasitesi ve yolaklarıyla doğrudan ilişkilidir. Karsinojen maddenin enterohepatik dolaşıma girmesi, yağda çözünürlüğünün yüksek, metabolizma ve eliminasyon oranının ise düşük olması karsinojenik etkinliği şiddetlendirmektedir. Yağda çözünürlüğü yüksek olan 3-MC gibi kimyasal maddeler vücuttan daha yavaş ekskrete edilirler. 3-MC'nin metabolizmasında rol oynayan karaciğer ve böbreklerin sağlıklı olup olmadığı da toksisitesinin ortaya çıkmasında etkili olmaktadır. 3-MC Faz I reaksiyonları ile toksik reaktif ürünlere dönüştürüldükten sonra konjugasyonla detoksifiye ürünler halinde atılır. Atılım organlarında reaktif ürünler ve parçalanan konjuge metabolitler olumsuz etkilere neden olabilmektedir [1,2,3,5,16]. Enzim indüktörleri biyotransformasyonu hızlandırarak etkin bileşiklerin kanserojenik etkilerini azaltmaktadır [16]. Bu nedenle karsinojen etkinlik maddelerin farmakokinetik özellikleriyle de ilişkilidir. Danovan ve ark.[9] PAH'ların transplasental kanserojen ve mutajen olduklarını bildirmektedir. Dolayısıyla PAH'lar gebe hayvanlar tarafından alındıklarında fetüsta da kanser ve teratojenik bozukluklar yapabilirler. Bazı kanserlerin beslenmeyle doğrudan ilişkili olduğu ortaya konmuştur [16]. Kanserojen maddelerin gıdalardaki düzeylerinde sıfır tolerans kuralı uygulanmaktadır [3,5].

Sisteamin sisteinin dekarboksilasyonu ile oluşan bir biyojenamindir. Sistein taşıyan glutatiyon toksik maddelerle konjuge olarak idrarla atılmalarına, detoksifiye edilmelerine neden olur. Sistein sisteamin halinde koenzim A (CoA)'nın yapısına (sisteamin + beta-alanin + pantoin asit + adenozin) iştirak ettiğinden enerji metabolizmasında görev alır [1,17,18]. CoA aynı zamanda bir tampon görevi üstlenerek asetik asitin taşınmasına katkı yapmaktadır. Sisteamin sistinozisin tedavisinde 15 mg/kg dozda kullanılır [19-21]. Vecsei ve ark.[18] ve Wilmer ve ark.[21] sisteinin somatostatininin sentezini azaltığını bildirmektedirler. Bu nedenle büyümeyi teşvik etmede kullanılma potansiyeli taşımaktadır. Doğan ve ark.[1] sisteaminin farelerde 3-MC ile indüklenen fibrosarkoma riskini düşürdüğünü tespit etmişlerdir.

Putresin ornitinin dekarboksilasyonuyla oluşan bir biyojenamindir. Bayat et ürünleri, balık konserveleri ve kadavralarda bakteriler tarafından oluşturulması nedeniyle kadavra alkaloidi veya ptomain olarak da adlandırılır [1-3,5]. Putresin ve türevlerinden olan spermin ile spermidinin hücrelerin büyüme, gelişme ve farklılaşmasında rolleri olduğu bilinmektedir [22,23]. Poliaminlerin gençlerde büyüme açısından önemli olduğu düşünülmektedir. Poliamin sentezinde rol oynayan ornitin dekarboksilaz enzim inhibitörlerinin kolon kanseri riskini azalttığı bildirilmektedir [21]. Bazı araştırıcılar putresinin bitkilerde de bulunduğunu ve bazı analoglarının apoptozu indüklediğini ve oksidatif sistemle alakalı olarak antiproliferatif etki gösterdiğini bildirmiştir [24].

Sisteaminin hücre metabolizmasında rol oynadığı bilinmektedir. Putresin ve sisteaminin taşıdığı amin grupları ile hücre içinde tampon görevi üstlendiği tahmin edilmektedir. Bu bileşiklerin taşıdıkları fonksiyonel gruplarla hücrelerin enerji metabolizması ve proliferasyonunu baskılayacağı düşünülmektedir. Sisteaminin immun sistemi desteklediği de bilinmektedir. Bu araştırmada sisteamin, putresin ve sisteamin-putresin kombinasyonunun farelerde 3-MC ile indüklenen fibrosarkoma üzerindeki etkilerinin araştırılması amaçlanmıştır.

MATERYAL ve METOT

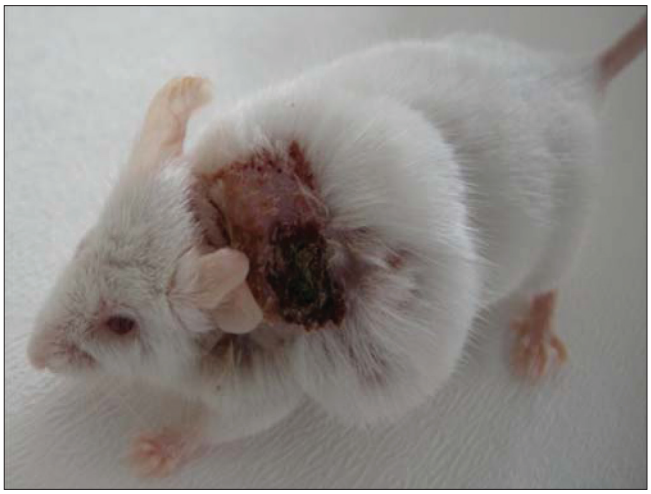
Bu araştırma Kafkas Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu'ndan izin alınarak yapılmıştır (KAÜ-HADYEK 26.11.2010/50). Araştırmada *Mus musculus albino* ırkına ait, 2-3 aylık ve 20±2.0 g ağırlığında olan toplam 135 adet erkek fare kullanıldı. Fareler her grupta 15 adet olacak şekilde 9 gruba ayrıldı. Bütün gruplar standart diyet ve su ile *ad libitum* olarak beslendi. Yem Erzurum Bayramoğlu Yem Fabrikasından, kimyasal maddelerden sisteamin (CAS:156-57-0) Fluka, putresin (P7505) Sigma, 3-MC (CAS: 56-49-5) ise Supelco firmasından temin edildi. 3-MC'nin 1 mg/0.2 ml susam yağı çözeltisi, sisteamin ve putresinin ayrı ayrı %0.1'lik içme suyu ile çözeltileri en fazla üç günlük olarak hazırlandı. Sisteamin ve putresin çözeltileri farelere içme suyuyla ad libitum olarak verildi

1. Grup negatif kontrol grubu olarak tutuldu. 2. Gruba deri altı yolla (toraks bölgesinin dorsalinden) 0.2 ml susam yağı, 3. gruba toraks bölgesinden deri altı yolla 0.2 ml 3-MC çözeltisi enjekte edildi. 4. Gruba sisteamin, 5. gruba putresin, 6. gruba sisteamin ve putresin çözeltilerinin eşit oranda karışımları *ad libitum* olarak içme suyu ile verildi. 7. Gruba toraks bölgesinden deri altı yolla 0.2 ml 3-MC çözeltisi enjeksiyonundan bir ay sonra sisteamin, 8. gruba toraks bölgesinden deri altı yolla 0.2 ml 3-MC çözeltisi

enjeksiyonundan bir ay sonra putresin, 9. gruba toraks bölgesinden deri altı yolla 0.2 ml 3-MC çözeltisi enjeksiyonundan bir ay sonra sisteamin ve putresin çözeltileri karışımı *ad libitum* olarak verildi. İlaç uygulamaları bütün gruplarda aynı zamana denk getirildi. Hayvanlar bir yıl süreyle her 12 saatte en az bir kere olmak üzere takip edildi. Bu süre sonunda yaşayan hayvanlar servikal dislokasyonla ötanazi edildi. Kendiliğinden ölen ve ötanazi edilen hayvanlar tartıldı ve dokuları mofolojik ve histopatolojik olarak araştırıldı [25]. Tümöral oluşumların boyutları ve ağırlıkları ölçüldü. Doku ve fibrosarkomalardan alınan örnekler formol-alkol solüsyonunda tespit edilip parafinde bloklandıktan sonra 6 mikrometre kalınlığında kesitler alındı. Hemotoksilen ve eozin ile boyanarak incelendi. Tümör sayılarının değerlendirilmesinde Minitab Realese 12.1 istatistik programı kullanıldı [26].

BULGULAR

Morfolojik muayenede grup 3, 7, 8 ve 9'da 3-MC'nin injeksiyon yerlerinde kıl dökülmesi, dermeatit ve irritasyon belirtiler tespit edildi. Bir yıl sonra grup 1'de 12, grup 2'de 11, grup 4'te 10, grup 5'te 11, grup 6'da 10 adet farenin yaşadığı tespit edildi. Grup 8'de 12 ay sonra sonra 3, grup 7 ve grup 9'da onbir ay sonra sırasıyla 3 ve 4 adet farenin tümör taşımadan yaşadığı belirlendi. Kontrol amacıyla 3-MC enjekte edilen farelerin hiç birinin 10 aydan daha uzun süre yaşamadığı tespit edildi. Makroskopik ve mikroskopik araştırmalarda grup 1, 2, 4, 5, 6'da hiç tümör gözlenmezken, grup 3'te 11 (%73.3), grup 7'de 8 (%53.3), grup 8'de 6 (% 40) ve grup 9'da 7 (%46.6) adet tümörlü fareye rastlandı. Grup 3'e ait tümörlü bir fare *Şekil 1*'de görülmektedir. Fibrosarkomalar mikroskopik olarak doğrulandı. Tümörlü fareye (grup 3) ait mikroskobik bulgular *Şekil 2* ve *Şekil 3*'te sunulmuştur. Grup 3'e ait bir farenin böbreğinde mononükleer hücrelerin baskın olduğu kronik miyelonefrit tespit edildi. Yine aynı gruptaki bir farede akciğerlerde yangı belirtileri gözlendi.



Şekil 1. Grup 3'e ait fibrosarkomalı bir fare
Fig 1. A mouse fibrosarcoma of Group 3

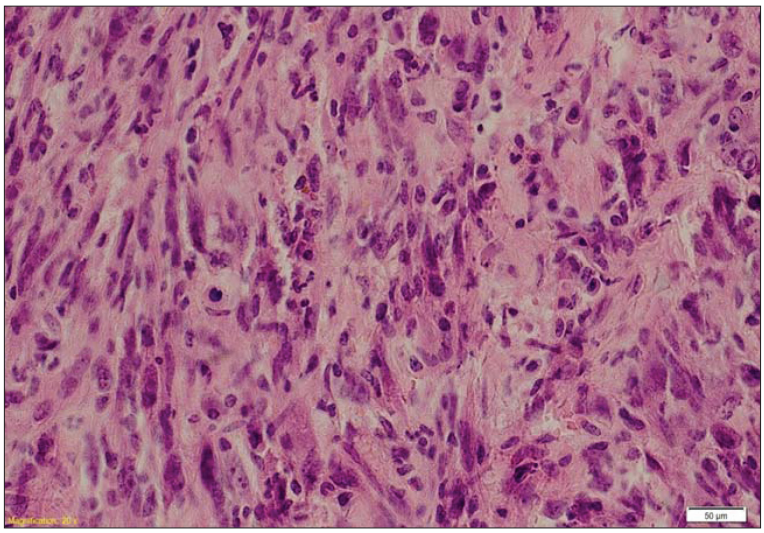
Farelerin ortalama ağırlıkları grup 1'de 27.2, grup 2'de 27.3, grup 4'te 26.2, grup 5'te 25.8, grup 6'da 26.9 olarak bulundu. Grup 3, 7, 8, 9'daki farelerin ağırlıkları, gözlenen tümörlerin sayı, ağırlık ve boyutları ölçülerek *Tablo 1*'de sunulmuştur. Hayvan ağırlığı ölen ve ötanazi edilen hayvanların tartılması ile elde edilmiştir. Sonuçlar istatistik açıdan ki kare testiyle değerlendirildiğinde grup 3 ve 5'te bulunan farelerin ağırlığı arasındaki fark diğer grupların aksine önemli bulundu ($P \leq 0.05$). Tümör ağırlık farkları ise bütün gruplarda önemsiz bulundu ($P \leq 0.05$). Oluşan tümör sayıları arasındaki fark kontrol grubu ile grup 3 arasında önemli ($P \leq 0.001$), grup 3, grup 7, 8 ve 9 arasında ise önemsiz bulundu ($P \leq 0.05$).

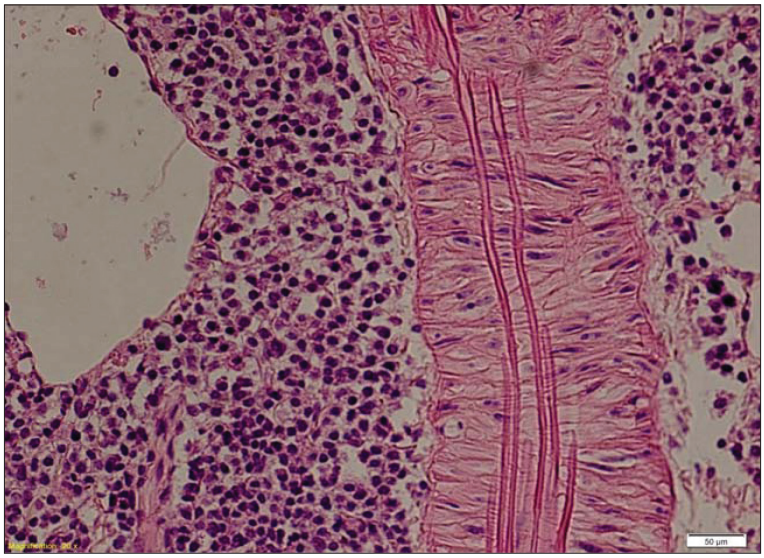
TARTIŞMA ve SONUÇ

Deney hayvanlarında yapılan kanser çalışmalarında mutajen ve karsinojen etkinliği bilinen 3-MC yaygın kullanılmaktadır [1,2,7,8,10,11]. Periton içi ve oral yolla 40-200 mg/kg dozda deney hayvanlarına verilen 3-MC kansere neden

Şekil 2. Grup 3'e ait bir fareden rezekte edilen fibrosarkoma'nın mikroskobik görünümü x200

Fig 2. Microscopic view of the resected from a mouse fibrosarcoma of Group 3 x200





Şekil 3. Grup 3'e ait bir farenin böbreğinde tespit edilen kronik nefritis x 200

Fig 3. Identified a mouse kidney chronic nephritis of Group 3 x200

Tablo 1. *Hayvanların ağırlıkları, tümörlerin gruplara göre sayı, ağırlık ve boyutları (G: Grup, HA: Hayvan ağırlığı, TA: Tümör ağırlığı, TB: Tümör boyutları, ağırlıklar gram, boyutlar cm olarak verilmiştir)*

Table 1. *Weights of the animals, according to the groups of tumors number, weight and size (G: Group, HA: Animal weight, TA: Tumor weight, TB: Tumor sizes, weights, grams, dimensions are given in cm)*

Fare No	G 3			G 7			G 8			G 9		
	HA	TA	TB	HA	TA	TB	HA	TA	TB	HA	TA	TB
1	28.4	-	-	29.5	8	2x3x2	27	-	-	27.7	-	-
2	32.3	5	2x2x2	30.5	7	2x2x2	36.4	8.4	2x3x2	25	-	-
3	34.5	7	2.5x2x2	28.3	8	3x2.5x2	27.5	-	-	26.9	-	-
4	25.9	-	-	25.6	-	-	38.1	5.4	2x2x2	29.8	6.4	2x2x2
5	30.1	-	-	28.1	-	-	25.3	-	-	40.6	6	2x2x2
6	28.2	7.5	3x2x2	25	-	-	28.2	6.5	2x2x2	24	-	-
7	25.6	6.2	2x2x2	35.6	4	3x2x1	29.5	5.1	2x2x2	26.3	5	2x2x2
8	26.8	5.1	2x3x1	32.1	-	-	25.4	-	-	31.3	3	2x1x1
9	28.4	-	-	29.8	5	3x2x1	30.1	-	-	36.1	8	2x2x3
10	32.5	4.8	2x3x1	24.3	-	-	30	-	-	24.5	-	-
11	34.5	7.4	2x3x2	28	-	-	32.3	6	2x2x2	25.6	-	-
12	29.7	3.5	2x1.5x1	33.7	6	2x2x2	25	6.1	2x2x2	34.2	5	2x2x2
13	29.6	9	2z3x2	26.1	-	-	30.6	-	-	31.3	5.4	2x2x2
14	25.3	8.1	2x3x2	24.1	6.4	2x2x2	28	-	-	27.8	-	-
15	27.6	7.3	2x2.5x2	31.3	7	2x2.5x2	29	-	-	23.6	-	-
Ortalama	29.3	6.4		28.8	6.4		29.5	6.3		29	5.5	

olmaktadır. Farelere deri altı yolla 1 mg dozda verilen 3-MC ile bir ay içerisinde tümör oluştuğu gözlenmiştir [1,7,11]. Bu araştırmada aynı dozda kullanılan 3-MC ile farelerde %73.3 oranında fibrosarkoma oluştuğu tespit edilmiştir. 3-MC'nin deri altı yolla enjekte edilmesiyle 3 ay içerisinde akciğer ve karaciğerde metastaza rastlanılmıştır [1,11]. Bu araştırmada metastaz görülmemiştir. Fibrosarkomaların fazla metastaz yapmadığı bilinmektedir [7,8]. Keshava [11] 3-MC ile yaptığı tümör indüksiyonunda akciğerde kanama odaklarına rastlamıştır. Bu araştırmada da 3-MC verilen grupların alveollerinde kanama ve eksudat birikimi gözlenmiştir. Bu bulgular ile araştırma sonuçları bir paralellik göstermektedir.

Yapılan çalışmalarda sisteaminin antimutajenik etkili olduğu bildirilmektedir [1,27]. Sisteamin 3-MC'nin oluşturduğu kanser oranını düşürdüğü tespit edilmiştir [1]. Bu çalışmada 3-MC verilmeyen kontrol gruplarında kanser gözlenmezken, 3-MC verilen grupta %73.3, 3-MC ve sisteamin verilen grupta ise %53.3 oranında tümöre rastlanmıştır. Bu sonuçlar Doğan ve ark.[1] yaptığı araştırma sonuçlarını desteklemektedir. N-asetilsistein gibi sistein bileşiklerinin

3-MC'nin meydana getirdiği hücre proliferasyonunu engellediği bildirilmektedir [28]. Serbest sisteaminin hidroksile bazlarla birleşerek genetik toksisiteyi azalttığı tespit edilmiştir [27]. CoA vücutta bir çeşit sisteamin deposu olarak görev yapmaktadır. Panteteinaz AsetilCoA'yı hidrolize ederek pantetonik asit ve serbest sisteamin açığa çıkarmaktadır. Serbestleşen sisteaminin glutationa dönüşüp hücre savunma sistemini güçlendirdiği bilinmektedir [29]. Panteteinaz enzimi inhibitörleri iodoasetat ve iodoasetamid gibi alkile ajanlar genotoksik etkiye neden olmaktadırlar [30]. Sisteamin daha çok CoA'nın sentezini doğurarak serbet asetik asiti bağlar. Sonuçta enerji metabolizması bloke olur. Bu durumda normal hücrelere göre hızlı çoğalan tümör hücrelerinin daha fazla etkilendiği düşünülmektedir. Sisteamin CoA'nın yapısında adenozini bağlayarak aynı zamanda serbest adenozin düzeyini düşürür ve genetik yapı ile enerjideki diğer andenozin yapıların (NAD, FAD) sentezini azaltır. Bu durumun hücre proliferasyonunun önlenmesinde görev üstlendiği düşünülmektedir. Willmer ve ark.[21] yaptıkları araştırmada sistinoziste enzimlerin inhibe olması sonucu ATP düzeyinin azaldığını ifade etmektedirler. Sisteaminin intrasellüler sistein, N-asetilsistein, ornitin ve glutatiyon düzeyini yükseltiğini belirlemişlerdir.

Nükleofilik antimutajenler bleomisin gibi elektrofilik maddelerin etkilerini azaltabilmektedir [31]. Sisteaminin eritroblastik lösemide stokrom oksidazdaki nitöz oksitle kompleks yaparak apoptozu indüklediği de bilinmektedir [32]. Gebhard [33] 3-MC'nin klastojenik etkinlikte olduğunu tespit etmiştir. Sisteamin, sistein gibi maddeler mutajenik maddelerin hücre içi dağılımını değiştirdikleri ya da doğrudan klastojenlerle birleşerek etkili oldukları ileri sürülmektedir. Antimutajenik özelliğinin kanser oluşumunu engellemede etkili olabileceği düşünülmektedir. Gebhard [33] sisteaminin antiproliferatif etkinlikte olduğunu bildirmektedir. Bu sonuçlar araştırma bulgularını destekler niteliktedir.

Bu araştırmada 3-MC verilen gruba göre 3-MC ile birlikte putresin verilen grupta (Grup 8) tümör gelişiminin daha az olduğu belirlenmiştir. Grup 8'de %40 oranında tümör tespit edilmiş olup, bu sonuç Grup 7'den daha azdır. Putresinin hücrelerin büyümesi, olgunlaşması ve farklılaşmasında rolü olduğu bilinmektedir [22,23,34,35]. Ruiz-Cano ve ark.[22] poliaminlerin bebeklerin gelişiminde rol oynayabileceklerini ileri sürmektedir. Putresinin kolon kanserinde tümör miktarını artırdığı bildirilmektedir [36]. Kanser hastalarında putresin miktarının yüksek, radyoterapi alanlarda ise düzeylerinin azaldığı belirlenmiştir [37]. Bu durum tümörlerin erken teşhisinde ve tedavinin izlenmesinde kullanılabilir. Tümörlü hastalarda miktarlarının artması putresinin hücreler tarafından bir savunma mekanizması olarak mı yoksa tümör hücrelerinin gelişmesini teşvik etmek için mi ortaya çıktığı tam olarak açık değildir. Vargas ve ark.[23] yaptıkları çalışmada kolon adenokarsinom riski ornitin dekarboksilaz enzim inhibitörleri ile düşürüldüğünü belirlemişlerdir. Takao ve ark.[35] yaptıkları çalışmada poliaminlerin sentezinde rol oynayan ornitin dekarboksilaz enzim inhibitörlerinin (difloromet ilornitin) hücrelerin büyümesini azalttığı, ancak artan putresin konsantrasyonunun apoptozu indüklediği ve proliferasyonu azalttığını bildirmektedir. Putresinin hücrelerin üremesi için gerekli olduğu ancak hücre ölümü de yaptığı ifade edilmektedir [38]. Putresin ve anologlarının apoptozu indüklediği bilinmektedir [24,34,35]. Russo ve ark.[24] yaptıkları çalışmada putresinin etkilerini NO ile ilişkilendirmişlerdir. Putresinin reaktif oksijen (ROS) düzeyinde artış yaparak tümör hücrelerinde nekroza neden olduğunu bildirmektedir. Blachier ve ark.[39] yaptıkları çalışmada sodyum nitropurissidin hücre proliferasyonunu ve putresin sentezini inhibe ettiğini açıklamaktadır. Putresin redoks reaksiyonlarını engellemektedir [40]. Ayrıca asetik asit konjugatları halinde atılması asetik asit düzeyini azaltarak enerji metabolizmasının bloke olmasına katkı yapmaktadır. Putresinin doğrudan kanserojen maddelerle bağlanarak da etkili olduğu düşünülmektedir. Peterson ve ark.[41] yaptıkları çalışmada putresin ve spermidinin furanın toksik metabolitleriyle reaksiyona girerek henüz mekanizması tam olarak açıklanmamış karsinojenik etkinliği bastırdığı ifade edilmektedir.

Bu araştırmada sisteamin ile putresinin birlikte verildiği grupta 3-MC ile indüklenen fibrosarkoma oranı %46.6 olarak bulunmuştur. Bu oran grup 7'den düşük ama grup 8'den daha yüksektir. Tümör hücreleri daha hızlı ürediğinden daha fazla enerji ile enerji üretimi ve genetik sentezde kulanılan materyale (NAD, FAD, Adenozin) ihtiyaç duymaktadır. Enerji ve üreme ilişkisinin aşağıdaki gibi olduğu düşünülmektedir.

Adenozin ↔ ATP ↔ NAD ↔ FAD ↔ CoA ↔ Genetik bazlar

Adenozinin CoA halinde bağlanması ise diğer yapılara ihtiyacı artıracaktır. Sisteamin adenozini CoA halinde bağladığından ATP üretimi için gereken baz, NAD ve FAD düzeyleri azalır. Sonuçta tümör hücreleri NAD kazanmak için glukozu laktik asite kadar yıkımlamaktadır. Glukozun anaerop şartlarda parçalanmasıyla ortaya çıkan asitler CoA ile taşınır. AsetilCoA asetik asiti serbestleştirerek hücrelerin enerji kazanmasına neden olur. Sisteamin AsetilCoA düzeyini artırmak ve doğrudan asetik asiti bağlayarak enerjide kullanılmasını sınırlayabilir. Bu durum, glutation sentezindeki artış ve taşıdığı SH gruplarının doğrudan ksenobiotikleri bağladığı ile bir arada düşünüldüğünde sisteaminin antiproliferatif etkinliğini açıklamada kullanılabilir. Sisteamin şeker baz çiftlerini CoA şeklinde bağlayarak genetik yapının sentezinde baskılanmaya neden olur. Putresin de sisteamin gibi amin grubuyla CoA'ya bağlanır. Bağlanmanın putresin + beta-alanin + pantoin asit+adenozin ya da putresin + pantoin asit + adenozin şeklinde olduğu düşünülmektedir. Bu durumda CoA'nın aktif grubu SH yerine amin grubu olur. Bu bileşiğin iki molekül asetik asit bağladığından enerji üretimini azaltabileceği düşünülmektedir. Khuhawan ve Qureshis [42] yaptıkları çalışmada putresinin asetik asit ile konjuge olduğunu bildirmektedir. Araştırmasında kanser hastalarında asetil

konjugasyonun arttığı, tedavi edildiğinde ise azaldığı ifade edilmektedir. Alifatik aminlerin vücutta asetil konjugasyona girdikleri bilinmektedir [2,3,42]. Bu veriler ileri sürülen hipotezi destekler niteliktedir. Putresinden dolayı CoA'nın sentezinin artışı ATP ve NAD düzeylerini azaltarak enerji üretimini baskılanır. Bu etkiler hücrelerin proliferasyonunu zayıflatır. Putresin CoA'ya karşı sisteamin ile yarıştığı düşünülmektedir. Serbest kalan sisteamin sistein üzerinden glutatiyon sentezinde kullanılır. Bu hipotez araştırmada tespit edilen putresinin antiproliferatif etkisinin sisteaminden daha kuvvetli olmasını açıklamaktadır. Nagele [43] yaptığı araştırmada bakır-putressin-piridin bileşiğinin NADH oranında artış yaptığını ifade etmektedir. Aynı araştırmada ADP-ribolizasyonun inhibe olduğu, piridin dinükleotid ve adenilat miktarlarının etkilenmediği, hücrelerde subletal hasarların görüldüğü bildirilmektedir. Bu veriler putresinin enerji ve genetik materyal sentezini engellediği şeklinde ileri sürdüğümüz hipotezi kısmen destekler niteliktedir. Ornitinin de putresin gibi karsinojenik furan metabolitleriyle çapraz bağlantılar yaptığı bildirilmektedir [41]. Antitümöral ilaç olarak putresin ve analoglarının kullanımı üzerinde benzer çalışmalar yapılmaktadır [44]. Bu araştırma sonucu ile elde elden veriler oldukça dikkat çekici olup, yeni tip antitümöral ilaçların geliştirilmesine farklı bir bakış açısı getirebileceği düşünülmektedir.

Sonuç olarak, 3-MC injeksiyonundan bir ay sonra verilen putresin, putresin-sisteamin kombinasyonu ve sisteaminin 3-MC ile indüklenen fibrosarkomaya karşı farelerde kısmen de olsa koruyucu etki gösterdiği söylenebilir. İleri sürülen hipotezin ve araştırma sonuçlarının doğrulanabilmesi için daha geniş çaplı çalışmaların yapılması gerekmektedir.

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RESEARCH ARTICLE

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Özet

Bu çalışmada sık kuzulatma yöntemi kullanılarak koyunlardan bir yılda elde edilecek kuzu sayısını ve buna bağlı olarak işletmenin karlılığını arttırma olanaklarının araştırılması amaçlanmıştır. Materyal olarak Özel sektörde Anadolu Merinosu ırkı 525 baş koyun ve 40 baş koç ve Bahri Dağdaş Uluslararası Tarımsal Araştırma Enstitüsü'nde (Kamu sektörü) 199 baş koyun ve 15 baş koç kullanıldı. Özel sektördeki ve Kamu sektöründeki koyunlar sık kuzulatma ve kontrol olmak üzere iki gruba ayrıldı. Özel sektörde 200, Kamu sektöründe ise 75 baş koyun normal olarak yılda bir kuzu elde etmek için kontrol olarak ayrılırken, Özel sektörde 325, Kamu sektöründe ise 124 baş koyun çalışma grubunu oluşturdu. Kontrol grubu koyunlar her yıl geleneksel tohumlama dönemi olan Ağustos-Eylül aylarında yılda bir çiftleştirilirken, çalışma grubunda bulunanlara 2 yılda 3 kuzulatma uygulandı. Çalışma grubu koyunlar ilk tohumlamadan itibaren 5 ay gebelik, 40 gün laktasyon ve 20 gün sütten kesmeyi takiben 1 aylık tohumlama periyoduna alındı. Çalışma grubunda sık kuzulatma için östrüs indüksiyonu ve senkronizasyonu amacıyla koç etkisi, koç etkisi + flushing ve hormon uygulamalarını içeren beş farklı protokol kullanıldı. Sonuç olarak hormon kullanılan sık kuzulatma uygulamalarıyla yılda bir kuzulatmaya göre daha fazla kuzu verimi ve kuzu üretkenliği sağlandı. Sık kuzulatma uygulamalarında elde edilen kuzu verimi ve kuzu üretkenliği üzerinde uygulanacak senkronizasyon yöntemlerinin önemli derecede etkili olduğu görüldü. Ancak bu çalışmada uygulanan senkronizasyon yöntemleri ile elde edilen kuzu veriminin kontrol grubuna göre karlı olmadığı tespit edildi.

Anahtar sözcükler: *Anadolu Merinosu, Sık kuzulatma, Koyun, Kuzu verimi, Ekonomik analiz*

Investigation of Accelerated Lambing Possibility of Anatolian Merino Sheep

Summary

Investigation of the possibilities to increase the number of lamb gained in a year via using the accelerated lambing method and, the profitability of a farm related to this is aimed with this study. As material, 525 Anatolian Merino ewes and 40 rams, aged at 2-4, in field conditions, and 199 ewes and 15 rams at Bahri Dağdaş International Agricultural Research Institute were used. Ewes in the field condition and at the Institute were divided in to two groups as accelerated lambing and control and, 200 ewes in the field condition and 75 ewes at the Institute were remained as control to get one lamb per year while 325 in the field condition and 124 at the Institute were formed treatment group. The control ewes were bred in August and September, the traditional breeding season, in a 12 month interval while 3 lambings in 2 years were applied to the ewes in the treatment group and they were bred for one month again following the period of 5 months of pregnancy, 40 days of lactation and 20 days of weaning. Ram effect, ram effect + flushing and some different protocols were used for induction and synchronization of estrus for accelerated lambing in the treatment group. As a result, more fecundity and lamb productivity achieved by accelerated lambing than once a year lambing. Synchronization methods were found to be effective on fecundity and lamb productivity in accelerated lambing applications. However, lamb yield obtained by synchronization methods used in this study were not profitable.

Keywords: *Anatolian Merino, Accelerated lambing, Sheep, Lamb productivity, Economic analysis*

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GİRİŞ

Koyunlar genellikle mevsime bağlı poliöstrik hayvanlardır ve gebelik süresi 5 ay olmasına rağmen genellikle yılda bir defa doğum olmaktadır [1]. Bir koyunculuk işletmesinde karlılık, yılda koyun başına elde edilen kuzu sayısı ile yakından ilişkilidir ve bunun arttırılması başta yeterli düzeyde döl verimiyle mümkündür [2]. Döl verimi özelliklerinin kalıtım derecesi düşük olduğundan koyunculukta döl veriminin artırılmasında genetik çalışmalara ilaveten, bakım-besleme ve sürü yönetimiyle ilgili çalışmalara ağırlık verilmektedir. Çevresel iyileştirme çalışmalarının içerisinde ek yemleme, erken kuzulatma, eksojen hormon ve suni ışık uygulaması sayılabilir [3]. Hayvan başına düşen ortalama verimin arttırılmasında kullanılan uygulamalardan birisi de sık kuzulatma sistemleridir [4]. Bu amaçla yılda iki kuzulatma, iki yılda üç kuzulatma, üç yılda dört kuzulatma, Camal sistemi ve Yıldız sistemi gibi değişik sık kuzulatma sistemleri geliştirilmiştir [5]. Geliştirilen bu yöntemlerle koyunlardan tüm yaşamı boyunca daha fazla yavru elde etmek mümkün olabilmektedir [3]. Sık kuzulatma sistemleri içerisinde en fazla uygulama sahasına sahip olan iki yılda üç kuzulatma sisteminde koyunların 8 ayda bir kuzulamaları öngörülmektedir [5]. Koyunlar doğumdan sonra postpartum anöstrüse girmektedirler [6]. Kuzular doğumdan sonra ikinci ayda sütten kesilirler ve koyunlar sütten kesimi takip eden ayda çiftleştirilirler [5]. Bununla birlikte, mevcut sık kuzulatma sistemlerinin başarısı ırka ve mevsime bağlı olarak değişmektedir [5,7]. Ayrıca sık kuzulatma sistemlerinin ekonomik olması uygulanabilirliği açısından önemlidir [8]. Yapılan bazı çalışmalarda kuzu veriminin Kıvırcık [9] ve East Friesian Composite [7,10] ırkı koyunlarda arttığı, Romney [7,10] ırkı koyunlarda ise azaldığı bildirilmektedir. Zarkawi [11] İvesi koyunlarında sık kuzulatmanın kuzu verimini artırdığını, aynı ırk üzerinde çalışan Gül ve Keskin [12] ise artış olmadığını belirtmektedirler.

Sık kuzulatma sistemlerinde östrüs senkronizasyonu önem taşımaktadır [1]. Bu amaçla koç etkisi, progestagenler, melatonin, prostaglandin F_{2a} (PGF_{2a}), pregnant mare serum gonadotropin (PMSG), follicle stimulating hormone (FSH), human chorionic gonadotropin (hCG) ve gonadotropin releasing hormone (GnRH) sezon içinde ya da dışında yalnız başlarına ya da birbirleriyle kombinasyonlar şeklinde kullanılabilmektedir [13,14]. Üreme sezonu dışında belirli bir dönem izole edilip sonrasında koçlar ani olarak koyunlar arasına katılırsa ovulasyon şekilleneceği [15], bununla birlikte bu etkinin sezona bağlı olarak değişebileceği kimi araştırmacılar tarafından bildirilmektedir [16,17]. Sezon dışında östrüs senkronizasyonu amacıyla 7 [18], 7-12 [19], 10-12 [20] ya da 12 [21] gün süreyle vaginal sünger yoluyla progesteron ve vaginal süngerin çıkarılması ile birlikte ya da çıkarılmadan 24-48 saat önce PMSG, bazen de PGF_{2a} uygulamalarının etkili olduğu bildirilmiştir. Koyunlarda sezon içinde GnRH enjeksiyonunun mevcut dominant follikülün ovulasyonu ya da regresyonuna neden olarak yeni bir folliküler dalga başlattığı [22], bunu takip eden 5. gün yapılacak PGF_{2a} uygulaması ile senkronize östrüslerin geliştiği bildirilmektedir [2,23].

Başarısını birçok faktörün etkilediği ve en çok uygulama alanına sahip sık kuzulatma yöntemlerinden olan iki yılda üç kuzulatma için en uygun senkronizasyon protokolünün ve bu yöntemin ekonomik açıdan uygulanabilirliğinin araştırılması bir gerekliliktir. Sunulan bu çalışma ile, özel sektör ve kamu şartlarında farklı östrüs senkronizasyonu yöntemleri kullanılarak sık kuzulatma yöntemi ile koyunlardan bir yılda elde edilecek kuzu sayısı ve buna bağlı olarak işletmenin karlılığını arttırma olanakları araştırıldı.

MATERYAL ve METOT

Materyal: Materyal olarak Eskişehir-Mahmudiye'de 4 ayrı işletmede (Özel sektör) Anadolu Merinosu 525 baş koyun ve 40 baş koç ve Bahri Dağdaş Uluslararası Tarımsal Araştırma Enstitüsü'nde (Kamu sektörü) 199 baş koyun ve 15 baş koç kullanıldı. Çalışma Bahri Dağdaş Uluslararası Tarımsal Araştırma Enstitüsü Hayvan Deneyleri ve Yerel Etik Kurulu'nun onayı ile (No: 22.07.2013/1) yapıldı.

Metot: Özel sektör ve Kamu sektöründeki koyunlar sık kuzulatma (Grup I, II, III, IV ve V) ve kontrol olmak üzere ayrıldı. Gruplar oluşturulurken her grupta koyunların yaş ortalamalarının birbirine benzer olmalarına özen gösterildi. Çalışma grubundaki koyunların birinci tohumlaması ilk yıl temmuz ayında, ikinci tohumlaması takip eden yıl mart ayında ve üçüncü tohumlaması ise takip eden kasım ayında yapıldı. Koyunlar ilk tohumlamadan itibaren 5 ay gebelik, 40 gün laktasyon ve 20 gün sütten kesmeyi takiben bir sonraki 1 aylık tohumlama periyoduna alındı.

Grup I, II ve III'de bulunan koyunlar üç tohumlama döneminde de hormon kullanılarak senkronize edildi. Koyunlara sezon dışında (Mart) senkronizasyon amacıyla 6 (Grup I), 8 (Grup II) ya da 10 (Grup III) gün süreli 40 mg progesteron içeren vaginal sünger (fluorogestene acetate, Chronogest, Intervet, Türkiye) uygulandı. Vaginal sünger çıkarılmadan bir gün önce im olarak 125 mg PGF_{2a} (D-kloprostenol, Reprodin, Bayer, Türkiye) ve 400 IU PMSG (Choronogest/PMSG, Intervet, Türkiye) enjeksiyonu yapıldı. Sezon içinde (Temmuz ve Kasım) ise 0. gün 10 mg GnRH (buserelin asetat, Receptal, Intervet, Türkiye) ve bundan 5 (Grup I), 6 (Grup II) ya da 7 (Grup III) gün sonra im yolla PGF_{2a} + PMSG enjeksiyonu ile senkronizasyon yapıldı. Grup I, II ve III'deki koyunların sezon içinde ve dışında son uygulamayı takiben 5 gün boyunca 12 saat aralıklarla 30'ar dk olmak üzere kızgınlık kontrolleri yapıldı ve kızgınlık gösterenler elde sıfat yöntemiyle tohumlandı. Grup IV (koç etkisi) ve V'te (koç etkisi + flushing) bulunan koyunlar çiftleştirme yapılan aylarda (Temmuz, Mart, Kasım) 1 ay süreyle koçlarla birlikte tutuldu. Grup V'te ise koyunlara çiftleştirmeden 4 hafta öncesinden başlanarak

çiftleştirmeye kadar flushing uygulandı. Bu amaçla koyun başına 500 g/gün ilave konsantre yem verildi. Kontrol grubu koyunlar her yıl Ağustos-Eylül aylarında yılda bir kez çiftleştirildi *(Tablo 1)*. Kızgınlık kontrolleri ve tohumlama amacıyla Grup IV, V ve kontrol grubunda 20 baş koyun için 1 baş koç, Grup I, II ve III'de ise 10 baş koyun için 1 baş koç kullanıldı.

Senkronizasyon uygulanan dönemlerdeki ovaryum aktivitesini belirlemek amacıyla her dönemde (Mart, Temmuz ve Kasım ayında) Özel sektör ve Kamu sektöründe 20'şer baş koyundan (her dönemde 40, toplamda 120 baş) 5 gün arayla 3 kez (0, 5 ve 10. günler) kan alınarak kan progesteron seviyesi belirlendi. Bu amaçla koyunların vena jugularisinden 10 ml'lik heparinli tüplere alınan kanlar 5.000 devirde 5 dk santrifüj edilerek serumları ayrıldı ve progesteron analizi yapılıncaya kadar -20°C'de saklandı. Kan progesteron seviyesi 0.5 ng/ml ve üzerinde olan koyunlar siklik olarak aktif kabul edildi [19].

Gerçekleştirilen tohumlamalara ilişkin döl verimi özelliklerinin ve sık kuzulatmanın etkinliğinin tespitine östrüs (östrüs gösteren koyun sayısı/koçaltı koyun sayısı), gebelik (gebe kalan koyun sayısı/koçaltı koyun sayısı), doğum (kuzulayan koyun sayısı/koçaltı koyun sayısı), çoklu kuzulama (çoklu kuzulayan koyun sayısı/kuzulayan koyun sayısı) ve 40 ve 80. gün kuzu yaşama oranları (%, yaşayan kuzu sayısı/doğan kuzu sayısı) ile bir doğumda ortalama kuzu sayısı (doğan kuzu sayısı/doğuran koyun sayısı), kuzu verimi (doğan kuzu sayısı/koçaltı koyun sayısı) ve kuzu üretkenliği (koçaltı koyun başına 80. gün kuzu ağırlığı) göz önünde bulunduruldu.

Yetiştirici elinde işletmelerde uygulanan rutin besleme programlarına uyuldu. Koyunlar Haziran-Ağustos aylarında merada, Eylül ve Ekim aylarında meraya ek olarak işletmelerdeki mevcut kaba yemlerle, Kasım-Mart ayları arası sürede ağılda yine işletmedeki kaba yemlerle ve Mart tohumlamasından sonra da tamamen meraya dayalı olarak beslendi. Kamu sektöründe ise uygulanan rutin beslenme programına uyuldu. Koyunlar Nisan-Kasım dönemleri arası merada, kışın ise Enstitüde mevcut kaba yemlerle (yonca ve fiğ kuru otu ile) beslendi.

Tablo 1. *Çalışmada oluşturulan gruplar ve gruplardaki hayvan sayıları*
Table 1. *Groups in the study and the numbers of animals in each group*

Grup	n		Uygulanan Senkronizasyon Programı
	Özel Sektör	Kamu Sektörü	
Grup I	65	24	Temmuz ve Kasım: GnRH + PGF_{2a} + PMSG Mart: Vaginal sünger + PGF_{2a} + PMSG
Grup II	65	25	
Grup III	65	25	
Grup IV	65	25	Koç etkisi
Grup V	65	25	Koç etkisi + flushing
Kontrol	200	75	Yılda bir Ağustos-Eylül aylarında çiftleştirme

Özel sektör ve Kamu sektöründe koç katım öncesi koç etkisi + flushing grubu haricinde flushing beslemesi yapılmadı. Koçlara ise aşım sezonunda 1 kg/baş/gün ek yem verildi. Koyunlara gebeliğin son 6 haftasında kaba yeme ilave olarak 500 g/baş/gün kesif yem, doğum sonrası ise sütten kesime kadar 1 kg/baş/gün koyun süt yemi verildi. Kuzulara sütten kesime kadar serbest miktarda standart kuzu başlangıç yemi verildi.

Ekonomik Analiz: İşletme karlılığını belirlemek amacıyla 2 yıl süresince elde edilen kuzuların sayısı ve 80. gün canlı ağırlıklarına bağlı olarak Gayri Safi Üretim Değeri (GSÜD) üzerinden karşılaştırmalar yapıldı. Her grupta 80. güne kadar yapılan kuzu bakım masraflarının aynı olduğu, masraflardaki farklılığın sadece sık kuzulatma çalışmasına bağlı olarak yapılan ilave hormon ve/veya yemleme masrafının kuzu başına düşen miktarlarından kaynaklandığı dikkate alındı. Aynı zamanda sık kuzulatma çalışmasının kuzu doğum ağırlıklarına da etkisi olabileceği düşünüldüğünden [7] yapılan analizlerde kuzu doğum ağırlığı kovaryant olarak alınmadı. GSÜD değeri, 80. gün kuzu canlı ağırlıklarının Kasım 2012 tarihinde Bahri Dağdaş Uluslararası Tarımsal Araştırma Enstitüsü'nün kuzu canlı ağırlık fiyatı olan 11 TL/kg ile çarpılması sonucu belirlendi. Çalışmada, yılda bir tohumlama uygulanan kontrol grubu ve sık kuzulatma gruplarında elde edilen kuzu miktarı ve 80. gün canlı ağırlıklarına bağlı olarak hesaplanan GSÜD üzerinden Kısmi Bütçe Analizi hesaplandı [24,25]. Bu hesaplamada yalnızca çiftçilerin yapacağı değişik seçimlerden etkilenen maliyetler yani değişen masraflar göz önünde tutuldu. Sabit masraflar kısmi bütçeleme hesaplarında göz önüne alınmadı [26]. Kısmi bütçe analizi tekniği uygulanırken yapılmayan masraflar kısmı tüm uygulamalar için aynı olduğundan sonuçları etkilemeyeceği için göz önüne alınmadı ve "0" olarak değerlendirildi.

İstatistik Analizler: Döl verimi, kuzuların yaşama gücü ve sütten kesilen kuzu oranlarının karşılaştırılmasında ki-kare testinden, kuzuların canlı ağırlıklarına ait özelliklerin karşılaştırılmasında ise Varyans ve Regresyon analizi tekniklerinden yararlanıldı. İncelenen faktörlerde grup sayısı üçten fazla olan ve istatistiki olarak önemli bulunan grupların farklılıkların karşılaştırılmasında Çoklu Karşılaştırma Testlerinden Least Significant Difference (LSD) kullanıldı.

BULGULAR

Çalışmada sık kuzulatma gruplarında Temmuz 2009, Mart ve Kasım 2010 tarihlerinde, kontrol grubunda ise 2009 ve 2010 yılları Ağustos aylarında olmak üzere tohumlamalar gerçekleştirildi. Doğumların ardından kuzu ağırlıkları tartıldı ve 80. gün ağırlıkları belirlendi.

Koyunların Siklik Aktivite Durumu: Sık kuzulatma uygulanan dönemlerde hem Kamu sektörü hem de Özel

sektörde kan progesteron seviyelerine göre siklik aktivite oranları belirlendi (P<0.01) *(Tablo 2)*.

Senkronizasyon Sonuçları: Tohumlama dönemlerinde elde edilen östrüs, gebelik ve doğum oranları Kamu sektörü ve Özel sektörde birbirine paralel oldu (*Tablo 3* ve *Tablo 4*). Çalışma gruplarında tohumlama dönemlerine göre bazı farklılıklar tespit edilirken, Kontrol grubunda elde edilen değerler 1 ve 2. yıllarda birbirine yakın oldu.

Kuzulama Aralığı: Çalışma gruplarında 1 ile 2. ve 2 ile 3. doğumlar arası ortalama süre Kamu sektöründe 245 ve 250 (ortalama 248) gün, Özel sektörde ise 249 ve 248 (ortalama 249) gün oldu.

İki Yıllık Toplu Sonuçlar: Proje süresince kaşeksi, pneumoni, aktinomikoz ve mecburi kesim gibi nedenlerden projeden çıkarılan koyun sayıları açısından gruplar arasında istatistiki fark saptanmadı.

İki yıllık toplu sonuçlar *Tablo 5* (Kamu sektörü) ve *Tablo 6*'da (Özel sektör) değerlendirildi. Kontrol grubunda elde edilen sonuçlar 100 kabul edildiğinde diğer gruplarda saptanan kuzu verimi ve kuzu üretkenliği farkları *Tablo 7*'de özetlendi.

Ekonomik Analiz Sonuçları: Çalışmada tekiz kuzulamada kuzu başına düşen ilave hormon ve/veya yemleme masrafı Kasım 2012 tarihi fiyatları esas alındığında 1, 2, 3, 4, 5 ve 6. gruplarda sırasıyla 19.67 TL, 19.67 TL, 19.67 TL, 0 TL, 15.00 TL ve 0 TL oldu. Çalışmada gruplarda saptanan GSÜD değerleri ve buna göre yapılan Kısmi Bütçe Analizinde çalışmadaki bütün gruplarda sık kuzulatma uygulamasının karlı olmadığı tespit edildi *(Tablo 8)*.

Tablo 2. *Kamu sektörü ve Özel sektörde tohumlama dönemlerinde kan progesteron seviyelerine göre koyunlardaki siklik aktivite oranı (%)*
Table 2. *Cyclic activity rates (%) in ewes at the breeding periods according to blood progesteron levels in field and Institute*

İncelenen Özellik	Sektör	Dönemler		
		Temmuz (n=20)	Mart (n=20)	Kasım (n=20)
Siklik olarak aktif koyun oranı (%)	Kamu sektörü	70[b]	5[c]	100[a]
	Özel sektör	65[b]	10[c]	100[a]

[a,b,c] *Aynı satırdaki farklı harfler arasındaki fark istatistiki açıdan önemlidir (P<0.01)*
[a,b,c] *Different superscripts in lines differ significantly (P<0.01)*

Tablo 3. *Kamu sektöründe elde edilen östrüs (senkronizasyonu takiben ilk 5 gün içinde), tohumlama (senkronizasyonu takiben 1 ay içinde), gebelik (1 aylık tohumlama döneminin sonunda), doğum oranları (%) ve kuzu sayıları*
Table 3. *Oestrus (5 days following the synchronization), insemination (1 month following the synchronization), pregnancy (at the end of the 1 month breeding period) and lambing rates (%) and number of lambs obtained at the Institute*

Sık Kuzu (Kamu Sektörü)	Grup	n	Östrüs (ilk 5 gün)		Tohumlama (1 ay)		Gebelik (usg)		Doğum		Kuzu Sayısı			Doğan Kuzu		Kuzu Verim
			n	%	n	%	n	%	n	%	Tek	Çoklu		n	Ort.	
												n	%			
Çalışma grubu 1. tohumlama (Temmuz 2009)	1	20	7	35[a]	19	95	17	85	17	85	14	3	18[b]	20	1.18[b]	1.00
	2	23	11	48[a]	21	91	19	83	18	78	11	7	39[ab]	25	1.39[ab]	1.09
	3	24	11	46[a]	23	96	22	92	22	92	15	7	32[ab]	29	1.32[ab]	1.21
	4	23	4	17[b]	22	96	20	87	20	87	14	6	30[ab]	26	1.30[ab]	1.13
	5	24	4	17[b]	24	100	21	88	21	88	10	11	53[a]	32	1.52[a]	1.33
Kontrol (Ağustos 2009)	6	69	20	29	67	97	66	96	61	88	44	17	28	78	1.28	1.13
Çalışma grubu 2. tohumlama (Mart 2010)	1	20	18	90[a]	18	90[a]	11	55[ab]	8	40[ab]	4	4	50	12	1.50	0.60[ab]
	2	23	19	83[a]	19	83[a]	12	52[ab]	12	52[ab]	6	6	50	19	1.58	0.83[ab]
	3	24	21	88[a]	21	88[a]	17	71[a]	14	58[a]	8	6	43	23	1.64	0.96[a]
	4	23	0	0[b]	6	26[b]	6	26[b]	6	26[b]	2	4	67	10	1.67	0.43[b]
	5	24	0	0[b]	10	42[b]	8	33[b]	8	33[ab]	5	3	38	11	1.38	0.46[b]
Kontrol (Ağustos 2010)	6	69	22	32	69	100	68	99	64	93	50	16	25	80	1.25	1.16
Çalışma grubu 3. tohumlama (Kasım 2010)	1	20	17	85[a]	17	85[a]	14	70[ab]	14	70[ab]	12	2	14	16	1.14	0.80
	2	23	21	91[a]	22	96[a]	18	78[a]	17	74[a]	14	3	18	20	1.18	0.87
	3	24	21	88[a]	21	88[a]	16	67[ab]	16	67[ab]	11	5	32	21	1.31	0.88
	4	23	3	13[b]	11	48[b]	8	35[b]	8	35[b]	5	3	38	11	1.38	0.48
	5	24	4	17[b]	12	50[b]	11	46[b]	11	46[b]	7	4	36	15	1.36	0.63

[a,b] *Aynı sütunda farklı harf taşıyan değerler istatistiki açıdan farklıdır (her tohumlama dönemi kendi içinde değerlendirilmiştir) (P<0.05)*
[a,b] *Different letters in columns differ significantly (each breeding period evaluated in itself) (P<0.05)*

Tablo 4. *Özel sektörde elde edilen östrüs (senkronizasyonu takiben ilk 5 gün içinde), tohumlama (senkronizasyonu takiben 1 ay içinde), doğum oranları (%) ve kuzu sayıları*

Table 4. *Oestrus (5 days following the synchronization), insemination (1 month following the synchronization),and lambing rates (%) and number of lambs obtained in field*

Sık Kuzu (Özel Sektör)	Grup	n	Östrüs (ilk 5 gün)		Tohumlama (1 ay)		Doğum		Kuzu Sayısı			Doğan Kuzu		Kuzu Verim
			n	%	n	%	n	%	Tek	Çoklu		n	Ort.	
										n	%			
Çalışma grubu 1. tohumlama (Temmuz 2009)	1	59	24	41^{a}	53	90	48	81	36	12	25^{ab}	61	1.27^{ab}	1.03^{ab}
	2	61	26	43^{a}	56	92	51	84	36	15	29^{ab}	67	1.31^{ab}	1.10^{ab}
	3	58	27	47^{a}	53	91	48	83	38	10	21^{b}	58	1.21^{b}	1.00^{b}
	4	60	8	13^{b}	57	95	53	88	39	14	26^{ab}	67	1.26^{ab}	1.12^{ab}
	5	62	10	16^{b}	59	95	56	90	33	23	41^{a}	80	1.43^{a}	1.29^{a}
Kontrol (Ağustos 2009)	6	184	47	26	174	95	160	87	124	36	23	197	1.23	1.07
Çalışma grubu 2. tohumlama (Mart 2010)	1	59	49	83^{a}	50	85^{a}	26	44^{ab}	14	12	46	38	1.46	0.64^{abc}
	2	61	52	85^{a}	54	89^{a}	30	49^{ab}	15	15	50	45	1.50	0.74^{ab}
	3	58	51	88^{a}	51	88^{a}	32	55^{a}	17	15	47	49	1.53	0.84^{a}
	4	60	2	3^{b}	15	25^{b}	14	23^{c}	6	8	57	23	1.64	0.38^{c}
	5	62	4	6^{b}	24	39^{b}	20	32^{bc}	10	10	50	30	1.50	0.48^{bc}
Kontrol (Ağustos 2010)	6	184	55	30	177	96	167	91	125	42	25	210	1.26	1.14
Çalışma grubu 3. tohumlama (Kasım 2010)	1	59	47	80^{a}	50	85^{a}	42	71^{a}	37	5	12^{b}	47	1.12^{b}	0.80^{a}
	2	61	50	82^{a}	55	90^{a}	39	64^{a}	32	7	18^{ab}	46	1.18^{b}	0.75^{a}
	3	58	49	84^{a}	51	88^{a}	40	69^{a}	33	6	15^{ab}	45	1.13^{b}	0.78^{a}
	4	60	9	15^{b}	28	47^{b}	21	35^{b}	14	7	33^{a}	28	1.33^{ab}	0.47^{b}
	5	62	12	19^{b}	32	52^{b}	26	42^{b}	16	9	35^{a}	35	1.35^{a}	0.56^{ab}

^{a,b,c} Aynı sütunda farklı harf taşıyan değerler istatistiki açıdan farklıdır (her tohumlama dönemi kendi içinde değerlendirilmiştir) (P<0.05)
^{a,b,c} Different letters in columns differ significantly (each breeding period evaluated in itself) (P<0.05)

Tablo 5. *Kamu sektöründe iki yılın sonunda elde edilen toplu sonuçlar*

Table 5. *The whole results in two years at the Institute*

İncelenen Özellikler	Gruplar					
	1	2	3	4	5	6
Projeden çıkarılan koyun sayısı	4	2	0	2	1	6
Koç altı koyun sayısı	20	23	24	23	24	69
İki yıllık koç altı koyun sayısı	60	69	72	69	72	138
Tohumlanan koyun sayısı*	54^{b}	62^{b}	65^{b}	39^{c}	46^{c}	136^{a}
Doğum yapan koyun sayısı*	39^{bcd}	47^{bc}	52^{b}	34^{d}	40^{cd}	125^{a}
Doğan kuzu sayısı	48	64	73	47	58	158
İkizlik oranı (%)*	23.08^{ab}	34.04^{ab}	34.62^{ab}	38.24^{ab}	45.00^{a}	26.40^{b}
Kuzu verimi	2.40	2.78	3.04	2.04	2.42	2.29
80. gün yaşama gücü (%)	83.33	82.81	86.30	89.36	84.48	89.87
Doğum ağırlığı ortalaması*	4.04^{bc}	4.29^{ab}	3.96^{c}	4.27^{abc}	4.38^{a}	4.53^{a}
40. gün ağırlık ortalaması (kg)*	11.81^{ab}	11.77^{ab}	11.26^{b}	11.88^{ab}	12.07^{ab}	12.25^{a}
60. gün ağırlık ortalaması (kg)*	14.96^{b}	15.2^{b}	14.28^{b}	15.19^{b}	14.84^{b}	16.23^{a}
80. gün ağırlık ortalaması (kg)*	17.86^{bc}	18.51^{bc}	17.42^{c}	18.97^{b}	18.23^{bc}	20.53^{a}
Kuzu üretkenliği	42.86	51.51	52.99	38.76	44.06	47.01

** İstatistiki açıdan %95 güven sınırında önemlidir; ^{a,b,c,d} Aynı satırda farklı harf taşıyan değerler istatistiki açıdan farklıdır (P<0.05)*
** Statistically significant at 95% confidence interval; ^{a,b,c,d} Different superscripts in lines differ significantly (P<0.05)*

Tablo 6. *Özel sektörde iki yılın sonunda elde edilen toplu sonuçlar*
Table 6. *The whole results in two years in field*

İncelenen Özellikler	Gruplar					
	1	2	3	4	5	6
Projeden çıkarılan koyun sayısı	6	4	7	5	3	16
Koç altı koyun sayısı	59	61	58	60	62	184
İki yıllık koç altı koyun sayısı	177	183	174	180	186	368
Tohumlanan koyun sayısı*	153^{b}	165^{b}	155^{b}	100^{c}	115^{c}	351^{a}
Doğum yapan koyun sayısı*	116^{b}	120^{b}	120^{b}	88^{c}	102^{c}	327^{a}
Doğan kuzu sayısı	146	158	152	118	145	407
İkizlik oranı (%)*	25.00^{b}	30.83^{ab}	25.83^{b}	32.95^{ab}	41.18^{a}	23.85^{b}
Kuzu verimi	2.48	2.59	2.62	1.98	2.34	2.21
80. gün yaşama gücü (%)*	84.93^{ab}	82.28^{b}	85.53^{ab}	86.44^{ab}	84.14^{b}	90.42^{a}
Doğum ağırlığı ortalaması*	4.58^{a}	4.34^{b}	4.51^{ab}	4.48^{ab}	4.45^{ab}	4.36^{b}
40. gün ağırlık ortalaması (kg)*	12.26^{ab}	11.77^{b}	12.15^{ab}	12.62^{a}	11.95^{b}	11.88^{b}
60. gün ağırlık ortalaması (kg)*	16.27^{ab}	15.79^{b}	16.26^{ab}	17.14^{a}	15.59^{b}	16.09^{b}
80. gün ağırlık ortalaması (kg)*	20.45^{ab}	20.04^{b}	20.25^{b}	21.47^{a}	18.93^{c}	20.69^{ab}
Kuzu üretkenliği	50.61	51.90	53.08	42.23	44.28	45.77

** İstatistiki açıdan %95 güven sınırında önemlidir; ^{a,b,c,d} Aynı satırda farklı harf taşıyan değerler istatistiki açıdan farklıdır (P<0.05)*
** Statistically significant at 95% confidence interval; ^{a,b,c,d} Different superscripts in lines differ significantly (P<0.05)*

Tablo 7. *Kamu sektörü ve Özel sektörde kontrol grubuna göre gruplarda elde edilen kuzu verimi ve kuzu üretkenliği farkları (%)*
Table 7. *Lamb yield and productivity difference between groups according to controls at the Institute and in field*

İncelenen Özellikler	Gruplar											
	Kamu Sektörü						Özel Sektör					
	1	2	3	4	5	6	1	2	3	4	5	6
Kuzu verimi	104.8	121.4	132.8	89.1	105.7	100	112.2	117.2	118.6	89.6	105.9	100
Kuzu üretkenliği	91.2	109.6	112.7	82.5	93.7	100	110.6	113.4	116.0	92.3	96.7	100

Tablo 8. *Gruplara göre yapılan Kısmi Bütçe Analizi*
Table 8. *Partial Budget Analysis made according to groups*

Grup	Yeni Masraflar (A)	Vazgeçilen Gelir (B)	Yapılmayan Masraflar (C)	Yeni Elde Edilen Gelir (GSÜD) (D)	A+B	C+D	Sonuç
1	19.67	182.37	0.00	126.95	202.04	126.95	Karlı Değil
2	19.67	182.37	0.00	128.17	202.04	128.17	Karlı Değil
3	19.67	182.37	0.00	133.53	202.04	133.53	Karlı Değil
4	0.00	182.37	0.00	109.76	182.37	109.76	Karlı Değil
5	15.00	182.37	0.00	107.61	197.37	107.61	Karlı Değil
6	0.00	182.37	0.00	182.37			

TARTIŞMA ve SONUÇ

Sık kuzulatma uygulanan dönemlerde hem Kamu sektörü hem de Özel sektörde en yüksek siklik aktivite oranı Kasım ayında, en düşük siklik aktivite oranı ise Mart ayında belirlendi. Elde edilen bu sonuç koyunların mevsime bağlı poliöstrik hayvanlar olduğunu ve 35. Kuzey paralelin üzerinde ve 34. Güney paralelin altındaki bölgelerde, gün ışığı alma süresi (fotoperiyot), düşük çevre ısısı, ırk, koç katımı ve beslenmenin üreme sezonunun başlamasında etkili olduğunu bildiren, Türkiye'de ise aşım sezonunun, günlerin kısalmaya başladığı Haziran ayında başlayıp Kasım ayı sonuna kadar sürdüğünü belirten literatür bilgiyle uyumludur [1]. Bununla birlikte çalışmada Temmuz ayında tespit edilen siklik aktivite oranı (%65-70) belirtilen dönemin bir geçiş dönemi özelliği gösterdiğini düşündürmektedir.

Tohumlama dönemlerinde elde edilen östrüs, gebelik ve doğum oranları Kamu sektörü ve Özel sektörde birbirine benzer oldu. Çalışma gruplarında 1. tohumlama

(Temmuz) döneminde elde edilen östrüs oranları son uygulamadan sonraki 5 gün dikkate alındığında 1, 2 ve 3. gruplarda 4 ve 5. gruplardan yüksek oldu ($P<0.05$). Bununla birlikte 1 aylık östrüs oranları bütün gruplarda benzer oldu. Çalışmada bu dönemde belirlenen siklik aktivite ve hormon kullanılarak uygulanan senkronizasyon yöntemi (GnRH + PGF_{2a} + PMSG) dikkate alındığında, koç etkisi ve flushing yöntemlerinin geçiş döneminde östrüsü uyarmada yetersiz kaldığı, fakat 1 aylık bir dönemde bunun telafi edildiği düşünülmektedir. Mart ve Kasım dönemlerinde (2 ve 3. tohumlamalar) ise hem 5 günlük hem de 1 aylık östrüs oranları 1, 2 ve 3. gruplarda 4 ve 5. gruplardan yüksek oldu ($P<0.05$). Çalışma gruplarındaki 2. tohumlamalarda (Mart) 1, 2 ve 3. gruplarda koyunlar için çiftleştirme sezonu dışı olmasından dolayı senkronizasyon amacıyla kullanılan progesteron içeren sünger, koç etkisi ve flushing gruplarından daha yüksek östrüs uyarımı sağladı. Elde edilen bu sonuç, koyunlarda siklik aktivitenin sezona bağlı olduğunu [15] ve sezon dışında koç etkisi ile yavru alınmakla birlikte bunun yeterli olmayacağını [16,17] bildiren araştırmacılarla uyumlu oldu. Çalışma gruplarında Kasım ayında (3. tohumlama) planlanan tohumlamalar ise kuzulama aralığının 8 aydan daha uzun olmasından dolayı Kasım sonu - Aralık başında gerçekleştirildi. Bu dönemde ilk 5 gün dikkate alındığında senkronizasyon için kullanılan hormonal yöntem (GnRH + PGF_{2a} + PMSG), koç etkisi ve flushing uygulamalarından daha yüksek oranda östrüsü uyardı. Tohumlamanın sürdüğü 1 aylık dönemde de koç etkisi ve flushing gruplarında östrüs yeterince uyarılamadı. Koç etkisi kullanılarak yapılan senkronizasyonlarda ilk siklusta fertilitenin düşük olduğu, sürüdeki ilk fertil östrüslerin 16-24. günler arasında dağılım gösterdiği araştırmacılar tarafından vurgulanmaktadır [15]. Çalışmada belirtilen tohumlama döneminde, normalde koç etkisi sonucu oluşması gereken fertil östrüslerin üreme sezonunun sonuna denk gelmesi sonucu fertilitenin yeterince uyarılamadığı düşünülmektedir. Nitekim Gül ve Keskin [12], iki yılda üç kuzulatma uygulamalarında en azından bir ya da iki tohumlamanın çiftleştirme sezonu dışına denk geldiğini, bu dönemlerde fertilitenin negatif yönde etkilendiğini bildirmektedirler.

Kontrol gruplarında tohumlama oranları ise 1. ve 2. yıllarda birbirine benzer oldu. Kamu sektörü (%97 ve %100) ve Özel sektördeki (%95 ve %96) sürülerde 1 aylık dönemde saptanan östrüs/tohumlama oranları Konya merinosu [27] ve Karacabey Merinosu [3] üzerinde üreme sezonunda yapılan diğer bazı çalışmalarda elde edilen sonuçlarla yakın oldu.

Kaşeksi, pneumoni, aktinomikoz ve mecburi kesim gibi nedenlerden dolayı çalışmadan çıkarılan koyun sayıları yönünden ne Kamu sektörü ne de Özel sektördeki gruplar arasında istatistiki fark saptanmadı. Bununla birlikte, iki yıl süren çalışmada sık kuzulatma uygulamasının sürüden çıkarılan koyun oranı üzerine bir etkisi saptanmamış olsa da, sık kuzulatmanın daha uzun sürmesi durumunda sürüden uzaklaştırılan koyun oranının artıp artmayacağı ile ilgili ilave çalışmaların yapılması gerektiği düşünülmektedir.

Ortalama kuzulama aralığı çalışma gruplarında Kamu sektöründe 248, Özel sektörde ise 249 gün oldu. Çalışmada hedeflenen 240 günlük kuzulama aralığından yaklaşık 8-9 gün kadar sapılmış olmakla birlikte önceden yapılan çalışmalarda bildirilen yaklaşık 280-305 günlük aralıklar [3,27,28] dikkate alındığında önemli derecede planlamaya uyulduğu düşünülmektedir. Bununla birlikte, her ne kadar sunulan çalışmada sıkı takip sonucu hedeflenen kuzulama aralığı yeterince uygulamaya aktarılabilmiş olsa da, yetiştiricilerin özellikle kuzuların yeterince süt ememediği bahanesiyle bu süreye uymama eğiliminde oldukları gözlendi.

İki yıllık veriler birlikte değerlendirildiğinde, hem Kamu sektörü hem de Özel sektörde kuzu verimi ve kuzu üretkenliği en yüksek 3. grupta, en düşük ise 4. grupta tespit edildi. Kontrol grubuna göre kuzu verimi 1, 2, 3 ve 5. gruplarda Kamu sektöründe %4.8-32.8 ve Özel sektörde %5.9-18.6 yüksek olurken 4 grupta %10.9 ve %10.4 daha düşük oldu. Kuzu üretkenliği ise kontrol grubuna göre Kamu sektöründe 2 ve 3. gruplarda %9.6 ve %12.7 daha yüksek, 1, 4 ve 5. gruplarda ise %6.3-17.5 daha düşük, Özel sektörde ise 1, 2 ve 3. gruplarda %10.6-16.0 yüksek, 4 ve 5. gruplarda ise %3.3 ve %7.6 düşük oldu. Hem Kamu sektörü hem de Özel sektörde özellikle sezon dışında saptanan kuzu verimi, koç etkisi ve flushing yöntemleri ile sezon dışında da yavru alınabileceğini fakat belirtilen yöntemlerin sık kuzulatma amacıyla yeterli olmadığını bildiren Yılmaz ve ark.'nın [16] bulgularıyla paralellik göstermektedir. Kıvırcık koyunları üzerinde yürütülen bir çalışmada Koyuncu [9], yıllık kuzulama sayısı ve bir doğuma düşen ortalama kuzu sayısının sık kuzulatma uygulandığında 1.33 ve 1.77; yılda bir kuzulatma uygulandığında ise 1.00 ve 1.56 olduğunu, toplamda ise sık kuzulatma ile doğumda %15 ve sütten kesimde %19 daha fazla verim alındığını bildirmiştir. Yeni Zelanda'da yapılan çalışmalarda ise DeNicolo ve ark.[7,10], kuzu verimi ve kuzu üretkenliğinin sık kuzulatma uygulanan koyunlarda daha yüksek olduğunu, ayrıca sık kuzulatma uygulanan East Friesian Composite ırkı koyunlarda %26 daha yüksek kuzu verimi alınmasına karşın, Romney ırkı koyunlarda kuzu veriminin %8 daha düşük gerçekleştiğini ve koyun ırkının da bu uygulamada önemli olduğunu vurgulamışlardır. Suriye İvesi koyunlarında iki yılda üç kuzulatma uygulayan Zarkawi [11] kuzu veriminin arttığını bildirirken, Gül ve Keskin [12] ise mevsimin İvesi ırkı koyunlarda fertilite ve kuzu verimi üzerine negatif etkisinden dolayı çalışmalarında sık kuzulatmanın kuzu sayında artışa neden olmadığını bildirmişlerdir. Çalışmalar arasındaki farklılıkların, sık kuzulatma uygulamalarının farklı ırk ve bölgelerde yapılması, kullanılan farklı senkronizasyon yöntemlerinden farklı dölverimi sonuçlarının alınmasından kaynaklanmış olabileceği düşünülmektedir.

GSÜD değerinin bir karlılık göstergesi olmamasına rağmen, diğer tüm masrafların aynı olduğu kabulünden yola çıkıldığında uygulamaların ekonomik analizinde bir

karşılaştırma göstergesi olarak kullanılabileceği bildirilmektedir [25]. Ayrıca Kısmi bütçelerin, yeni girdilerin, yeni işletme tekniğinin veya pazarlama imkanlarının kullanılıp kullanılmaması konularında işletmecinin karar vermesine ışık tutabileceği vurgulanmaktadır. GSÜD bir girdinin diğerine ikamesi veya tarım tekniğinin değiştirilmesi durumlarında da kullanılabilmektedir [26]. Sunulan çalışmada iki yıl sonunda kontrol grubuna göre Kamu sektöründe 2 ve 3. gruplarda sırasıyla 4.5 ve 5.98 kg, Özel sektörde ise 1, 2 ve 3. gruplarda sırasıyla 4.84, 6.13 ve 7.31 kg daha fazla kuzu üretkenliği sağlanmakla birlikte, Kısmi Bütçe Analizinde değerlendirilen bütün çalışma gruplarında yapılan sık kuzulatma uygulamasının karlı olmadığı tespit edildi. Suriye İvesi ırkı koyunlarda iki yılda üç kuzulatma uygulayan Zarkawi [11] elde ettiği sonuçları ekonomik analize tabi tutmamış fakat sık kuzulatmanın kuzu verimini artırdığını bildirmiştir. Bu konu ile ilgili olarak yaptıkları çalışmada Keskin ve ark.[5], İvesi koyunlarında iki yılda üç kuzulatmanın kuzu verimini artırdığını, bununla birlikte sık kuzulatmanın ekonomik açıdan günün şartlarına göre değerlendirilmesi gerektiğini, elde edilecek kuzu geliri yapılacak masrafları karşılıyorsa bunun uygulanması gerektiğini önemle vurgulamaktadırlar.

Sonuç olarak sık kuzulatma uygulamasıyla yılda bir kuzulatmaya göre daha fazla kuzu verimi ve kuzu üretkenliği sağlanabileceği, bununla birlikte sık kuzulatma uygulamalarında elde edilecek kuzu verimi ve kuzu üretkenliği üzerinde uygulanacak senkronizasyon yöntemlerinin önemli derecede etkili olduğu tespit edildi. Ayrıca, sunulan çalışmada uygulanan senkronizasyon yöntemleri sonucu elde edilen farkın ekonomik olarak yeterince karlı olmadığı sonucuna varıldı.

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RESEARCH ARTICLE

Effect of Cereal Grains on the Total Lipid, Cholesterol Content and Fatty Acid Composition of Liver and Muscle Tissues in Native Geese

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Summary

In this study we evaluated the effect of cereal grains on total lipid, cholesterol content and fatty acid composition of liver and muscle tissues in native geese. The thirty five geese used in the study were divided into five groups of seven. The groups of geese feed with barley, wheat, rye and corn, respectively. The first group was used as the control group. Water and feed were provided for ad libitum consumption for 6 weeks. While the level of total cholesterol in the liver tissue decreased (P<0.001) in barley and wheat groups, it of the thigh muscle decreased (P<0.001) in all groups. While the total lipid content of the liver tissue increased in rye and corn groups (P<0.001), it of the thigh muscle increased (P<0.001) in wheat and rye groups. While the total lipid content of the back muscle decreased in barley and wheat groups (P<0.001), it of the breast muscle decreased (P<0.001) in wheat, rye and corn groups. Although the amount of palmitic acid in the liver tissue increased (P<0.001) in the rye group, the amount of stearic decreased (P<0.001) in the barley group. The amount of arachidonic, docosahexaenoic, PUFA, n-3 and n-6 acids in the liver tissue increased (P<0.001) in the wheat, rye and corn groups. Consequently, in addition to other foods, rye could be used as a valuable nutrition of the geese diet.

Keywords: *Cereal Grains, Cholesterol, Total lipid, Liver, Muscle, Geese Diet*

Yerli Kazlarda Karaciğer ve Kas Dokularının Total Lipit, Kolesterol İçeriği ve Yağ Asidi Kompozisyonu Üzerine Tahıl Tanelerinin Etkisi

Özet

Bu çalışmada yerli kazlarda karaciğer ve kas dokusunun total lipit, kolesterol içeriği ve yağ asidi kompozisyonu üzerine tahıl tanelerinin etkisini inceledik. Çalışmada kullanılan 35 kaz 5 gruba ayrıldı. Kaz grupları sırasıyla arpa, buğday, çavdar ve mısır ile beslendi. İlk grup kontrol grubu olarak kullanıldı. Su ve besin 6 hafta boyunca ad libitum olarak verildi. Karaciğer dokusundaki total kolesterol seviyesi arpa ve buğday gruplarında azalış (P<0.001) gösterirken, but kasında tüm gruplarda (P<0.001) azalmıştır. Karaciğer dokusundaki total lipit içeriği çavdar ve mısır gruplarında artış (P<0.001) gösterirken, but kasında buğday ve çavdar gruplarında (P<0.001) artmıştır. Sırt kasındaki total lipit içeriği arpa ve buğday gruplarında azalış (P<0.001) gösterirken, göğüs kasında buğday, çavdar ve mısır gruplarında (P<0.001) azalmıştır. Karaciğer dokusunda palmitik asit miktarı çavdar grubunda arttığı (P<0.001) halde, stearik asit miktarı arpa grubunda (P<0.001) azalmıştır. Karaciğer dokusundaki araşidonik, dokosahekzaenoik, PUFA, n-3 ve n-6 asitleri buğday, çavdar ve mısır gruplarında artmıştır (P<0.001). Sonuç olarak, diğer besinlere ek olarak çavdar kaz diyetinde değerli bir besin olarak kullanılabilir.

Anahtar sözcükler: *Tahıl taneleri, Kolesterol, Total lipit, Karaciğer, Kas, Kaz diyeti*

INTRODUCTION

Poultry meat is a consumption material which is preferred by a lot of people. There has been growing interest over recent years in the modulation of the cholesterol content and fatty acid composition in poultry products because occurrences of cardiovascular heart disease have been closely related to the dietary intake of cholesterol and saturated fatty acid (SFA) content [1]. To lead a healthy life is to reduce intake of cholesterol and saturated fatty acids [2].

The study of avian nutrition, which was the focus of much biochemistry for the early part of this century, was carried out of mainly using chicks, ducks, quail and geese.

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Geese are commercially produced for meat, fatty liver and feathers [3]. Geese production is broadly free-range production system in Turkey [4].

From 1940s to nowadays, nutritional science has been neglected by the majority of biochemists; contents of the diet have been defined and in most cases their biochemical role is known. Although with humans the emphasis is on improving health and longevity, in the domestic poultry and the other domesticated birds, the emphasis of nutritional studies has been largely directed towards optimizing growth [5].

A controlled feeding can modulate the fat development. The studies of the nutritionist are to define means to achieve a good of carcass fattening and to increase output in lean meat while considering the impact of genetic, nutritional and economic factors [6]. These factors contribute to the tendency of boiler and geese to accumulate excess body fat. Therefore, improving carcass quality with feed additives has become a main focus of nutrition research [7]. Depending on the source of energy in the diet (starch or lipids) the fatty acid profile of muscle in fowl reflects a balance between hepatic steatosis and dietary lipids [6]. In all birds, fatty acids are mainly synthesized in the liver and then exported to peripheral tissues, including the muscles [8].

In domestic birds, i.e. geese and duck, they are used to commercial production of fatty liver ('foie gras') which has their specific capacity [9]. In the goose, the liver weight may increase 10-fold in two or three weeks and account for up to 10% of body weight [10] . Actually, the relative importance of the liver steatosis and the peripheral fat deposition results from a poise which may be controlled by the efficiency of lipid transfer between adipose tissues and muscles [11].

The mechanism of dietary-induced fatty liver of birds remains puzzling. In response to overfeeding, de novo hepatic lipogenesis from dietary carbohydrates is sensationally made better in the goose [12].

Cholesterol is a very important organic molecule to cellular function [13].

The aim of the present study was to compare the effects of feeding different cereal grains on the total lipid, cholesterol and fatty acid composition of muscle and liver tissues in native geese.

MATERIAL and METHODS

Animals

All experiments were conducted in accordance with the principles and guidelines approved by the Fırat University of Technology Animal Care and Use committee (No: MKÜ-HADYEK-2012/30). Thirty-five adult male and female native geese *(Anser anser)* were bought in Kars/Turkey. At the start of the experiment, the geese weighed 2450-2850 g. Geese were randomly assigned to five groups. They were placed in groups of 7 geese in metal cages (200 cm long x 200 cm wide x 200 cm high). The environmental conditions were control for ventilation. The room temperatures were set at 31 and 30°C, respectively, during the six weeks. They were provided ad libitum access to drinking water and experimental diets for 6 weeks. The weekly amount of feed provided was 7 kg for each group *(Table 1)*. The first group was used as control (CON) and fed with fresh meadow grass. We choose fresh meadow grass in control group for geese is fed naturally in grasslands of Kars. The second group was only fed with barley (BA), the third group with wheat (WH), the fourth group with rye (RY) and the fifth group with corn (COR).

Table 1. *Percent composition of diets, (%)*
Tablo 1. *Diyetin yüzde kompozisyonu*

Feed Ingredients	Groups				
	Control	Barley	Wheat	Rye	Corn
Fresh meadow grass	99.40	-	-	-	-
Barley	-	99.10	-	-	-
Wheat	-	-	99.10	-	-
Rye	-	-	-	99.10	-
Corn	-	-	-	-	99.10
Salt	0.60	0.60	0.60	0.60	0.60
Vitamin[1]	-	0.20	0.20	0.20	0.20
Mineral[2]	-	0.10	0.10	0.10	0.10

[1] ***Vitamin added per kilogram:*** *vitamin A, 2.000.000 IU; Vitamin D_3, 400.000; D-Biotin, 6.50 mg Vitamin E,; 2.600 mg; Vitamin B_1, 520 mg; Vitamin B_2, 1.320 mg; Vitamin B_5, 2.000 mg; Vitamin B_6, 660 mg; Vitamin B_{12} 2.50 mg*
[2] ***Vitamin added per kilogram:*** *Niacin, 2.600 mg; Mn, 6.500 mg; Fe, 6.500 mg; $C_5H_{14}CINO$, 26.65 mg; Zn, 6.500 mg; Cu, 1.320 mg; Na, 180.000 mg; I, 100 mg; Se,26.50 mg; Co, 26.50 mg*

At the end of the feeding period, geese were slaughtered muscle and liver tissues were removed, weighed and refrigerated at 4°C. After weighing of the tissues, a ~10 g sample was taken from part of the thigh, back, and breast of each group and were kept at -20°C until the lipid extraction and further analysis.

Preparation of Fatty Acid Methyl Ester and Gas Chromatographic Analysis

Total lipids were extracted with hexane-isopropanol (3:2 v/v) as described by Hara and Radin [14]. About 1 g of the samples were taken and homogenized with homogenizer (Bosch, Germany).

Fatty acids in the lipid extracts were converted into methyl esters by means of 2% sulphuric acid (v/v) in methanol [15]. The mixture was incubated at 55°C for 17 h. Nonlipid contaminants in lipid extracts were removed by NaCl solution. Fatty acid methyl esters were treated with 1 ml 2% KH_2CO_3 solution. The hexane phase was evaporated by the nitrogen flow and then by dissolving in 1 mL fresh hexane, they were taken to auto sampler vials.

Then the methyl esters were separated and quantified by gas chromatography and flame- ionization detection (Shimadzu GC 17 Ver. 3) coupled to a Glass GC 10 software computing recorder. Chromatography was performed with Machery-Nagel (Germany) capillary column (25 m in length) and 0.25 mm in diameter. The temperatures of the column was kept at 120-220°C, injection temperature was kept at 240°C and the detector temperature was kept at 280°C. The colon temperature program was adjusted from 120-20°C and then 5°C min until 200 and 4°C min from 200-220°C. It was kept at 220°C for 8 min and the total duration was set as 35 min and nitrogen gas was used as the carrier gas. Identification of the individual methyl esters was performed by frequent comparison with authentic standard mixtures that were analyzed under the same conditions [16].

Cholesterol Analysis

Total lipids were extracted with hexane-isopropanol (3:2 v/v) as described by Hara and Radin [14]. About 1g of the samples were taken and homogenized.

The 5 ml supernatant was taken to tubes and 5 ml KOH was added to it. The mixture was incubated for 30 min at 85°C. It was then cooled under room condition and 5 ml of distilled water was added. Hexane-isopropanol extracts were combined and evaporated. The residue was dissolved in 1 ml of acetonitrile: methanol centrifuged and the supernatant was used for cholesterol content determination.

For the analysis cholesterol content were used the fully automatic High Performance Liquid Chromatography equipment (HPLC) [17]. The equipment for HPLC consisted of a pump (LC-10ADVP), a UV-vis detector (SPD-10AVP) a column oven (CTO-10ASVP), an auto sampler (SIL-10ADVP) a degasser unit (DGU-14A) and a computer system with Class VP software (Shimadzu, Kyoto, Japan). Discovery RP-Amide C16 column (150 mm×4.6 mm, 5µm; Sigma, USA) was used as the HPLC column and 50 mM $NaClO_4$ 0.1% H_3PO_4 was used as the mobile phase and flowrate 1 ml/min. Detection was performed at 215 nm by UV-vis detector and 40°C column oven.

Statistical Analysis

Values have been reported as the mean±SEM. Statistical analysis was done with SPSS 16.0 software. Analysis of variance (ANOVA) and Duncan test were used to compare the experimental groups with the controls.

RESULTS

The purpose of this study in the province of Kars/Turkey, geese reared in different foods (barley, wheat, rye, and corn), some biochemical parameters of nutrition as a result of changes in muscle and liver tissue was investigated.

Fatty Acid Content of Diet

Palmitic acid (16:0) level was found to be rather low compared to other groups in control. Barley, wheat and rye show a significant difference is determined. However, a significant increase was observed in corn. Palmitoleic acid (16:1n-7) compared to the control level except for rye was increased. This increase was found to be quite high, especially corn.

Stearic acid (18:0) significantly reduced the amount of wheat was compared with the control. Barley and maize were increased compared to the control. Oleic acid (18:1n-9) levels of corn and rye, is very high compared to other groups *(Table 2)*.

Linoleic acid (18:2n-6) compared to control all the feed increased the amount of this increase, especially in corn were found to be more specific. Linolenic acid (18:3n-3) according to the amount of other feeds in the control was very much reduced.

Total Lipid and Cholesterol Content

In this study, it was found that the total lipid content of liver tissue was between 137.14±1.87-267.96±2.14 mg/100 g and total cholesterol content was 63.94±1.52-87.54±1.22 mg/100 g wet tissue. The content of total lipid and cholesterol in muscle and liver tissues of native geese are shown in *Table 3*.

While the cholesterol levels and the lipid content of liver tissue decreased in barley (BA) and wheat (WH) groups, it increased in rye (RY) and corn (COR) groups. The cholesterol levels of thigh tissue decreased in all groups.

Table 2. *Fatty acid content of diets (mg/100 g, n=7)*
Tablo 2. *Diyetlerinin yağ asidi içeriği (mg/100 g, n=7)*

Fatty Acids	Control	Barley	Wheat	Rye	Corn
16:0	9.89	36.64	38.23	30.80	83.11
18:0	1.03	21.81	0.22	1.65	11.42
16:1n-7	1.27	5.00	9.30	1.11	22.00
18:1n-9	2.00	2.93	6.10	38.88	179.50
18:2n-6	10.94	104.26	149.05	120.42	470.33
18:3n-3	44.41	13.56	15.36	20.41	12.08
18:4n-3	1.00	1.83	2.05	3.57	1.62

Table 3. *Total lipid and cholesterol content in liver and muscle tissues of native geese (n=7)*
Tablo 3. *Yerli kazların karaciğer ve kas dokularındaki total lipit ve kolesterol içeriği (n=7)*

Parameters	Groups				
	Control	Barley	Wheat	Rye	Corn
Liver (mg/100 g)					
Total lipid	211.22±1.84^{d}	137.14±1.87^{e}	197.99±2.11^{c}	267.96±2.14^{a}	215.80±1.92^{b}
Cholesterol	74.41±1.33^{b}	64.99±1.33^{c}	63.94±1.52^{c}	87.54±1.22^{a}	86.54±4.34^{a}
Muscle Total Lipid (mg/100 g)					
Thigh	302.97±3.67^{d}	209.98±3.20^{e}	948.32±1.67^{a}	449.26±2.47^{c}	848.87±1.77^{b}
Back	256.63±0.69^{a}	490.23±1.90^{c}	500.53±2.12^{c}	559.82±3.60^{c}	729.94±2.48^{cd}
Breast	489.79±2.79^{a}	597.62±3.04^{c}	481.86±1.94^{a}	988.89±2.83^{cd}	739.04±2.86^{cd}
Muscle Cholestrol (mg/100 g)					
Thigh	29.30±1.16^{a}	27.11±0.29^{b}	24.24±1.81^{c}	23.31±0.81^{c}	25.29±1.12^{b}
Back	29.17±2.63^{d}	20.84±2.37^{c}	22.47±2.04^{b}	30.05±5.42^{a}	26.00±3.19^{a}
Breast	37.41±0.25^{c}	35.35±0.64^{c}	30.83±0.25^{c}	32.69±0.15^{a}	26.72±0.70^{b}

Differences between the groups comprising different letters in the same line is statistically significant (P<0.001)

While the cholesterol levels of back tissue decreased in BA and WH, it of breast tissue decreased in WH, RY and COR. While the lipid content of thigh tissue decreased in BA, it increased in WH, RY and COR. Total lipid content of back tissue was increased (P<0.001) in all groups. Total lipid content of breast tissue was increased (P<0.001) in BA, RY and COR *(Table 3)*.

The present data confirm that, when food intake is kept identical in native geese, lipid metabolism and amount of the total cholesterol is very different as compared to control (CON) group. They effect markedly in the proportions and the total lipid content of liver and muscle tissue.

The total cholesterol level of all muscles slightly decreased (P<0.001) in WH and COR, at the end of the feeding period. Fournier et al.[18] was reported that there was no significant difference between Landes and Poland geese, with the exception of the percentage of cholesteryl esters, which was higher in the Landes goose.

Fatty Acid Content of Liver and Muscle Tissue Lipids

The influence of the dietary treatment on the liver and muscle tissues of individual fatty acids is shown in *Table 4*. In liver tissues, the amount of palmitic acid increased significantly in RY, but stearic acid decreased significantly in BA. The amount of oleic acid increased significantly in WH, RY and COR. The amount of linoleic acid increased (P<0.05) significantly in WH and COR when compared to COR. The amount of arachidonic, docosahexaenoic, poly-unsaturated, n-3 and n-6 acids decreased significantly in BA when compared to CON, but they increased significantly in WH, RY and COR.

The amount of fatty acids in thigh muscle is shown in *Table 5*. In thigh muscles, the amount of palmitic, stearic, oleic, linoleic, linolenic, arachidonic, SFA, PUFA and n-3 acids decreased significantly (P<0.001) in BA when compared to control group but they increased significantly in WH, RY and CORN. The amount of palmitoleic acid increased in BA and COR when compared to CON. The amount of docosahexaenoic acid increased in WH when compared to control group.

The amount of fatty acids in back muscle is shown in *Table 6*. In back muscles, the amount of oleic, linoleic, SFA, MUFA, PUFA and n-6 acids increased significantly

Table 4. *Amounts of fatty acids in liver (mg/100 g, wet tissue)*
Tablo 4. *Karaciğerdeki yağ asidi miktarı (mg/100 g, yaş ağırlık)*

Fatty Acids	Groups				
	Control	Barley	Wheat	Rye	Corn
16:0	41.41±3.28^{bc}	27.64±2.38^{c}	37.87±3.14^{b}	46.92±1.59^{a}	39.58±3.23^{b}
18:0	39.85±2.16^{ab}	25.04±1.72^{c}	34.52±3.36^{b}	49.13±2.73^{a}	42.91±2.00^{a}
SFA[1]	81.25±5.27^{a}	52.68±3.88^{c}	72.39±0.92^{b}	96.05±3.01^{a}	82.49±5.13^{a}
18:1n-9	38.60±0.94^{c}	34.58±1.48^{c}	47.28±1.81^{ab}	55.10±2.18^{a}	45.08±2.62^{b}
18:2n-6*	26.86±1.33^{b}	32.22±1.32^{ab}	33.86±1.25^{a}	29.80±3.15^{ab}	37.22±2.44^{a}
20:4n-6	42.50±2.40^{bc}	24.39±1.54^{d}	38.73±2.73^{c}	52.00±2.61^{a}	46.66±1.20^{ab}
22:6n-3	13.99±1.47^{b}	6.87±0.64^{c}	9.72±1.11^{c}	20.24±1.79^{a}	20.69±2.34^{a}
PUFA[2]	77.33±0.59^{d}	63.49±1.10^{e}	82.32±0.87^{c}	102.04±1.02^{a}	118.55±1.56^{a}
Σn-3	13.99±1.13^{b}	6.87±0.20^{d}	9.72±0.85^{c}	20.24±0.47^{a}	20.69±1.05^{b}
Σn-6	69.37±2.90^{c}	56.61±1.94^{d}	72.60±1.10^{c}	81.79±2.78^{b}	97.85±3.09^{a}

** P<0.05, Other groups are P<0.001, [1] **SFA**= Saturated fatty acid, [2] **PUFA**= Polyunsaturated fatty acid*

Table 5. *Amounts of fatty acids in thigh muscle (mg/100g, wet tissue)*
Tablo 5. *But kasındaki yağ asidi miktarı (mg/100g, yaş ağırlık)*

Fatty Acids	Groups				
	Control	Barley	Wheat	Rye	Corn
16:0	53.17±3.44^{d}	43.60±2.92^{e}	120.85±2.58^{b}	89.48±2.16^{c}	197.88±2.45^{a}
18:0	25.59±1.02^{c}	18.24±2.70^{d}	40.88±2.14^{b}	40.81±2.73^{b}	61.04±1.33^{a}
SFA[1]	78.79±1.65^{d}	68.26±9.07^{d}	161.73±3.66^{b}	130.29±3.22^{c}	258.92±8.30^{a}
16:1n-7	12.46±0.47^{b}	8.53±1.52^{bc}	26.48±1.97^{a}	7.31±1.04^{c}	25.60±0.95^{a}
18:1n-9	83.00±1.21^{e}	77.61±2.67^{d}	197.65±3.58^{b}	154.93±1.76^{c}	312.83±1.70^{a}
MUFA[2]	95.47±2.13^{e}	86.15±2.83^{d}	224.13±7.19^{b}	162.25±4.42^{c}	340.86±2.71^{a}
18:2n-6	44.53±0.70^{d}	28.68±2.52^{e}	69.82±3.67^{b}	56.87±1.45^{c}	102.75±5.13^{a}
18:3n-3	20.42±1.99^{c}	12.79±0.83^{d}	24.93±1.43^{b}	25.06±0.98^{b}	31.75±1.47^{a}
20:4n-6	20.70±2.80^{c}	13.11±0.22^{d}	38.40±2.05^{b}	37.61±2.33^{b}	48.77±2.69^{a}
22:6n-3	3.80±1.74^{bc}	1.17±1.10^{c}	19.98±1.47^{a}	8.14±1.72^{bc}	8.09±1.25^{b}
PUFA[3]	89.44±1.35^{e}	55.76±2.70^{d}	153.13±7.19^{b}	126.26±4.09^{c}	184.35±4.70^{a}
Σn-3	24.21±4.65^{c}	14.08±3.35^{d}	44.91±1.83^{a}	33.20±5.08^{c}	39.11±1.91^{b}
Σn-6	65.23±8.27^{d}	41.68±9.94^{e}	108.22±6.53^{b}	94.48±9.71^{c}	145.23±3.76^{a}

Differences between the groups comprising different letters in the same line is statistically significant (P<0.001)
*[1] **SFA** = Saturated fatty acid, [2] **MUFA** = Monounsaturated fatty acid, [3] **PUFA** = Polyunsaturated fatty acid*

(P<0.001) in all groups when they compared to CON. The amount palmitoleic acid were not statistically significant (P>0.05) in all groups when they compared to CON. The amount of stearic acid increased significantly in BA, WH and COR. While arachidonic acid increased in BA, RY and COR, docosahexaenoic acid increased (P<0.01) in WH and COR when compared to CON.

The amount of fatty acids in breast muscle is shown in *Table 7*. In breast muscles, the amount of stearic, linoleic and docosahexaenoic acids increased significantly (P<0.001) in RY and COR when they compared to CON. While palmitoleic acid decreased in BA and RY, linolenic acid increased significantly (P<0.05) in RY and COR. The amount of palmitic acid decreased significantly in WH but it increased in BA, RY and COR. Arachidonic, MUFA and n-6 increased in all groups. While the amount of SFA increased significantly in BA, RY and COR, the amount of PUFA increased significantly in WH, RY and COR.

DISCUSSION

The fatty acid components are in general C_{16} and C_{18} fatty acids and a typical proportion of saturated: monosaturated: polyunsaturated fatty acids in broilers is 0.31:

Table 6. *Amounts of fatty acids in back muscle (mg/100 g, wet tissue)*
Tablo 6. *Sırt kasındaki yağ asidi miktarı (mg/100 g, yaş ağırlık)*

Fatty Acids	Groups				
	Control	Barley	Wheat	Rye	Corn
16:0	50.09±0.45^{d}	111.23±0.72^{c}	107.36±2.02^{c}	114.79±2.10^{b}	156.41±3.17^{a}
18:0	26.46±0.88^{c}	41.50±0.50^{b}	40.22± 1.96^{b}	29.59±2.68^{c}	49.97±1.42^{a}
SFA	76.55±1.31^{d}	153.22±1.68^{b}	147.58±1.54^{bc}	144.38±1.88^{c}	203.38±3.34^{a}
16:1n-7*	4.38±0.42^{b}	6.92±1.15^{ab}	7.19±1.15^{ab}	5.96±1.18^{ab}	8.56±1.37^{a}
18:1n-9	76.83±0.42^{d}	159.72±2.18^{a}	170.49±0.96^{a}	111.45±2.09^{c}	153.71±1.65^{b}
MUFA	78.00±1.04^{d}	166.63±1.57^{b}	177.68±2.82^{a}	117.68±3.69^{c}	162.27±2.78^{b}
18:2n-6	36.68±1.76^{d}	56.53±3.86^{c}	64.94±0.86^{b}	58.69±3.37^{bc}	75.77±1.08^{a}
18:3n-3	14.72±1.70^{bc}	11.42±2.41^{c}	34.26±2.14^{a}	19.34±1.20^{b}	15.40±1.46^{bc}
20:4n-6	23.40±1.72^{c}	35.10±1.56^{b}	26.15±0.55^{c}	36.63± 0.82^{b}	45.52±1.07^{a}
22:6n-3**	3.14±1.48^{b}	3.83±2.42^{b}	3.59±1.75^{b}	10.25±1.98^{a}	8.91±0.44^{a}
PUFA	77.94±1.19^{d}	105.87±0.87^{c}	128.94±1.34^{b}	124.90±0.97^{b}	172.59±3.69^{a}
Σn-3	17.86±0.87^{c}	14.25±1.58^{d}	37.85±2.44^{a}	29.58±2.27^{b}	24.31±1.74^{b}
Σn-6	60.08±1.26^{c}	91.62±1.31^{b}	91.09±1.21^{b}	95.32±1.40^{b}	148.90±2.48^{a}

** P>0.05, ** P<0.01, The other groups are P<0.001*

Table 7. *Amounts of fatty acids in breast muscle (mg/100g, wet tissue)*
Tablo 7. *Göğüs kasındaki yağ asidi miktarı (mg/100g, yaş ağırlık)*

Fatty Acids	Groups				
	Control	Barley	Wheat	Rye	Corn
16:0	112.60±1.98^{d}	139.19±1.67^{bc}	101.60±3.41^{cd}	211.47±1.77^{a}	139.76±3.53^{b}
18:0	42.53±1.50^{c}	47.25±1.17^{a}	42.87±2.70^{c}	77.15±2.96^{c}	62.23±1.44^{b}
SFA	155.14±0.90^{d}	186.44±1.17^{bc}	130.47±1.28^{cd}	288.62±1.74^{a}	201.75±4.35^{b}
16:1n-7*	14.24±1.42^{a}	9.21±0.40^{b}	6.93±1.06^{b}	13.16±2.18^{ab}	11.28±1.08^{ab}
18:1n-9	155.14±2.22^{d}	204.56±1.5^{bc}	144.21±2.15^{cd}	340.64±2.78^{a}	211.59±3.30^{b}
MUFA	166.55±2.58^{d}	213.76±3.38^{bc}	151.14±2.60^{cd}	353.80±2.06^{a}	222.88±2.94^{b}
18:2n-6	68.21±2.80^{c}	70.85±1.80^{bc}	68.12±1.02^{bc}	123.98±2.75^{a}	81.45±0.94^{b}
18:3n-3	29.81±2.27^{b}	20.17±1.15^{c}	29.58±3.13^{b}	40.02±2.23^{a}	22.75±2.25^{c}
20:4n-6	27.99±2.49^{c}	42.00±0.66^{b}	36.99±1.58^{b}	65.10±1.82^{a}	45.07±1.30^{b}
22:6n-3	4.54±1.24^{d}	6.74±1.33^{cd}	7.11±1.85^{c}	27.21±1.07^{a}	15.98±0.33^{b}
PUFA	130.54±1.10^{d}	139.77±1.26^{d}	141.81±2.43^{c}	160.69±2.20^{b}	165.54±1.66^{a}
Σn-3	34.35±1.74^{bc}	26.91±2.02^{c}	36.69±2.54^{b}	67.23±1.93^{a}	38.73±0.30^{b}
Σn-6	96.19±1.48^{c}	112.85±3.32^{b}	105.12±3.51^{b}	189.07±2.13^{a}	126.81±1.40^{b}

** P<0.05, The other groups are P<0.001*

0.45:0.23 [19]; a similar distribution is found in jungle poultry and dove [20]. This is a considerably higher proportion of polyunsaturated fatty acids than occurs in mammalian tissues. Shifts in favor of polyunsaturated fatty acids can be accomplished by feeding domestic poultry a high proportion of dietary polyunsaturated fats. In quail, the proportion of saturated to unsaturated fatty acids is also affected by environmental temperature [21,22]. The most ample fatty acids in quail are palmitic, stearic, myristic, palmitoleic, oleic, linoleic, eicosatrienoic and arachidonic acids, and the same is probably true for other domesticated birds [23].

Fatty acids influence many structural metabolic and regulatory components of cells [24]. As essential amino acids and fatty acids cannot be synthesized by animals and humans, they are taken from diets and have to be turned into within the body [25]. Hermier et al.[26] reported that

geese contained mostly oleic (18:1n-9, ~45%), palmitic (16:0, ~30%) and stearic (18:0, ~11-14%) acids, which are the main products of the de novo hepatic lipogenesis. In contrast, polyunsaturated fatty acids (PUFA) are essential and must be provided by the diet. The increase in total lipid content caused by genotype or by overfeeding mainly resulted from a deposition of triglycerides and MUFA. Oleic and palmitoleic acids are the primary fatty acids synthesized per birds [27]. SFA also increased, chiefly palmitic and stearic acids rendered per feed. In spite of large amounts of linoleic acid representing 57% of total fatty acids rendered by the overfeeding diet [28], PUFA amounts in thigh muscle slightly increased. Lipid composition in muscle was therefore mainly influenced by lipogenesis than feed composition.

Mourot et al.[29] was reported that *P. major* (breast) muscle was higher in 18:0 and lower 14:0 and 16:1n-7 in Landes geese. The fatty acids disclosed an increase in saturated fatty acids and a decrease in linoleic acid content [30,31]. The data shown that palmitic (16:0), oleic (18:1) and linoleic (18:2) acids formed a majority of the fatty acid content in muscle tissues *(Table 4-7)*.

Gabarrou et al.[32] reported that the concentration of stearic acid decreased, whereas oleic acid increased noticeably from 10 to 26.3%. The concentrations of (n-6) polyunsaturated fatty acid concentration decreased from 5 to 3.7% for linoleic acid and, more sensationally, from 18.1 to 7.1% for arachidonic acid. Our data found that the fatty acid composition of muscle tissue contains greater n-6 than n-3 fatty acids *(Table 4-6)*. In addition, the n-6 fatty acid content of muscle tissues were higher than liver.

The present study demonstrated that the nature of one's habitual diet with respect to both the amount and type of fatty acids related to the fatty acid composition of structural and stored lipids in liver and muscle tissue. Rye-based diet is resembled with corn-based diet, and rye is one of overfeeding ingredients instead of corn. More research is needed to determine the physiological mechanism by which rye affects geese growth and to identify the optimum method to include rye in geese diets so as to improve bird development. This research can be for the public knowledge regarding an important international meat source for many and the healthfulness of that product.

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RESEARCH ARTICLE

An Epidemiological Study on Prevalence of Goat Warble Fly Infestation (GWFI) from Punjab Province, Pakistan

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Summary

The purpose of the present study was to determine the prevalence of Warble Fly Infestation (WFI) in goats of Punjab Province, Pakistan. Goat warble fly infestation is caused by *Przhevalskiana silenus* (Diptera: Oestridae). There were a total of five hundred animals examined from July 2012 to January 2013 from Khoshab and Chakwal districts of Punjab province for the prevalence of warble. The larvae were collected from the infested goats and identified as *P. silenus*. The results showed that the prevalence of GWFI was 17.8% (89/500). The number of nodules in the infested animals ranged from 1-14 (6.61±2.4). The breed wise prevalence was in beetle breed (13.2%), local breed (18%) and desi breed (22.9%), respectively. The sex wise prevalence was in male (15.3%) and in female (19.4%). The prevalence based on age showed that the rate of infestation in animals having age group (1-3 year) was 20.9%, (4-6 year) was 14.6% and (>6 year) was18.1%, respectively. The present study showed that these epidemiological factors have a significant effect on the prevalence of WFI in goats of Punjab Province. The results showed the effect of different treatments given to animals on the basis of sex, age groups, infested and non-infested animals. The results of this survey showed that the fly is active from March to June. It was first study on GWFI in Punjab Province; northern part of Pakistan. It would be very helpful in devising the future strategies towards the eradication and control of warble fly in other endemic areas of Pakistan.

Keywords: *Goat Warble Fly Infestation, GWFI, Prevalence, Przhevalskiana silenus, Khoshab, Chakwal districts, Pakistan*

Pakistan Punjab Eyaletindede Keçi Nokra Enfestasyonunun Prevalansı Üzerine Epidemiyolojik Bir Çalışma

Özet

Bu çalışmanın amacı Pakistan'ın Punjab Eyaletinde Keçi Nokrasının prevalansını tespit etmektir. Keçi Nokrası *Przhevalskiana silenus* (Diptera: Oestridae) tarafından meydana getirilir. Prevalansın tespiti amacıyla Punjab'ın Khoshab ve Chakwal bölgelerinde Temmuz 2012 ile Ocak 2013 tarihleri arasında toplam 500 adet hayvan incelendi. Larvalar enfekte keçilerden toplandı ve *P. silenus* olarak identifiye edildi. Keçi Nokrasının prevalansı %17.8 (89/500) olarak tespit edildi. Hayvanlardaki nodüllerin sayısı 1-14 (6.61±2.4) olarak belirlendi. Türlere göre prevalans beetle ırkında %13.2, lokal ırklarda %18 ve desi ırklarda %22.9 olarak tespit edildi. Tekelerde prevalans %15.3 iken dişilerde %19.4 idi. Enfestasyon; 1-3 yaş arası keçilerde %20.9, 4-6 yaş arası olanlarda %14.6 ve 6 yaş üzerilerde %18.1 oranlarında mevcuttu. Araştırılan epidemiyolojik faktörlerin Punjab Eyaletinde Keçi Nokrasının prevalansı üzerinde önemli etkisi olduğu tespit edildi. Çalışmanın sonuçlarına göre nokranın Mart ayından Temmuz ayına kadar aktif olduğu belirlendi. Bu çalışma Keçi Nokrası üzerine Punjab Eyaletinde yapılan ilk çalışmadır. Çalışmanın Pakistan'ın diğer endemik bölgelerinde nokranın eradikasyonu ve kontrol edilmesine yönelik stratejileri belirlemede yararlı olacağı görüşündeyiz.

Anahtar sözcükler: *Keçi Nokra Enfestasyonu, GWFI, Prevalans, Przhevalskiana silenus, Khoshab, Chakwal bölgeleri, Pakistan*

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INTRODUCTION

Pakistan is an agricultural country and livestock acts as the backbone of agriculture. Milk, meat, hides and wool obtained from the livestock help to increase the export of Pakistan as well as prosperity of the farmer.

Parasitism is one of the major problems of low productivity in livestock sector of the world [1]. One of these is WFI, which cause infection in cattle, buffaloes, sheep and goats [2-4]. Hypodermosis prevalence is common in semi-hilly, mountainous and riverine areas of Pakistan [3]. Due to its high prevalence, it exists in many parts of the world. It was found that the prevalence of warble fly infestation was almost 80% in Czech Republics, 49.2% in Greece, 85% in Italy, 52.3% in Spain, 40% in United Kingdom and 32-43% in Romania [5]. The prevalence of Warble Fly Infestation (WFI) was 3.2%, 18.4% in buffalo and cattle of Pakistan [6,7]. Previous studies showed that hypodermosis is one of the major parasitic infection in many countries of the northern hemisphere. This menace not only causes the physical damage to the animal, but also affects the internal organs and damages the host immune system. In many European and North American countries, chemotherapy treatments used against adult fly and first larval stage, have significantly reduced the infestation of this disease [8]. The infestation rate was in cattle (14.1%), sheep (2.1%) and goats (24.9%) respectively in Green mountains, Libya. The goats were infested by *P. silenus* [9]. The adult fly is active from April to June in different areas of world. The adult fly lacks mouthparts and survives on resources accumulated during the larval period. During the periods that the fly is active, the first instar larvae emerge from eggs laid directly on the hairs of the hind legs (mainly tarsal and femoral regions) of the goat. The larvae then penetrate the epidermis and dermis to enter into the subcutaneous tissue to migrate for a short distance to reach the flanks and sacrum. The migration pattern inside the body of animals seems to be exclusively subcutaneous [10,11]. Leather industry is one of the major industrial units working in Pakistan and producing large export products but, due to this parasite, this industry is suffering from economic losses. The losses due to this menace cannot be calculated due to a number of factors, while hide damage was the most important consequence of the infestation resulting in low price on account of holes formed by the warble fly. Pakistan produces 7.5 million hides and 36.3 million skins, annually. The estimated losses in D. G. Khan and Rajanpur districts were Rs: 12.9, 9.9 million, respectively. The total losses were Rs 22.8 million from cattle and Rs 2.2 million from buffaloes [12]. Although Pakistan is an agricultural land having a large number of livestock; warble fly is continuously attacking the livestock products but no important work has been done in this regard to calculate damage caused by this notorious parasite.

The purpose of present study is to determine the prevalence of Warble Fly Infestation (WFI) in district Khoshab and Chakwal of Punjab province, Pakistan. The objectives of the present were (1) Treatments given to different animals in different herds and their effectiveness. (2) Sex & breed wise prevalence of Warble Fly Infestation (WFI) in goats of different areas of Punjab Province (Khoshab and Chakwal).

MATERIAL and METHODS

Location

Punjab is the Pakistan's second largest province at 205.344 km^2 (79.284 sq^2 miles) after Balochistan and is located at the northwestern edge of the geologic Indian plate in South Asia. The geographical location of the Chakwal is 32° 56′ 0″ North, 72° 52′ 0″ East and of Khoshab is 32° 17′ 48″ North, 72° 21′ 9″ East in Punjab Province, Pakistan.

Topography

The Punjab province is bordered by Kashmir (Azad Kashmir, Pakistan and Jammu and Kashmir, India) to the north-east, the Indian states of Punjab and Rajasthan to the east, the Pakistani province of Sindh to the south, the province of Baluchistan to the southwest, the province of Khyber Pakhtunkhwa to the west, and the Islamabad Capital Territory to the north. Undivided Punjab is home to six rivers, of which five flows through Pakistani Punjab. From west to east, these are: the Indus, Jhelum, Beas, Chenab, Ravi and Sutlej. Nearly 60% of Pakistan's population lives in the Punjab. It is the nation's only province that touches every other province; it also surrounds the federal enclave of the national capital city at Islamabad. This geographical position and a large multi-ethnic population strongly influence Punjab's outlook on National affairs and induces in Punjab a keen awareness of the problems of the Pakistan's other important provinces and territories. The landscape is amongst the most heavily irrigated on earth and canals can be found throughout the province. Weather extremes are notable from the hot and barren south to the cool hills of the north. The foothills of the Himalayas are found in the extreme north as well.

Climate

The habitat of the warble fly is hilly and semi-hilly areas. According to it those areas are selected that have hilly or semi-hilly conditions like Chakwal and Khoshab. These areas have suitable temperature conditions and other ecological factors like high altitudes that are ideal for the growth and development of the warble fly. Moreover; these areas also have large number of livestock that help to further increase the living conditions and host of the warble fly.

Experimental Design

This epidemiological survey was conducted from

September, 2012 to March, 2013. These months are selected because warbles present on the back of the animals start developing from September and last till February. The larvae were collected from infested animals.

Palpation Method

The animals of these areas were examined on monthly basis by palpation method. The nodules were counted by using visual and hand palpation method. The counting of nodules on animal started from anterior portion leading to the posterior portion. The animals were examined on monthly basis to count the numbers of nodules and all this was recorded on a separate data sheet. Initially some of the larvae were directly collected from the upper dorsal part of the animal near the vertebral column. These were collected with the help of hands. The larvae were collected by picking them from the ground, when they dropped. The larvae from animal skin dropped on the ground during the months of January to onward to form mature fly which starts the life cycle again. So during these months (February, March) larvae are collected from the ground instead of animal skin directly. The larvae were collecting in bottle containing 70% ethanol and kept in freezer at -20°C.

Statistical Analysis

The Statistical analysis (Chisquare) was done by using the statistical package SPSS for Windows 20.0.

RESULTS

Out of five hundred goats, 89 (17.8%) were found to be infested by *Przhevalskiana silenus*. The number of nodules in the infested animals ranged from 1-14 (6.61±2.4). The nodules were observed on the back of infested goats. The warble started to appear by the start of September and skin perforation started from end of October to December. The larvae collected from infested goats were identified as *P. silenus* according to Zumpt [13]. This is the first report of *P. silenus* in goats of Khoshab and Chakwal district, Pakistan *(Fig. 1)*.

The present study was conducted in 10 villages, 40 herds of Khoshab and Chakwal district to determine the prevalence of warble fly infestation in the goats from July 2012 to January 2013. The results of present study revealed that the rate of infestation was 17.8% (89/500).

The village wise prevalence was determined from the ten villages. The prevalence in villages of Khoshab district as in Dhokri (12.7%), Ghatti (13.2%), Jabbi sharif (9.1%), Warcha (0%) and Chohasharif (6.7%). In district Chakwal it was in village Manara, (25.5%), Runsial, (34.9%), Bhone, (30%), Tala gang, (14.3%) and Choa Saidan shah, (9.1%). The statistical analysis has showed the significant differences ($P<0.05$) in the prevalence of GWFI in different villages of Punjab Province, Pakistan.

The goats of three breeds (Beatle, Desi breed and local breed) were examined in the present study on monthly basis. The statistical analysis shows that prevalence in

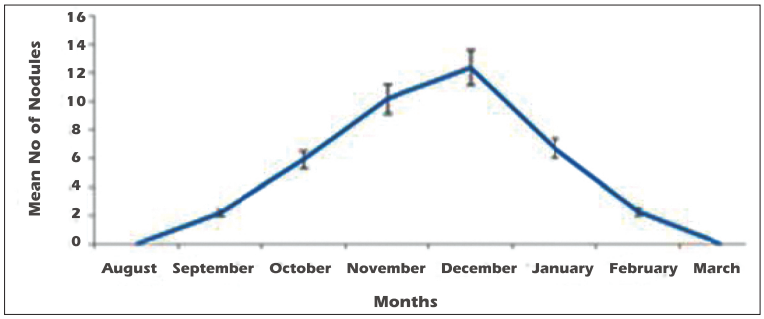
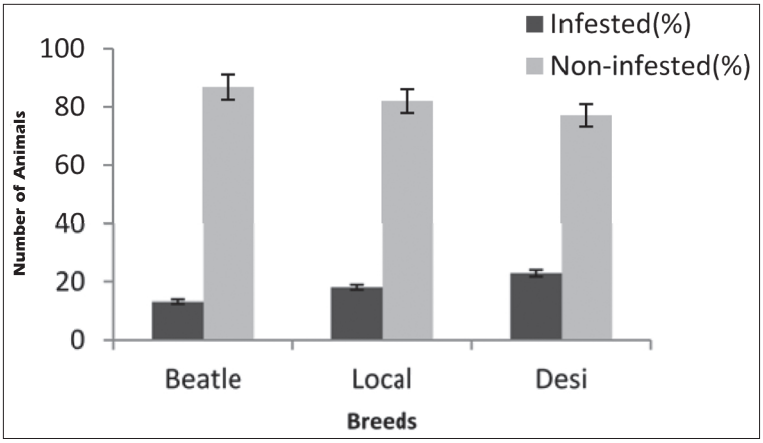


Fig 1. Month wise intensity of Infestation of WFI in goats

Şekil 1. Keçi nokra enfestasyonunun aylara göre dağılımı

Fig 2. Breed wise prevalence of WFI in goats of Punjab Province, Pakistan

Şekil 2. Pakistan'ın Pencap Eyaletinde Keçi nokra enfestasyonunun ırklara göre dağılımı



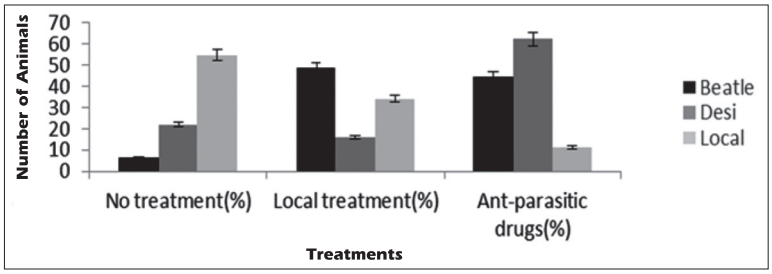
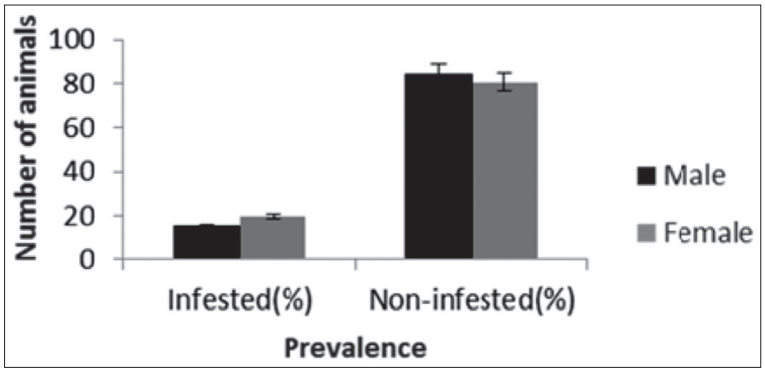


Fig 3. Effect of treatments given to different breeds of goats of Punjab Province, Pakistan

Şekil 3. Pakistan'ın Pencap Eyaletindeki farklı ırk keçilerde tedavinin etkisi

Fig 4. Sex wise prevalence of WFI in goats of Punjab Province, Pakistan

Şekil 4. Pakistan'ın Pencap Eyaletindeki keçilerde Keçi nokrasının cinsiyet üzerindeki yaygınlığı



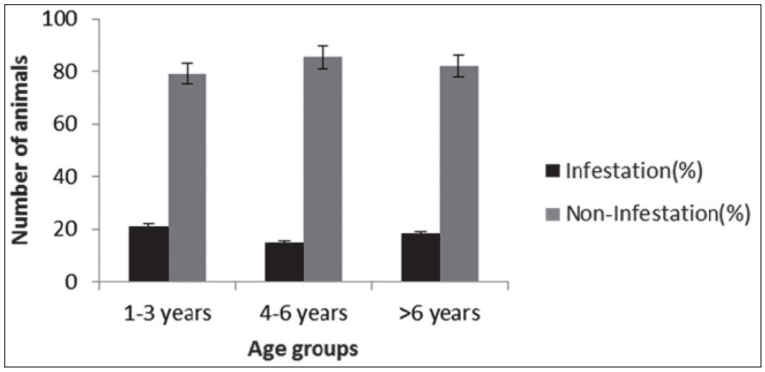
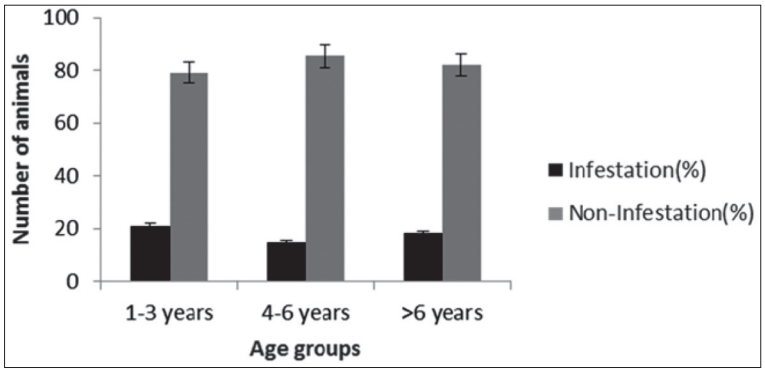


Fig 5. Use of Medication in different sexes examined for WFI in goats of Punjab Province, Pakistan

Şekil 5. Pakistan'ın Pencap Eyaletinde Keçi nokrası için muayene edilen farklı cinsiyetteki keçilerde ilaç kullanımı

Fig 6. Age wise prevalence of WFI in goats of Punjab Province, Pakistan

Şekil 6. Pakistan'ın Pencap Eyaletinde nokralı keçilerin yaş prevalansı



beetle breed was (13.2%), local breed (18%) and desi breed (22.9%), respectively. Among all three breeds the highest infestation was observed in desi breed (22.9%) *(Fig. 2)*.

The results showed that majority of the non-infested animals were medicated {local treatment (29.4%), Anti-parasitic drugs (43.1%)} as compared to non medicated goats (27.5%) The medication schedule of all the examined goats was recorded consisting of non-medicated, local treatment and anti-parasitic drugs. In beatle breed the non-medicated was 13/197 (6.6%), local treatments 96/197 (48.7%) and anti-parasitic drugs 88/197 (44.7%). In desi breed, non-medicated was 29/133 (21.80%), local treatments 21/133 (15.78%) and anti-parasitic drugs 83/133 (62.4%). In local breed non-medicated was 93/170 (54.7%), local treatments 58/197 (34.1%) and anti-parasitic drugs 19/170 (11.2%). There are 27% (135/500) goats were non medicated, 35% (175) were given local treatment and 190 (38%) were given anti-parasitic drugs *(Fig. 3)*.

Table 1. *Showing the statistical analysis of different epidemiological factors on the prevalence of WFI in goats of Punjab Province, Pakistan*
Tablo 1. *Pakistan'ın Punjab Eyaletinde Keçi nokrasının prevalansı üzerine değişik epidemiyolojik faktörlerin istatistiksel analizi*

S. No	Factors	Groups	Prevalence		Statistical Analysis (Chi-square)
			Infested	Non-Infested	
1	Age	1-3	33 (20.9%)	125 (79.1%)	χ^2=2.037 df=2 p=0.329
		4-6	25 (14.6%)	146 (85.4%)	
		>6	31 (18.1%)	140 (81.9%)	
2	Sex	Male	29 (15.3%)	161 (84.7%)	χ^2 = 1.348 df = 1 p = 0.246
		Female	60 (19.4%)	250 (80.6%)	
3	Breed	Beatle	26 (13.2%)	171 (86.6%)	χ^2=5.928 df=2 p=0.05
		Desi breed (Taedi)	39 (22.9%)	131 (77.1%)	
		Local breed	24 (18%)	109 (82%)	
4	Villages (District)	Dhokri, (Khoshab)	7 (12.7%)	48 (87.3%)	χ^2=47.107 df=9 P=0.00
		Ghatti, (Khoshab)	7 (13.2%)	46 (86.8%)	
		Jabbi sharif, (Khoshab)	5 (9.1%)	50 (90.9%)	
		Warcha, (Khoshab)	0 (0%)	48 (100.0%)	
		Chohasharif, (Khoshab)	3 (6.7%)	42 (93.3%)	
		Manara, (Chakwal)	14 (25.5%)	41 (74.5%)	
		Runsial, (Chakwal)	38 (34.9%)	71 (65.1%)	
		Bhone, (Chakwal)	9 (30.0%)	21 (70.0%)	
		Tala gang, (Chakwal)	4 (14.3%)	24 (85.7%)	
		ChoaSaidan shah, (Chakwal)	2 (9.1%)	20 (90.9%)	
5	Medication	Non Medicated	22 (24.7%)	113 (27.5%)	χ^2=0.729 df=2 P=0.396
		Local treatment	54 (60.7%)	121 (29.4%)	
		Anti-parasitic drugs	13 (14.6%)	177 (43.1%)	

The results showed that female 60/310(19.4%) and male 29/190 (15.3%) goats were infested *(Fig. 4)*. The medication schedule was also recorded in both the sexes *(Fig. 5)*. The prevalence in goats having age group (1-3 year) was 33/158 (20.9%), in age group (4-6 year) was 25/171 (14.6%) and in age group (> 6 year) 31/171 (18.1%) were infested. The results showed that younger animals were more infested as compared to older animals *(Fig. 6)*. The statistical analysis showed that there is significant difference between infested and non-infested animals in all age groups *(Table 1)*.

DISCUSSION

The prevalence in goats of Khoshab and Chakwal districts was 17.8%. Our results were correlates as 25% goats were infested with WFI in Pakistan [2]. In Rakhi Manu and Rakhi Guage area the rate of infestation was 41% and 40% in goats [4]. Similarly, Otify and Mansour reported 24.9% [9], in northern Jordan 10% goats were infested from WFI [11] and in Iran 7% to 18.9% [14]. These results contradictions with present research results might be due to the use of antiparasitic drugs in the study area. As far as the prevalence of warble fly infestation in district Khoshab and Chakwal is concerned, this is the first report related to goat warble fly infestation.

The female (19.4%) were more infested as compared to male (15.3%). The statistical analysis showed no significance differences (P<0.05) between two sexes. Our results were similar to as prevalence rate was same in male and female [11], there was no significant difference between male and female (P<0.05) [15]. Similarly, no significant difference among male (47.81%) and female (46.82%) in Jammu province of India [16]. Likewise, Mohammad Hossein Radfar investigated that the difference in the prevalence of the infection between males and females was not significant (P>0.05) [17].

The present study shows that highest infestation was observed in desi (Taedi) (22.9%) breed as compare to local (18%) and beatel breed (13.2%) due to the poor immune response. Our results were in accordance with Yadav *et al*.[16] reported the significantly higher infestation rate among Bakerwali (51.51%) breed as compare to the Beetle (42.59%).

The prevalence in goats having age group (1-3 year)

was 33/158 (20.9%), in age group (4-6 year) was 25/171 (14.6%) and in age group (>6 year) 31/171 (18.1%) were infested. The results showed that younger animals were more infested as compared to older animals. Similarly, the statistical analysis in relation to age showed significant (P<0.01) difference among different age groups <1 year (2.81%), 1-3 years (51.17%), and >3 years (43.16%) [16].

It is concluded from the present study that WFI is serious threat in goats of Pakistan. So it is strongly recommended that due to the economic significance of this parasitic disease, it should be explored in different areas of Pakistan and its effects and damages must be studied for its control.

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RESEARCH ARTICLE

Occupational Stress and Risk Factors in Veterinary Surgeons [1]

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[1] *This study was presented as poster at the XV. National Public Health Congress on 2-6 October, Bursa, Türkiye, 2012*

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Summary

The aim of this study was to investigate occupational stress and risk factors among veterinary surgeons. The present cross-sectional study was performed in 2012 via a web-based survey. Data of 223 individuals who responded to the survey are presented. In order to evaluate the occupational stress Turkish Version of "Swedish Demand-Control-Support Questionnaire" was used. Mean age of participants was 37.45±9.11 and 28.3% of subjects were female. Work load, work control, skill, decision latitude, and social support mean points with standard deviations were found to be 9.42±1.86, 10.27±2.72, 7.06±1.78, 3.21±1.64 and 11.26±3.94, respectively. It was reported that 92 subjects were working for public institutions and 131 were working for private sector. Decision latitude and social support levels in surgeons working for public institutions were statistically significantly lower than their counterparts in the private sector. There was no statistically significant difference in work load, work control and skill use between two groups. Of participants 54.3% reported that they had car accidents, 19.3% reported that they had the accident in the last one year, and 9.9% reported that they had the accident during a patient visit. Majority of Turkish veterinary surgeons in our study group reported that they experienced occupational stress. Occupational stress and related factors in work environment can influence work health negatively by causing physical, mental and social problems.

Keywords: *Veterinary surgeon, Occupational stress, Work load, Work control, Social support*

Veteriner Hekimlerde İş Stresi ve Risk Faktörleri

Özet

Bu çalışmada, veteriner hekimler arasında iş stresinin ve risk faktörlerinin incelenmesi amaçlandı. Kesitsel tipteki bu çalışma, 2012 yılında web-tabanlı anket aracılığı ile yapıldı. Ankete yanıt veren 223 bireyin verileri sunuldu. İş stresini değerlendirmek için "İsveç İş Yükü-Kontrol-Destek Anketi"nin Türkçe Sürümü kullanıldı. Katılımcıların yaş ortalaması 37.45±9.11'di ve %28.3'ü kadındı. Katılımcıların iş yükü, iş kontrolü, beceri, karar serbestliği ve sosyal destek ortalama puanları ile standart sapmaları sırasıyla 9.42±1.86, 10.27±2.72, 7.06±1.78, 3.21±1.64 ve 11.26±3.94 olarak belirlendi. Kamuda çalışan hekimler arasında karar serbestliği ve sosyal destek düzeyleri özel sektörde çalışanlara göre istatistiksel olarak anlamlı düzeyde daha düşüktü. İş yükü, iş kontrolü ve beceri kullanımı yönünden iki grup arasında istatistiksel olarak anlamlı bir fark saptanmadı. Katılımcıların %54.3'ü araba kazası yaşadıklarını, %19.3'ü son bir yıl içinde kaza yaptıklarını ve %9.9'u kazaların hasta ziyareti sırasında olduğunu bildirdi. Çalışma grubumuzda yer alan veteriner hekimlerin çoğu iş stresi yaşadıklarını bildirdi. İş stresi ve işyeri ortam faktörleri, fiziksel, ruhsal ve sosyal sağlık sorunlarına yol açarak çalışanların iş sağlığını olumsuz yönde etkileyebilir. İş sağlığı ve güvenliği uygulamalarının henüz her meslek grubuna yansımadığı Türkiye gibi ülkelerde veteriner hekimler arasında iş stresi gibi mesleksel risklere karşı koruyucu, hizmet içi eğitimlerin yapılması yararlı olacaktır.

Anahtar sözcükler: *Veteriner hekim, İş stresi, Risk faktörleri, İş yükü, İş kontrolü, Sosyal destek anketi*

INTRODUCTION

According to World Health Organization (WHO), occupational stress is a response, which appears when levels of knowledge, skill, and coping with of subjects in order to work and solve problems under high workload

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and occupational stress [1]. Occupational stress is tolerable only if it is stimulating, motivating, and encouraging for learning innovations. However, this delicate balance may be affected favourably or unfavourably by work-related (duration of work, workload, duration of pauses, social support etc.) and individualized (socio-economical state, family-surrounding relationships, personality etc.) factors. When the change starts to develop in an unfavourable direction (high workload, weak social support, low work control etc.), work stress continuously strengthens. When this high work stress reaches a level which cannot be managed by the worker and becomes chronic, it causes decreased work efficiency by causing physical, mental, and social health problems [1-4]. Work load-work control and support model is one of the best methods, which indicate these components acting in development of work stress [2-4].

Occupational stress and mood disorders like depression and anxiety are also observed widely among veterinary surgeons, which affect health unfavourably [5,6]. Recent studies have especially indicated that occupational stress, anxiety and depression related to various physical and mental reasons caused increased suicidal risk [7-11]. It has been defined that suicide related mortality rate was 3-4 folds increased among veterinary surgeons than the population as well as 2 folds higher than other healthcare personnel [8,11]. Workload-work control and support model are generally employed in studies, which investigate occupational stress among veterinary surgeons [12]. According to studies performed on veterinary surgeons, main risk factors triggering occupational stress are extended working hours, inefficient time for family and for resting and having no enough break, financial problems, difficulty in following up new developments and innovations, requirement of limitless working period, high expectations of clients/animal owners, intense physical workload, low rating of veterinary occupation by population, and disrespectful behaviour among veterinary surgeons [12-17]. In addition, several factors like private life conditions of staff, absence of occupational controls, inability to contribute in decision making steps, low social support at working environments, unclear and incomprehensible job descriptions can also trigger occupational stress [15,18].

In the present study, it was aimed to investigate occupational stress and risk factors among veterinary surgeons in Turkey.

MATERIAL and METHODS

This present cross-sectional study was performed between January and June in 2012. Study data were collected via a web survey prepared by investigators. The web-based survey was transferred to chambers of veterinary surgeons and veterinary surgeons via internet. Data from 223 responders were presented. The Swedish Demand-Control-Support Questionnaire [19-21], which was tested for validation and reliability in Turkish by Demiral et al.[22] was used to measure occupational stress of veterinary surgeons. High scores from the scale indicated high occupational stress, high work control and high social support. Occupational stress was evaluated as ratio of workload to work control. Socio-demographic data, risk factors related to occupation, and some health behaviour were also investigated in this present web-based study.

Statistical analyses were performed by using SPSS 15.0 package program. Mean, standard deviation, median, and ratio values were used for descriptive analysis of data. T test and One Way Variation analysis were used for comparisons of means in normally distributed data analysis, whereas non-parametric statistical tests (Mann-Whitney U and Kruskal-Wallis) were used in analysis of abnormally distributed data. Mann Whitney U test was used in comparison of occupational stress levels of subjects working for the public and private sector. The level of significance was accepted at $P<0.05$.

RESULTS

In the present study, 245 subjects were enrolled and response of 223 participants, who completed the survey were examined. Mean age of participants was 37.45±9.11 years. Of participants, 71.7% were males. Of participants, 34.1% (n=76) reported that they had their own office and average duration in occupation was 12.9±9.5 years. The demographic data of the study group were presented in *Table 1*.

Participants expressed that they worked 5.9±0.8 day in a week on average. Daily working, while standing up, while sitting and duration of computer use were evaluated as work-related factors. Reported work-related factors by veterinary surgeons on *Table 2*. When the correlation between occupational stress level and work-related factors was investigated no statistically significant difference was detected (between subjects working for public and private sector).

Of participants 19.7% (n=44) declared that they exercised regularly and 57.8% (n=129) reported that their working environments affected them negatively. Of participants, 51.5% (n=115) reported that they experienced physical disturbances in the last one month at the work or because of the work *(Table 3)*. When physical disturbances with respect to gender were examined, chronic fatigue was the 3rd in order, whereas the 5th in order for males. Physical health problem, which was first in order was lower back and back pain for both females and males. The most commonly reported mental health problem was stress (19.7%), *(Table 3)*.

Of participants, 51.1% (n=114) reported that they kept

Table 1. *Sociodemographic characteristics of the study participants*
Tablo 1. *Çalışmaya katılanların sosyodemografik özellikleri*

Factors	**Mean±SD**	**Min-Max.**
Age	37.45±9.11	24-66
Average duration in occupation (years)	12.9±9.5	1- 43
Gender	Number (n)	Percentage (%)
Male	160	71.7
Female	63	28.3
Total	223	100.0
Mariatal status		
Married	163	73.1
Single	60	26.9
Total	223	100.0
Number of children		
1	60	26.9
2	66	29.6
3 and more	12	5.4
Total	138	100.0
Working for		
Veterinary clinic/hospital	83	37.2
Private sector	48	21.5
University	41	18.4
Ministry of Agriculture	39	17.5
Municipality	10	4.5
Turkish Armed Forces	2	0.9
Total	223	100.0
Having own workplace		
Yes	76	34.1
No	147	65.9
Total	223	100.0

Table 2. *Reported work-related factors by veterinary surgeons*
Tablo 2. *Veteriner hekimlerin bildirdiği iş ile ilişkili faktörler*

Factors	**Mean±SD**	**Min-Max.**
Day work (week)	5.9±0.8	4-7
Use cell phone (minutes)	70.41±61.14	10-300
Daily working (h/day)	Number (n)	Percentage (%)
4-5 h	5	22.2
6-7 h	18	8.1
8-9 h	101	45.3
10 h and more	99	44.4
Total	223	100.0
Daily working durations while standing up (h/day)		
0-1 h	15	6.7
2-3 h	33	14.8
3-4 h	35	15.7
4-5 h	39	17.6
5-6 h	36	16.1
6 h and more	65	29.1
Total	223	100.0
Daily working durations while sitting down (h/day)		
0-1 h	26	11.7
2-3 h	34	15.2
3-4 h	26	11.7
4-5 h	50	22.4
5-6 h	39	17.5
6 h and more	48	21.5
Total	223	100.0
Use computer (h/day)		
0-1 h	47	21.1
2-3 h	49	22.0
3-4 h	45	20.2
4-5 h	25	11.2
5-6 h	23	10.3
6 h and more	34	15.2
Total	223	100.0

animals, and 31.6% of those subjects keeping animals declared that they kept cats (n=62), and 18.4% (n=43) reported that they kept dogs. Of 114 subjects, who responded the survey and kept animals, 79.8% (n=91) reported that keeping animals decreased occupational stress. Veterinary surgeons, who participated in the study, reported stressors related to occupation by veterinary surgeons *(Table 4)*. When investigated with respect to gender, mistreatment/death of animal was at the first line in order (Male = 19.0%, Female = 20.6%). The second most commonly reported stressor among males was animal owner pressure or super pressure (12.7%) whereas it was transmission risk of zoonosis diseases among females (17.5%). Moreover, mistreatment/death of animal stressor was defined to be decreased with the increasing age *(Fig. 1)*.

Of participants, 54.3% (n=121) reported that they had a car accident; 19.3% of them (n=43) had an accident in the last one year, and 10.8% of them had the traffic accident.

Moreover, 82.1% of veterinary surgeons (n=183), who completed the survey, responded as "Yes" to the question "Do you believe that you experience occupational stress?". Of participants, 47.5% (n=106) responded as "I am, but it is not enough", and 36.8% (n=82) responded as "No" to the question "Are you able to spare enough time to yourself or to your family?"

Mean stress scores were higher in subjects working for public sector than for the private sector (42.56 vs. 40.29, respectively). There was a statistically significant difference in decision latitude between subjects working for public and private sector (P<0.05) *(Table 5)*.

Table 3. *Reported physical and mental health problems by veterinary surgeons*
Tablo 3. *Veteriner hekimlerin bildirdiği fiziksel ve ruhsal sağlık problemleri*

Physical	**Number (n)**	**Percentage (%)**
Lower back and back pain	58	26.0
Chronic fatigue	10	4.5
Leg, foot pain	10	4.5
Head, neck pain	9	4.0
Skin infections and irritation	8	3.6
Animal attack, being bitten	7	3.1
Other	13	5.9
Total	115	100.0
Mental		
Unresponded	25	21.4
Stress	23	19.7
Short temper	18	15.4
Depression	15	12.8
Burnout	14	12.0
Unhappiness/restlessness	12	10.3
Chronic fatigue/insomnia	9	7.7
Attention deficit	1	.9
Total	117	100.0

Table 4. *Reported stressors related to occupation by veterinary surgeons*
Tablo 4. *Veteriner hekimler tarafından bildirilen iş ile ilgili stresörler*

Stressors	**Number (n)**	**Percentage (%)**
Unresponded	76	34.1
Mistreatment/animal death	45	20.2
Transmission of zoonosis disease	36	16.1
Animal owner pressure/superior pressure	27	12.1
Animal attack	20	9.0
Future/economic concerns	12	5.4
Other	6	2.7
Traffic accident	1	.4
Total	223	100.0

Moreover, there was a statistically significant difference in stress points between subjects who owned their businesses and who worked for employers (t = -3.204; P<0.05). Mean score of subjects working for employers were higher than those who owned their businesses (42.39 vs. 38.97). There were statistically significant differences in work control, skill and decision latitude when stress points were examined in subscales (P<0.05, P<0.05, P<0.05, t = -4.406, t = -3.089, t = -3.867). Means of subjects working for employers were higher in all three score examinations (work control = 9.29-10.78, skill = 6.60-7.29, decision latitude = 2.68-3.49). When stress points of married and

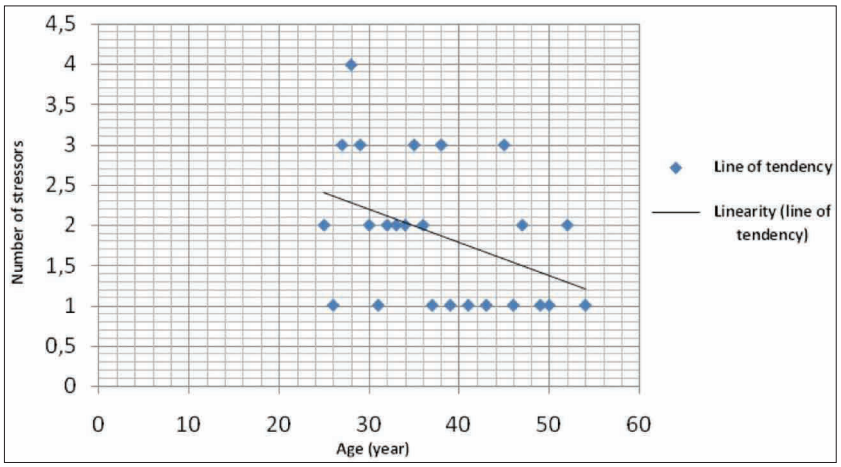

Fig 1. Relationship between mistreatment/animal death stressor and age

Şekil 1. Yanlış tedavi/hasta kaybı stresörleri ile yaş arasındaki ilişki

Table 5. *Mean points in subgroups of occupational stress scale among veterinary surgeons*
Tablo 5. *Veteriner hekimler arasında iş stresi ölçeği alt grupları ortalama puanları*

Swedish Demand-Control-Support Questionnaire	**Civil Servant (n:82)**	**Private Sector Employee (n:117)**	***P****
	Mean ± SD	**Mean ± SD**	
Work load	9.65±1.73	9.26±1.94	0.080
Work control	10.73±2.94	9.95±2.51	0.065
Skill use	7.12±1.92	7.02±1.68	0.969
Decision latitude	3.61±1.84	2.94±1.42	**0.008**
Social support	11.46±3.58	11.12±4.19	0.195

** Mann-Whitney U test, P <0.05 was considered*

single participants were compared, work load stress point of married ones was higher (9.73 vs 8.67), whereas mean scores of work control (10.09 vs 10.96) and decision latitude (3.10 vs 3.69) were statistically significantly lower than the single ones (P<0.001, P<0.05, P<0.05, t = -3.65, t = 2.02, t = 2.25). According to weekly working durations, the highest work load score was defined in 5-day working group (9.84±1.86). In further analyses performed by Tukey test result, there was a significant difference between subjects working 5 days and 7 days in a week (P<0.05).

There were statistically significant differences in work load, skill, and social media between subjects who believed that working environments had negative and no negative effects on the physical health (P<0.05). On the contrary, subjects who reported that their physical health states were not affected. Mean work load scores were higher. When groups were compared in skill, points of subjects, who said "Yes", were higher (7.30). When groups were examined for skill, there was a statistically significant difference between them (P<0.05). When groups were examined for social support, stress scores of subjects who said "Yes" were higher (11.71). There was a significant difference between groups (P<0.05). According to correlation analyses, it was defined that there was a positive correlation between age and work load (P<0.05), but a negative correlation between age and work control as well as decision latitude (P<0.05, P<0.05). There was a positive, statistically significant correlation between years in occupation practice and work load (P<0.05).

DISCUSSION

In the present study, it has been investigated how occupational stress is changed according to business place, gender, age and other risk factors, and also comparisons with the literature is presented in this section. There has been just few performed study on veterinary surgeons about occupational stress, which is one of the most important problems endangering occupational health. In a study performed on Belgian veterinary surgeons, which investigated occupational stress level and risk factors. Female/male ratio (24.5 vs. 75.5) was in line with our study results (28.3 vs. 71.7) [23]. According to studies investigating occupational stress in veterinary surgeons, the most important stress factor is extended working hours [14,23-25]. Weekly working hours were 54 h in the Belgian study [23] whereas 50 h in another study from Turkey, which was investigated occupational satisfaction and burnout levels of veterinary surgeons [15]. In our study, mean weekly working hours reported by veterinary surgeons was 45 h. Nearly half of veterinary surgeons (41.3%) reported that they have been working as a civil servant and 40 h in a week.

In a study performed on veterinary surgeons in Australia, it was reported that extended working duration, inefficient break-time and holiday durations, and attitudes and behaviour of animal owners increased occupational stress [13,24]. Veterinary surgeons participated in our study reported similar stressors affected negatively their physical and mental health states. Working stress studies indicated that occupational stress level was increased by low decision latitude, low social support and high work load, and caused physical and mental health problems [2-4,22]. Occupation related stressors, which were reported by vets, were mistreatment/animal death, transmission risk of zoonosis diseases, animal owner pressure/superior pressure, animal attack, future/economic concerns and traffic accident in decreasing order. In the German study, vets also reported that they had high levels of concerns because of financial and social insufficiencies [14]. In the Australian study, it was reported that approximately 1/3 of veterinary surgeons had psychologically bad health [6]. Moreover variables like advanced age, extended working duration, time from graduation, male gender are related to stress, anxiety and depression [6].

Veterinary surgeons face with many various occupational hazards during their working life. Being bitten, being scratched, other traumas, injuries, muscle-skeletal system diseases, car accidents, and having zoonosis diseases are the leading ones [13,26-29]. Data from Australia [13], New Zealand [25] and Germany studies [14,30] were similar to stressors reported from our study group. In a study performed on 160 vets in West Australia, 71% of veterinary surgeons were reported to be injured in the last decade. The most commonly reported injuries were cat-dog bites, cat scratching, incisions and lower back injuries due to lifting up heavy animals [27]. Chronic fatigue, leg-foot pain, head-neck pain, skin infections or skin irritation, animal attack and being bitten were other reported physical problems along with lower back and back pains, which were reported as occupation related physical problems by veterinary surgeons in our study, and they were placed in the first line (58%) in the list. When the literature is reviewed, factors like stress. X rays, anaesthetics, animal bites, cytotoxic drugs, exposure to pesticides, and radiation have been reported as risks endangering occupational health and safety in veterinary surgeon practice [27,31]. In our study group, although zoonosis diseases were reported in the leading orders as an occupational risk factor, differently from the literature, radiation, X rays and anaesthetics were not reported. This result suggested that veterinary surgeons in our study group did not perceive or value these risk factors as risks practically endangering occupational health and safety.

In studies investigating age and stress, stress levels in middle aged veterinary surgeon population were higher than those of advanced age [14,25]. No correlation was defined between stress and age in the Australia study. In our study, stressors of mistreatment/animal death were decreased in female and male veterinary surgeons as the

age was increased. In another Australian study, similar to the study from New Zeeland, occupational stress was higher in young veterinary surgeons when compared with the older ones, and in females when compared with the males [17]. In studies from Finland and Australia, similar to the study from New Zealand, young veterinary surgeons had higher occupational stress levels than those in older ones as well as females had higher levels than males [17,25,32]. In our study group, young veterinary surgeons were defined to be affected more from stressor factors than their older counterparts. In the study conducted by Smith et al.[13], a strong correlation was defined between gender and all components of stress. In our study group, similar results were obtained with studies from the New Zeeland and Australia [13,25]. Female veterinary surgeons are experiencing more stress in working hours. Employer/Colleague expectations, animal and animal owner expectations, communication, source support, upper management support, professional support and unexpected treatment outcomes than their male counterparts [30]. According to correlation analysis in our study, positive correlation was detected between age and work load ($P<0.05$) whereas a negative correlation in work control and decision latitude ($P<0.05$). Positive correlation was detected between time spent in the occupation and work load ($P<0.05$).

In the Australian study, it was reported that there was a correlation between working and stress level in small cattle veterinary surgery [13]. In the German study, stress levels in veterinary surgeons, who had their own clinics and were working in clinics, were defined higher [30]. Statistically significant difference was defined between veterinary surgeons, who had their own businesses and who worked for employers ($P<0.05$). Mean score of subjects working for employers were higher than the ones who owned their businesses (42.39 vs. 38.97). When stress score subscales were examined, statistically significant differences were defined in work control, skill, and decision latitude, and mean values of subjects who did not own their businesses were defined higher than the ones, who worked for employers. In the study from New Zeeland, many of veterinary surgeons declared that good communication with their families and friends helped them more in tackling with the stress [25]. In our study group, it was reported that enough time could not be spared for families. This problem arose as an important stress factor preventing subjects from dealing with occupational stress. In a study investigating psychological stress from Turkey, it was reported that keeping animals and regular physical exercising could be effective in dealing with stress [33]. In our study, veterinary surgeons reported that keeping animals reduced occupational stress, and 19.7% of participants performed physical exercises regularly. In a Canadian study, which was conducted on web-base similar to our study, 2% of veterinary surgeons reported they experienced no occupational stress; 5% experienced severe and almost half of them (53%) expressed moderate level of stress. No statistical difference was detected between median stress levels according to working environments [31]. In our study, 82% of the veterinary surgeons reported that they experienced occupational stress. Additionally, different from the literature, it was observed that there were differences in occupational stress subscale points according to working environments. According to results of occupational stress scale, decision latitude and social support levels of veterinary surgeons working for the government were statistically significantly lower than the ones working in the private sector. This result suggested that subjects working in the private sector had more latitude in making decision; and they gained more social support from their managers and colleagues. However, no statistically significant difference was detected when points of work load, work control and skill use were reviewed.

In recent years, occupational exposure studies showed that veterinary surgeons were exposed to many physical, chemical hazards while they were working, and also that they did not use at all or not effectively use preventive measures [29-34]. One of the important stressors investigated in occupational stress studies is car accidents. It was reported that car accidents happened while going to or returning from work, and they were related to working environment, weekly working hours, duration of lunch break, distance to the work, gender, and number of children [16]. It was reported in the German study that weekly working duration of veterinary surgeons was 44.2 h [16]. While 69.9% of veterinary surgeons in the German study reported that they did not have any accidents in the last one year, 61.9% of male and 34.9% of female veterinary surgeons reported that they had an accident in the last one year in our study [16]. In another study, it was reported that risk of having a car accident was reported to be high in subjects, who worked for more than 48 hours a week in line with high occupational stress [14]. In our study, more than half of participants (54.3%) had a car accident, and it was determined that 14.4% of those accidents happened during animal/clinical visits. Working hours and high occupational stress might increase accidents related to the work and especially risk of car accidents [16]. However, in our study, only one participant reported that traffic accident was a stressor related to occupation *(Table 4)*. This finding can be result from veterinary surgeons in our study group did not see occupational stress as a risk factor for traffic accident, although majority of the participants reported that they faced with at least one car accident.

Study Limitations

Our study has some limitations. This is a web-based study with a small sample size; therefore, any conclusions maybe limited in their implications. Further, we sent an e-mail to the all member veterinary chambers of the Turkish Veterinary Medical Association. However, the questionnaire could not be send to higher numbers

of veterinary surgeons because there was no healthy communication link between chambers of veterinary surgeons and vets, and also mutual feedback system was absent between them. Another limiting factor in our study is, we believe, that "Veterinary Occupation Health" concept and its perception have not been improved in our country yet, so vets did not show sufficient interest in the issue. Although our study had some limitations like it was performed on subjects with a few in number, and its representative strength was weak, we believe that, it would provide contributions into the literature as it was the first study evaluating occupational stress among veterinary surgeons by via of stress scale in Turkey. As insufficient awareness between veterinary surgeons about occupation health and safety could negative effects on results of this type of study. Therefore, on the job training programs would be beneficial in prevention from occupational exposures, which had negative effects on occupational health and safety, as occupational stress had the priority, among veterinary surgeons in Turkey.

Majority of Turkish veterinary surgeons enrolled in our study group reported that they experienced occupational stress. According to occupational stress scale points, decision latitude and social support levels of veterinary surgeons working for the government were statistically significantly lower than those in subjects working for private sector. There was no statistically significant difference in work load, work control and skill using points between subjects working for the government and private sector. Daily work and cellular phone use durations of participants were high. More than half of vets were determined to have a car accident. While lower back and back pains were the most commonly reported physical health problems; stress, quick temperament, and depression were the most commonly reported mental health problems by vets. The most commonly reported stressors related to occupation were observed as mistreatment, death of animal, transmission risk of zoonosis diseases, animal owner pressure and superior pressure at work environment. It was also determined that as age increases, stressors of mistreatment and animal loss were decreased.

Recommendations

Results of occupational stress studies performed on veterinary surgeons are consistent with our results, and results have indicated that interventions are required to decrease the occupational stress [7-9,12]. It is known that low job satisfaction and burnout appears to cause occupational stress in veterinary surgeons [35,36]. In a study performed on veterinary technicians, skill training programs performed in before and after graduation were emphasized to be beneficial in increasing job satisfaction and decreasing occupational stress [37]. Occupational risks observed in veterinary practices vary according to working environments and works performed. Therefore, considering these separate interventional studies should be planned and trainings should be performed directed to vets working for private sector and for the government in the clinic and field [32]. In the working environment sources of stress can be defined, and they may be eradicated or preventive measures can be developed for the working staff. During periodical examination of working staff, work related problems can be investigated, and required preventive measures can be defined. These measures are consideration of work load, working hours and management of work, individualization of work (ergonomy) and increasing social support. Training programs may be beneficial in development of skills related to work, communication, management of stress and conflict. These training programs and supportive applications will decrease occupational stress. Communication tools like social media and remote training services may decrease occupational stress experienced by vets in their working environments.

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RESEARCH ARTICLE

Comparison of the Growth Performance and Carcass Characteristics of Two Slow-Growing Broiler Genotypes Fed Diets Supplemented with Dry Oregano (*Origanum vulgare* L.) or Lemon Balm (*Melissa officinalis* L.) Leaves under the Organic System [1]

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Summary

This study was conducted to determine the growth performance and carcass characteristics of two slow-growing broiler genotypes (Hubbard S757 and Hubbard Grey Barred JA) fed diets supplemented with dry oregano (*Origanum vulgare* L. or lemon balm leaves (*Melissa officinalis* L.) as growth promoter source under an organic housing system. In this study 240 chicks (mixed-sex) were allocated randomly into 4 experimental groups according to a 2 x 2 factorial arrangement for 2 broiler genotypes and 2 diets. The effects of dry herb leaves and genotype x herb leaves interaction on studied parameters were not significant at all weeks of age (P>0.05), except neck (%). Body weight , body weight gain, feed efficiency, carcass weight and yield, leg weight and yield, breast weight and yield, back weight and yield, edible giblets weight and yield of Hubbard S757 genotype were higher (P<0.05) than those of Hubbard Grey Barred JA genotype. The female breast (P<0.01) and edible giblets yield (P<0.05) were superior to those of males. These results show that herb leaves used as a growth promoter source under organic housing system did not affect the studied parameters and that in terms of these parameters, and subsequent Hubbard S757 genotype were superior to Hubbard Grey Barred JA genotype.

Keywords: *Organic system, Slow growing chicken, Growing rate, Herb leaves, Carcass traits*

Organik Sistemde Kuru Kekik (*Origanum vulgare* L.) ve Melisa (*Melissa officinalis* L.) Katkılı Yemlerle Beslenen Yavaş Gelişen İki Etlik Piliç Genotipinin Büyüme Performansı ve Karkas Özelliklerinin Karşılaştırılması

Özet

Bu araştırma, organik sisteminde yavaş gelişen Hubbard S757 ve Hubbard Gri çubuklu JA etlik piliçlerin; diyetlerine kuru kekik (*Origanum vulgare* L.) ve oğul otu (*Melissa officinalis* L.) yaprakları ilave edilmesinin performans ve karkas özelliklerine etkisini saptamak amacıyla yürütülmüştür. Denemede toplam 240 adet günlük etlik civciv (karışık cinsiyet) 2 genotip, 2 diyet ve 3 tekerrürlü olarak faktöriyel deneme deseninde rastgele 4 gruba dağıtılmıştır. Boyun randımanı hariç bütün haftalarda araştırmadaki parametreler üzerine bitki yaprakları ve bitki yaprakları ile genotip interaksiyonunun etkileri gözlenmemiştir (P>0.05). Hubbard S757 genotipinin canlı ağırlık, canlı ağırlık artışı, yemden yararlanma, karkas ağırlığı ve randımanı, but ağırlığı ve randımanı, göğüs ağırlığı ve randımanı, sırt ağırlığı ve randımanı, yenilebilir iç organ ağırlığı ve randımanı Hubbard gri çubuklu JA genotipindekilerden yüksek bulunmuştur (P<0.05). Dişilerin oransal göğüs parçası (P<0.01) ve yenilebilir iç organları (P<0.05) erkeklerinkinden yüksek olmuştur. Bu araştırmanın sonucunda, büyümeyi teşvik edici olarak kullanılan bitki yapraklarının ele alınan özelliklere etkisi saptanmamıştır ve bu parametreler bakımından Hubbard S757 genotipi Hubbard gri çubuklu JA genotipinden üstün bulunmuştur.

Anahtar sözcükler: *Organik sistem, Yavaş gelişen genotip, Büyüme oranı, Bitki yaprakları, Karkas özellikleri*

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INTRODUCTION

Due to the recent consumer demands for organic foods, organic poultry production has become a growing segment of the poultry industry. The use of fast growing broilers in these systems causes physiologic and metabolic problems and criticism because of the lack of consideration of animal welfare. Slow growing chickens are more suitable for organic and free-range systems and they reach to 2.2-2.5 kg body weight (BW) in 80-120 days, because organs and muscles grow in harmony and the possible metabolic and physiologic problems caused by fast growing decreases [1].

In Europe, organic poultry production is regulated by different national and international rules regarding the choice of genotype. EC regulation 1804/99 and the Network for Animal Health and Welfare in Organic Agriculture's final recommendation [2] suggest using local, slow growing breeds for their higher rusticity and capacity to use outdoor areas and pasture [3]. Slow-growing genotypes, which were designed for outdoor production [4], have growing period of at least 81 days according to European organic programs [5]. Slow-growing birds are more adapted to natural systems, and the quality of their meat is more appropriate for a specialty or gourmet market [6,7].

Thus, some researchers [3,8] stated that slow-growing chickens possess a good aptitude for pasture, which enhances the dietary intake of bioactive substances (i.e vitamins, antioxidants, and fatty acids) contained in the forage. In addition, the free-range chicken can consume young vegetative plant material and live protein sources, such as insects, worms, and grubs, which could reduce the feed cost that accounts for approximately 70% of the total variable costs [9,10] stated that compared with conventional free-range and organic systems, the pastured poultry is likely to induce considerably greater levels of pasture consumption, and thus it is an ideal system to evaluate the nutritional impact of pasture intake in broiler performance and carcass quality.

The removal of antibiotic growth promoters from poultry diets has triggered researches for suitable natural alternatives to combat the increased potential for bacterial disease development in organic growing flocks. Actually, the ban of some feed additives (antibiotics, coccidiostatic, and the other artificial agents which are helpful to growing) in poultry nutrition and its subsequent associated concerns has created efforts to use different plant compounds as possible natural alternatives [11,12].

Recently, the researchers have endeavored to use some phytobiotics (herbs and herbal products) as alternatives to in-feed antibiotics for young animals and birds [13-15]. Phytobiotics are incorporated in poultry diets to replace synthetic products in order to stimulate or promote the effective use of feed nutrients, which may subsequently result in more rapid body weight gain (BWG), higher production rates, the stimulation of appetite, improved feed efficiency, the improvement of endogenous digestive enzyme secretion, activation of immune response and antibacterial, antiviral, antioxidant and antihelminthic actions [16,17].

Oregano (*Origanum vulgare* L., OV) is an aromatic plant with a wide distribution throughout the Mediterranean area and Asia [18]. It has been suggested that the oregano possesses in vitro antimicrobial [17,19], antifungal [20,21], insecticidal [22] and antioxidant [23] properties. Lemon balm (*Melisa officinalis* L., MO) is the most common herbs used in our traditional folk herbal medicine. However, little is known about the antioxidant properties of their extracts and essential oils in poultry [24].

Lemon balm is known as a herb with high content of antioxidant active substances [24] and the antimicrobial activity [25]. Some molecules responsible for the antioxidative properties of natural plant extracts are flavonoids and phenolic compounds [26]. Research is needed to determine the suitability of different slow-growing genotypes fed dietary herbal supplement for Turkey organic and natural production systems that provide outdoor access with regard to overall growth performance and consumer acceptability.

To our knowledge, the comparison of the performance and carcass characteristics of two different slow growing genotypes fed with supplementation of dry oregano (*Origanum vulgare* L.) or lemon balm (*Melissa officinalis* L.) leaves, which are among the alternative growth promoters, into compound feed has not been reported in the organic rearing system. Therefore, the present study was conducted to compare the growth performance and carcass characteristics of two different slow growing genotypes fed using dietary dry oregano or common balm leaves as growth promoter supplement.

MATERIALS and METHODS

The study was carried out at Cumhuriyet University. Two hundred and forty slow growing chickens consisting of equal numbers of Hubbard S757 (S757) and Hubbard Grey Barred JA (GB-JA) strains were utilized for the investigation. In the study, 240 male and female day old chicks were weighed, identified with a wing number and randomly allocated to 4 treatment combinations with 3 replications in a 2 (genotypes: S757, GB-JA) X 2 (diets: 10g *Origanum vulgare*/kg basal diet, + 10 g *Melissa officinalis*/kg basal diet) factorial arrangement in a complete randomized design. The experiment was approved by the Ethics Committee of the University of Cumhuriyet in Sivas, Turkey (20.06.2011/50).

There were 12 mobile chicken house (1.5 x 1.5 m),

each containing 20 birds (wing numbered) per replication with 10 birds/m^2 stocking density placed in each of the 100 m^2 (space outside at least 4 m^2/bird for EU standards) grazing area. Moving shelters are secure and allow chickens access to sunlight and fresh air, while allowing them to forage and scratch the ground for food. It is made from wood and includes adequate [27] drinkers, feeders, heater and perch. The surrounding of the current research area was covered by plastic netting material against predatory and foreign birds. The experiment was ended at the end of the 14th week.

Starter (0-28 days), grower (29-81 days) and finisher (82-98 days) diets were formulated to provide adequate levels of all nutrients for broilers *(Table 1)*. All birds used in the experiment were cared for according to applicable recommendations of the National Research Council [28]. The basal diets were supplemented with levels of OV and MO to provide 10 g/kg of total OV and MO in the diet from the first 15 day. The specified chemical compositions of the organic diets are presented in *Table 1*, and certified as organic feed materials used. Creating artificial poultry pasture, *Lotus corniculatus* (50%) and *Bromus inermis* (50%) are used a combination of both. Organically grown herbs of oregano (*Origanum vulgare* L.) or lemon balm (*Melissa officinalis* L.) were harvested and the leaves were separated from the twigs. The herb material consisted of leaves that were spread out on a concrete floor and allowed to dry for a period of 3-4 days under room temperature.

The research in regard to the animal production, feeds and pasture was carried out according to the principles and implementation of regulation on organic agriculture [27] published by the Republic Of Turkey, Ministry of Food, Agriculture and Livestock. Barley, wheat bran, white wheat, rye, corn, triticale, oat and soybean meal provided from Buğrahan Co.Ltd. as certified organic, and the research has been followed by an independent audit organization ORTAR control certification Co.Ltd..

Initially, 14 day-old chicks were housed in mobile housing, feed and water were provided *ad libitum*, and they were not allowed go out for grazing. After this period chicks were allowed to go out and graze freely and all basal feed and water were provided between the hours 07.00-19.00 *ad libitum* for all chicks during the experimental period. Natural day length lighting is provided for chickens from the first day to slaughter age without additional lighting. The research has been conducted from 14 June to 30 September 2012. Natural lighting time at the beginning and at the end of the experiment was recorded 15 h 8 min and 11 h 57 min, respectively.

Ceramic heaters are used for heating which are sources of Far Infrared Rays and do not spread light. As required by Türkoğlu *et al.*[29], temperature at chick level was decreased every week in line with their growth to reach 20°C in the fourth week, and was then maintained at this temperature until slaughter. The chicks were vaccinated against Newcastle diseases (day old and 14 day), gumboro (on 7 and 21 days) and Infectious Bronchitis virus (IBV, 28 day). Any drug or antibiotic was not used to increase efficiency or against diseases.

Table 1. *Ingredients and composition of experimental organic diets (%)*
Tablo 1. *Araştırmada kullanılan organik rasyonun yapısı ve içeriği (%)*

Feed Ingredients	0–28 days	29–81 days	82–98 days
Barley	3.45	4.50	4.50
Vegetable oil	4.36	5.00	5.00
Wheat bran	5.00	5.00	5.00
White Wheat	12.40	4.00	4.00
Rye	3.00	4.00	4.00
Corn	40.00	20.00	20.00
Triticale	-	22.00	32.00
Oat	2.10	5.00	-
Fish meal	7.30	5.00	-
Soybean meal	20.00	22.00	22.00
Dicalcium phosphate	1.10	2.10	2.10
Limestone	0.74	0.80	0.80
Salt	0.30	0.30	0.30
Vitamin-mineral premix*	0.25	0.30	0.30
Calculated nutrients composition (g/kg)			
ME (MJ/kg)	13.00	12.72	12.91
Dry matter	899.00	903.00	901.00
Crude protein	197.00	201.00	180.00
Crude ash	4.70	5.90	4.80
Lysine	10.80	10.60	8.50
Methionine + Cystine	6.60	6.70	5.90
Threonine	7.30	7.20	6.20
Calcium	10.00	11.60	9.00
Sodium	1.90	1.80	1.50
Tryptophan	2.40	2.60	2.50
Linoleic acid	31.9	32.1	31.3

** Each kg of vitamin-mineral premix contained: vit A, 4.400.000 IU; vit D_3, 1.600.000 IU; vit E, 20.000 mg; vit K_3, 1.600 mg; vit B_1, 1.200 mg; vit B_2, 3.200 mg; vit B_3, 20.000 mg; vit B_5, 6.000 mg; vit B_6, 1.600 mg; vit B_9, 800 mg; vit B_{12}, 8 mg; biotin, 80 mg; antioxidant dry, 50.000 mg; Cu, 6.000 mg; Fe, 20.000 mg; Mn, 48.000 mg; Se, 80 mg; Zn, 40.000 mg; Co, 80 mg; I, 500 mg*

Weekly BW of birds was individually measured, feed consumption (FC) and viability were calculated for each group during the 14th week of the study. The average feed consumption of the group was used to calculate the individual feed conversion ratio (FCR). A total of 48 chicks were randomly chosen and weighed by selecting two male and two female birds from each subgroup. After 10 h fasting, birds were slaughtered to determine the carcass traits in 14 weeks. In order to determine hot carcass weight, edible giblets and abdominal fat was removed. Cold carcass weight was determined after chilling the

carcasses at +4°C for 24 h. Cold carcass weight was divided by the slaughter weight to determine cold carcass yield. Carcass parts weights (legs, breast, wings, back, neck, edible giblets) were determined according to the Institute of Turkish Standards [30,31] scattering technique. Carcass yield (dressing percentage) and weight of edible giblets were expressed as a percentage of BW just before slaughter (g/100 g BW), and carcass parts were expressed as a percentage of the cold (chilled) carcass weight (CW) without giblets (g/100 g CW).

The 48 birds (fasted for 10 h with free access to water) were slaughtered without stunning under Turkish slaughter procedure (these birds were slaughtered under conditions acceptable to the appropriate ethics committee) by severing the throat and major blood vessels in the neck in local processing plant in organic system [31]. The significance of the mean scores between the treatments for BW, BWG, FC, FCR, CW and carcass yield were studied by multifactor ANOVA, including the effects of genotype, herb leaves, sex and genotype and their interaction. The statistical analysis was conducted using the SPSS 16.0 (Inc. Chicago. IL. USA) program. Treatment effects were considered to be significant at P<0.05. Data were expressed as mean values with pooled standard errors (standard errors of the mean, SEM).

RESULTS

During the entire experimental period birds showed excellent health (no signs of footpad burns and good plumage state). There were no reports of predation because the 12 experimental plots were fenced to keep out foxes and were covered with nylon nets to avoid avian predation.

In the present study, BW, BWG and FC and the feed efficiency of two different genotypes fed with two dry herb leaves during the experiment were presented in *Table 2* and *Table 3*, respectively. Significant differences were observed in BW, BWG and FCR, but not FC due to genotypes.

Mean values for carcass measurements derived from dissection and proportions of carcass parts are given in *Table 4* and *Table 5*. Weight of hot and cold carcass, legs, breast, wings, back, neck and edible giblets were significantly higher (P<0.01) for S757 genotype chicken than GB-JA genotype. Weight of abdominal fat have also significant difference between genotypes (P<0.05; *Table 4*).

Genotype by dietary dry herb leaves interaction was not significant at all weeks of age for BW, BWG, FC and FCR. All carcass parts yields did not effect by genotype *X* herb leaves interaction except neck yield (P>0.05). Concerning genotype *X* herb leaves and genotype *X* herb leaves *X* sex effects had no significant influence on mass of carcass parts (P>0.05; *Table 4* and *Table 5*).

Weight of hot and cold carcass, legs, breast, wings, back, neck and edible giblets and abdominal fat did not differ significantly between both dry herb leaves (P>0.05). As the weight of hot and cold carcass, legs, breast, wings, back, neck and edible giblets and abdominal fat yields

Table 2. *The BW and BWG of two different slow growing broiler genotypes*
Tablo 2. *Yavaş gelişen iki farklı etlik piliç genotipinde canlı ağırlık ve canlı ağırlık artışları*

Items			Body Weight, g				Body Weight Gain, g			
			Age (week)				Age (week)			
Genotype [1]	Herb [2]	Sex [3]	0	4	8	14	0-4	4-8	8-14	0-14
GB-JA	OV	M	35.26	216.50	494.28	2143.22	172.73	483.70	1438.57	2095.00
		F	34.48	178.17	416.30	1614.11	149.16	397.02	1020.95	1567.13
	MO	M	35.11	207.67	516.59	2173.00	168.80	499.21	1445.35	2113.36
		F	35.40	185.50	467.62	1641.06	168.95	427.17	1069.92	1666.04
S757	OV	M	36.63	306.55	749.63	2847.33	255.48	692.49	1876.35	2824.31
		F	35.63	273.83	648.93	2184.11	240.69	570.39	1368.48	2179.56
	MO	M	36.62	304.78	764.28	2792.83	267.17	686.70	1852.79	2806.66
		F	35.33	285.17	681.23	2256.33	257.42	587.51	1426.24	2271.17
SEM			0.175	9.043	21.610	67.79	8.287	17.248	43.954	66.678
Main and interaction effects										
Genotype			**	**	**	**	**	**	**	**
Herb leaves			-	NS	NS	NS	NS	NS	NS	NS
Sex			*	*	**	**	NS	**	**	**
[4] G X H			-	NS	NS	NS	NS	NS	NS	NS

*[1] **GB-JA**=Hubbard Grey Barred JA; **S757**=Hubbard S757; [2] **OV**= Origanum vulgare; **MO**= Melissa officinalis; [3] **M** = male; **F** = female; [4] Genotype X Herb leaves; *P<0.05; **P<0.01; **NS**= P>0.05; **SEM:** Standard eror of the mean*

Table 3. *The feed consumption and feed convertion ratio of two different slow growing broiler genotypes*
Tablo 3. *Yavaş gelişen iki farklı etlik piliç genotipin yem tüketimi ve yemden yararlanma oranları*

Items		Feed Consumption (g)				Feed Consumption Ratio (g feed:g weigth gain)			
		Age (week)				Age (week)			
Genotype [1]	**Herb [2]**	**0 – 4**	**4 – 8**	**8 – 14**	**0 – 14**	**0 – 4**	**4 – 8**	**8 – 14**	**0 – 14**
GB-JA	OV	687.40	1424.78	4945.45	6370.24	4.21	3.17	3.94	3.41
	MO	770.91	1461.50	5187.31	6648.81	4.77	3.26	4.16	3.58
S757	OV	719.06	1423.28	5125.32	6548.61	2.99	2.32	3.26	2.70
	MO	768.74	1428.00	5189.75	6617.75	2.95	2.31	3.29	2.70
SEM		13.739	18.548	64.863	80.921	0.251	0.149	0.129	0.130
Main and interaction effects									
Genotype		NS	NS	NS	NS	**	**	**	**
Herb leaves		*	NS	NS	NS	NS	NS	NS	NS
[3] G X H		NS	NS	NS	NS	NS	NS	NS	NS

*[1] **GB-JA**=Hubbard Grey Barred JA; **S757**=Hubbard S757; [2] **OV**= Origanum vulgare; **MO**= Melissa officinalis; [3] Genotype X Herb leaves; *P<0.05; **P<0.01; **NS**= P>0.05; **SEM:** Standard eror of the mean*

Table 4. *The carcass characteristics of two different slow growing broiler genotypes in organic system (g)*
Tablo 4. *Organik sistemde yavaş gelişen iki farklı genotipin karkas özellikleri (g)*

Items			Slaughter (g)	Hot Car. (g)	Cold Car. (g)	Legs (g)	Breast (g)	Wings (g)	Back (g)	Neck (g)	E. Giblets (g)	Abdominal Fat (g)
Genotype [1]	**H [2]**	**S [3]**										
GB-JA	OV	M	2113	1473	1438	469	279	151	441	72	79	34
		F	1628	1150	1112	347	264	121	343	44	63	28
	MO	M	2143	1480	1447	481	270	148	466	68	76	34
		F	1639	1154	1115	337	228	123	351	63	63	27
S757	OV	M	2821	1998	1951	614	420	204	641	73	94	46
		F	2151	1528	1497	450	356	157	473	53	80	38
	MO	M	2872	2048	1998	628	427	211	654	69	99	47
		F	2237	1549	1525	444	347	156	519	50	82	32
SEM			65.31	47,86	47,28	15,31	11,76	4,70	17,17	1,92	2,02	2,47
Main and interaction effects												
Genotype			**	**	**	**	**	**	**	**	**	*
Herb leaves			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sex			**	**	**	**	**	**	**	**	**	NS
[4] G X H			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
[5] G X S			*	*	*	NS	NS	**	NS	NS	NS	NS
[6] H X S			NS	NS	NS	NS	NS	NS	NS	*	NS	NS
[7] G X H X S			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

*[1] **GB-JA** = Hubbard Grey Barred JA; **S757** = Hubbard S757; [2]**OV**= Origanum vulgare; **MO**= Melissa officinalis; [3] **M** = male; **F** = female; [4] Genotype X Herb leaves; [5] Genotype X Sex; [6] Herb leaves X Sex; [7] Genotype X Herb leaves X Sex; *P<0.05; **P<0.01; **NS**= P>0.05; **SEM:** Standard eror of the mean*

did not differ significantly between both dry herb leaves (P>0.05). Wings and neck yield had significant differences in herb leaves *X* sex interaction (P<0.05).

DISCUSSION

Effect of Genotype

The BW and BWG of S757 chickens were higher than GB-JA genotype, while those of chickens whose diets were supplemented by dry oregano or lemon balm leaves did not differ significantly during all weeks in the organic system. The growth rates of genotypes studied under the conditions of the present experiment were accurately measured for the GB-JA and S757 and their live market weights were different from one another. From 3 to 14 weeks of age, S757 genotype grew faster (P<0.05) than GB-JA genotype. FC of two different slow growing broiler

Table 5. *The carcass characteristics of two different slow growing broiler genotypes in organic system (%)*
Tablo 5. *Organik sistemde yavaş gelişen iki farklı genotipin karkas özellikleri (%)*

Items			Hot Car. (%)	Cold Car. (%)	Legs (%)	Breast (%)	Wings (%)	Neck (%)	E. Giblets (%)	Abdominal Fat (%)
Genotype [1]	H [2]	S[3]								
GB-JA	OV	M	69.74	68.08	32.64	19.45	10.52	4.24	5.50	2.38
		F	70.62	68.23	31.18	23.64	10.92	3.57	5.76	2.57
	MO	M	69.03	67.51	33.26	18.65	10.27	4.32	5.33	2.34
		F	70.37	67.99	30.31	20.50	11.08	5.27	5.74	2.45
S757	OV	M	70.83	69.15	31.47	21.56	10.50	4.49	4.88	2.33
		F	70.98	69.55	30.07	23.67	10.55	4.01	5.41	2.60
	MO	M	71.29	69.53	31.49	21.38	10.63	3.85	4.98	2.44
		F	69.23	68.14	29.18	22.64	10.27	3.66	5.48	2.10
SEM			0.29	0.26	0.23	0.39	0.09	0.12	0.09	0.14
Main and interaction effects										
Genotype			NS	*	**	**	NS	NS	*	NS
Herb leaves			NS	NS	NS	NS	NS	NS	NS	NS
Sex			NS	NS	**	**	NS	NS	*	NS
[4]G X H			NS	NS	NS	NS	NS	*	NS	NS
[5]G X S			NS	NS	NS	NS	NS	NS	NS	NS
[6]H X S			NS	NS	NS	NS	*	*	NS	NS
[7]G X H X S			NS	NS	NS	NS	NS	NS	NS	NS

*[1] **GB-JA**=Hubbard Grey Barred JA; **S757**=Hubbard S757; [2]**OV**= Origanum vulgare; **MO**= Melissa officinalis; [3] **M** = male; **F** = female; [4] Genotype X Herb leaves; [5] Genotype X Sex; [6] Herb leaves X Sex; [7] Genotype X Herb leaves X Sex; *P<0.05; **P<0.01; **NS,** P>0.05; **SEM:** Standard eror of the mean*

genotypes and both herb leaves treatments were similar during all weeks in the organic system. Feed efficiency of S757 slow growing broiler genotype were greater than GB-JA genotype while those of chickens fed supplemented dry oregano or lemon balm leaves were similar during all weeks in the organic system. It seems possible that slow growing genotypes in organic system could be slaughtered at 12 weeks according to feed efficiency indicators. As it is seen *Table 2* and *Table 3*, slow growing S757 genotype fed with supplemented both dry herb leaves diet showed higher BW, BWG and feed efficiency which were associated with the growing rate. This increasing positive effect is not reflected to their FC. It may be that the genotype S757 have benefited effectively from pasture feeding. Unfortunately, the pasture consumption was not measured in this study, but this parameter may need to be measured in future research to provide better understanding. Fanatico *et al.*[7] claimed that outdoor access has many factors, such as temperature, photoperiod, and light intensity, which are not controlled and are inherently variable. Furthermore, chickens raised outdoors have access to pasture and the forages, insects, and worms that may be available.

These results are consistent with those of Castellini *et al.*[32] suggesting that there are negative effects of the organic rearing system of chickens on BWG and FCR for Ross cockerels aged 56 and 81 days. A possible explanation for this might be that the high fiber content of pasture biomass may limit nutrient utilization and could reduce growth rates and feed efficiency [10]. Sirri *et al.*[33] claimed that FCR, calculated on FC with the exclusion of pasture intake resulted in 4.42 for slow growing genotype was higher than that of current finding. Grashorn and Closterman [34], while studying performance and slaughter characteristics of five slow and one fast growing genotypes for extensive production up to 84 days reported that genotypes differed significantly in BW's (Ross being heavier in this respect). On the other hand, Santos *et al.*[35] and Ponte *et al.*[10] showed significantly higher BW in broiler chickens that had free access to pasture. Similar results were obtained by Bassler and Ciszuk [36], Santos *et al.*[35] and Mikulski *et al.*[37], in FCR.

Breast is the most valuable portion of the chicken carcass in the market; even small differences in breast yield among genotypes could have a significant economic impact. In our experiment, while slow growing chickens showed differences in all carcass characteristics induced by genotype *(Table 4)*, but only cold carcass, legs, breast, and edible giblets yields were significant (P<0.05; P<0.01; *Table 5)*.

The S757 genotype had higher cold carcass, edible giblets (P<0.05), legs and breast (P<0.05) yields compared to the GB-JA (P<0.01). Carcass yield was higher than that

(56.8%) of Sirri *et al.*[33] and lower than that (75.9%) of Şekeroğlu and Diktaş [38] in slow growing genotype, while it was similar to those reported by Castellini *et al.*[6], Fanatico *et al.*[7], Dou *et al.*[39], Poltowicz and Doktor [40]. Likewise, Castellini *et al.*[32] and Poltowicz and Doktor [40] showed that birds had access to free range achieved a higher percentage of breast muscle in the carcass. The present findings seem to be consistent with other research [39] which found the results of breast and legs yield (20.17% and 27.65%) of slow growing broiler in free-range system.

The abdominal fat yield of slow growing chickens was higher than those of reported by Castellini *et al.*[6] and Poltowicz and Doktor [40], while was similar than those of reported by Mikulski *et al.*[37] and Dou *et al.*[39]. Wang *et al.*[41] found that the abdominal fat yield of chickens in the free range system was significantly lower than chickens in the indoor treatment. Fat deposition is affected by many factors such as diet, age, genotype, environmental conditions, and sex [37]. On the other hand Narimani-Rad *et al.*[42] have reported that dietary supplementation of 1% oregano could improve broiler performance in conventional system and carcass quality via more weight gain, increase carcass yield and decrease abdominal fat deposition and might be a useful method to the production of organic broilers. It may be the case therefore that the dry lemon balm leaves also was the same effect just as dry oregano leaves on the decreasing abdominal fat of slow growing chickens in organic system.

The finding of genotype *X* sex and genotype *X* herb leaves *X* sex interactions had no significant influence on mass of major carcass parts yield as a percentage of BW.

Effect of Herb Leaves

This study demonstrated that supplementing dietary oregano or lemon balm have no significant effects on all growth performance parameters measured until the age of 14 weeks. Such a case can firstly be explained by the fact that all chickens growing performance showed similar effects in term of both herb leaves treatment. For this reason, using one of two dry herb leaves in organic system may have a positive effect on growing performance of chickens. The second reason for the lack of effects of supplements may be related to the environmental conditions [15]. Recent studies [12,43-45] have engaged fast growing broilers fed supplemented products (leaf, oil or extract) of the oregano or lemon balm in conventional systems, including slow-growing chicken employed in the present study, but the design used in their trial did not conform to the standard of organic production in the EU. Therefore, the results from the present study cannot be compared with the results of Botsoglou *et al.*[23], Roofchaee *et al.*[12], Marcinčák *et al.*[44,45]. Previous observations have shown that herbs, plants extracts, essential oil and/or the main components of the essential oil did not affect BWG, FC or feed efficiency of chickens in indoor conventional systems [11,46-51]. A possible explanation for some of our results may be the lack of adequate research in organic systems. There is a large volume of published studies describing the role of dried leaves, flowers, extracts, essential oil of oregano which possesses substantial antioxidative, antimicrobial and antifungal activity [19,23,52,53]. They actually suppress pathogenic microflora in the gastrointestinal tract of animals and thus reduce mortality during the fattening period, especially in stress period [45]. In fact, they used anticoccidial medication in their diets while it does not actually contain any of the drug in current organic diets. Therefore, Giannenas *et al.*[54] and Aparecida da Silva *et al.*[55] have stated that oregano essential oil exerted an anticoccidial effect which was similar to the ionophorous antibiotic verified through the intestinal morphometric and excretion of oocysts. Mortality can have a large impact on profitability.

The mortality percentage of S757 slow growing chickens fed supplemented oregano or lemon balm leaves was 5% and 5% while those of GB-JA slow growing chickens fed supplemented oregano or lemon balm leaves was 0% and 3.3%, respectively (P>0.05) from 0-14 weeks (for the entire growing periods). It can thus be suggested that viability of genotypes fed with dry lemon balm and oregano leaves were higher due to antimicrobial properties during the experiment. Although previous work has shown higher mortality in fast growing birds compared with slow-growing birds [6,56], there was not difference in mortality in this study, and all treatments had less than 5% mortality.

Effect of Sex

Contrary to expectations, this study did not find a significant difference in terms of BWG between sexes at 0 to 4 weeks. The result of this study indicates that BW in chickens was genotype and sex dependent, that is, birds' BW vary according to their sexes. In this study, males and females of S757 recorded significantly (P<0.01) higher mean values than the GB-JA' sexes in terms of BW and BWG at day old, 4, 8, 14 weeks and 4 to 8, 8 to 14, 0 to 14 weeks, respectively (*Table 2*; P<0.01) as expected.

It seems possible that these results are due to the fact that males consumed a higher level of diet than females for maximal BW and BWG [57]. This could be that generally male growing chickens gained weight faster than females due to higher daily FC in males [58] were also observed in this study. Males genetically have slow feathering growth than females which influence the different needs and use of dietary protein. In addition, it was shown by the study of Li and Nolan [59] that the daily protein synthesis and degradation rate for male broilers were higher than female broilers, suggesting that protein accumulation rate in males was also higher than females [60].

This present experiment showed that male chickens have significant differences on slaughter weight, hot and

cold carcass weight, legs, breast, wings, back, neck and edible giblets weight (P<0.01), but no differences in abdominal fat weight in regard to effect of sex on carcass parameters.

With regard to dressing weight and all carcass parts except abdominal fat weight, males and females of S757 chickens had highest mean values than both sexes of GB-JA chickens. This also implies that this trait is genotype and sex dependent, and that S757 performed better, and superior to GB-JA genotype at the same age, and under uniform organic management conditions. Many authors [6,40,61,62] stressed that free-range production system positively affects the quality of bird carcasses by reducing their fat content genotypes due to great locomotory activity and pasture aptitude continued to show low fat deposition [63].

The legs of males were higher than those of female (P<0.01). In terms of overall parts yield, the females had higher percentages of breast (P<0.01) and edible giblets (P<0.05) meat yields than males. Males had greater leg yield (P<0.01). This finding agrees with the studies of Young *et al*.[64], Fanatico *et al*.[7] who also found that females have higher breast meat yields than males, whereas males have higher leg yields. Concerning the cut-up yields, the breast meat and thigh and drumstick yields were higher than those (8.0% vs. 21.6%) of Sirri *et al*.[33]. In another study, Suto *et al*.[65] applied prolonged fattening of one broiler genotype when they measured various carcass traits and reported sex differences. Several factors have been shown to affect CW and carcass yield. These factors include genotype, nutrition, age, BW and sex [64,66]. Genotype x sex interaction have significant differences on slaughter weight, hot and cold carcass, wings weights (P<0.05), while other carcass parts weight were similar to those of both genotype and sex. Male chickens fed dry oregano leaf had the highest neck weight, and female chickens fed dry lemon balm leaf had the lowest neck weight (P<0.05), while for the slaughter weight and other carcass parts were not any significant differences (P>0.05).

The choice of different chicken genotypes in organic farming plays a key role in determining growth performance and carcass traits. The results of the present study suggest that the performance and carcass traits of the slow growing chicken S757 genotype grown under the standards of organic production according to the relevant EU legislation could be better than that of GB-JA slow-growing chickens fed with both supplemented dry oregano or lemon balm leaves.

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RESEARCH ARTICLE

Protective Role of Vitamin C on Sperm Characteristics and Testicular Damage in Rats Exposed to Radiation

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Summary

The aim of this study was to investigate the protective effects of vitamin C on sperm characteristics and testes in male rats exposed gamma radiation (2 Gy). A total of 21 adult male wistar albino rats (8 weeks of age, weighing 180-220 g) were divided into three groups. Control, radiotherapy (received scrotal γ-radiation of 2 Gy as a single dose) and radiotherapy + vitamin C treated rats (during the 55 days after irradiation, 500 mg vitamin C/500 ml water daily orally). Testes samples from all groups were taken at day 55 post-irradiation and epididymal sperm characteristics, all-genital organs weights and testes histology were evaluated. Radiotherapy decreased significantly the sperm motility, concentration, left testes and epididymis weights, Johnsen's biopsy score and seminiferous tubular diameter but it increased the sperm head defects as compared to the Control group ($P<0.05$). The administration of vitamin C only reduced the harmful effects of radiotherapy on the seminiferous tubular diameter ($P<0.05$). It has been concluded that the radiotherapy may cause alteration in the genital organ weights, spermatologic and hystologic parameters in rats and administration of vitamin C may be slightly beneficial for seminiferous tubular diameter following testicular irradiation during the radiotherapy.

Keywords: *Radiation, Vitamin C, Sperm, Testes, Rat*

Radyasyona Maruz Bırakılmış Sıçanlarda Spermatozoa Özellikleri ve Testislerde Yarattığı Hasar Üzerine Vitamin C'nin Koruyucu Rolü

Özet

Bu çalışmada, vitamin C'nin gamma radyasyon (2 Gy) uygulanmış erkek sıçanlarda spermatolojik özellikler ve testis üzerine olan koruyucu etkilerini saptamak amaçlandı. Çalışmada, 8 haftalık yaşta ve ortalama 180-220 g canlı ağırlığa sahip toplam 21 adet Wistar Albino ırkı erkek sıçanlar kullanıldı. Sıçanlar rastgele 3 eşit gruba ayrıldı. Gruplar; Kontrol, radyasyon tedavi (scrotal bölgeye tek doz 2 Gy radyasyon ışıması yapıldı) ve radyasyon tedavi + vitamin C grubu (radyasyon ışımasından sonraki 55 gün boyunca, günlük oral olarak, 500 mg/500 ml, vitamin C/su) şeklinde oluşturuldu. Tüm gruplardaki sıçanlardan radyasyon tedavisi sonrası 55. gününde genital organlar alınarak spermatolojik özellikler, genital organ ağırlıkları ile testis dokusuna ait histolojik özellikler değerlendirildi. Radyasyon tedavisi ve kontrol grubu, spermatolojik, morfometrik ve histolojik özellikleri bakımından karşılaştırıldığında, Radyasyon tedavisi uygulanan hayvanların motilite, konsantrasyon, sol testis ağırlığı, epididimis ağırlığı, seminifer tubul çapı ve Jhonsen biyopsi skorlarında istatistiksel olarak önemli düzeyde azalma ve anormal spermatozoon baş sayısında ise artış saptandı ($P<0.05$). Çalışmada vitamin C uygulamasının sadece seminifer tubul çaplarında radyasyonun zararlı etkisini önemli düzeyde azaltığı tespit edildi ($P<0.05$). Sonuç olarak, radyasyon tedavisinin sıçanlarda genital organ ağırlıkları, spermatolojik özellikler ve histolojik parametrelerde birtakım değişikliklere neden olduğu ve radyasyon uygulamasını takiben tedavi amaçlı vitamin C uygulamasının ise testislerdeki seminifer tubul çaplarındaki iyileşme gibi hafif düzeyde koruyucu etki gösterdiği tespit edildi.

Anahtar sözcükler: *Radyasyon, Vitamin C, Spermatozoa, Testis, Sıçan*

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INTRODUCTION

Exposure to a wide variety of external factors including certain drugs, enviromental pollutants, heavy metals and ionizing radiation has been linked to a variety of adverse health outcomes that may have significant animal and public health consequences. Various tissues and organ systems of an individual differ in their response to radiation and as a rule; systems with proliferating cells are most sensitive. The testes which generates male germ cells is known to be a radiosensitive organ in the body. Testicular damage after local or whole-body irradiation by external sources (gamma radiation) has been well documented in both animals and man [1-3]. Ionizing radiation damages the biological systems in a major way by generating reactive oxygen species (ROS). These ROS interact with biological molecules producing toxic free radicals leading to lipid peroxidation and DNA damage [4,5]. ROS can also alter the balance of endogenous protective systems, such as glutathione and enzymatic antioxidant defence systems [6,7]. The endogenous antioxidant defences are inadequate to reduce the radiation-induced free radicals. Appropriate antioxidant intervention may inhibit or reduce free radical toxicity and thus offer protection against radiation. Because exogenous compounds contribute to the antioxidant capacity of the system, dietary antioxidants supplementation may have the ability to decrease an individual's susceptibility to oxidative damage. As such, various antioxidant supplements such as vitamins, carotenoids, carnitine, alpha lipoic acid and polyphenols [8-10], as well as the dietary consumption of high amounts of antioxidant-rich foods [11,12] have been shown to decrease an individual's susceptibility to oxidative damage. Vitamin C, also known as ascorbic acid, is a very important water-soluble vitamin. It is essential for preserving optimal health and it is used by the body for many purposes. Vitamin C is a highly effective antioxidant. Even in small amounts vitamin C can protect indispensable molecules in the body, such as proteins, lipids (fats), carbohydrates, and nucleic acids (DNA and RNA) from damage by free radicals and reactive oxygen species that can be generated during normal metabolism as well as through exposure to toxins and pollutants (e.g., smoking). The radioprotective effect of ascorbic acid seems to be due to its interactions with radiation induced free radicals [13]. Ascorbic acid pre-treatment inhibited the radiation-induced elevation in lipid peroxidation [14]. It protected the mice against radiation induced sickness, reduced the mortality and improved the healing of wounds after exposure to whole body gamma-radiation [15]. But some reports indicate that the capacity of vitamin C to reduce harmful effects of radiation is inconclusive [16-18], therefore further studies are needed. The aim of the present study was to evaluate the radioprotective effect of vitamin C on gamma-radiation-induced damage to testes and epididimal spermatozoa.

MATERIAL and METHODS

The experiment was performed in accordance with guidelines for animal research from the National Institutes of Health and were approved by the Dicle University Ethics Committee on Animal Research (approval no: 2012/36).

Chemicals

Vitamin C (Redoxon Ampul 500 mg/5 ml) was obtained from Roche, İstanbul, Turkey. It was dissolved in tap water at concentrations of 500 mg/500 ml and *ad libitum,* were given orally before and after irradiation.

Animals

Wistar albino male rats were obtained from the Experimental Animal Center of Dicle University. The animals were housed at 21°C under a 12 h light-dark cycle and were allowed tap water and standard pellet diet for rats (Elazığ Yem Inc. Elazığ, Turkey).

Experimental Design

Twenty-one rats (8 weeks old, weighing 180-220 g) were randomly divided into three groups of seven rats each. Control group did not receive any treatment. The radiotherapy group received scrotal γ-radiation of 2 Gy and radiotherapy + vitamin C group received scrotal γ-radiation plus vitamin C. The dose of vitamin C used in the present study was based on previous reports [19]. Because of vitamin C has been reported to be well tolerated without any toxic effects over the dose range 100-200 mg/d [19], its middle dose was chosen in our study protocol.

Scrotal -Irradiation

Prior to radiotherapy, the rats were anesthetized with xylazine/ketamine (10/90 mg/kg, i.p.) and immobilized from their 4 extremities on a tray. Irradiation was delivered by an ALCYON-II model cobalt-60 teletherapy unit (General Electric/GE Healthcare) at a source-surface distance of 80 cm. A single dose of 2 Gy radiations was given at a depth of 1 cm (thicknees) with a dose rate of 0.4 Gy/min to an area of 30 x 30 cm of the scrotum in a supine position.

Sample Collection

All rats were kept under identical conditions for fifty days with free access to food and water. At the end of the fifty days, the rats were intraperitoneally administered a combination of 6 mg/kg of 2% xylazine HCl (Rompun, Bayer) and 75 mg/kg ketamine HCl (Ketalar, Pfizer) for anesthesia. Afterward, the testes of each rat were located and the testes, epididymis, seminal vesicles, and ventral prostate were removed, cleared of adhering connective tissue. The left testes, epididymis, seminal vesicles, and ventral prostate weight were evaluated along with epididymal sperm concentration, sperm motility, and sperm

morphology. One the other hand, right testes was fixed in 10% Bouin fixative for histopathologic examinations.

Epididymal Sperm Count

The epididymis was finely minced with anatomical scissors in 10 ml of physiologic saline, placed in a rocker for 10 min, and allowed to sit at room temperature for 2 min. After incubation, supernatant fluid was diluted 1:10 with a solution containing 5 g sodium bicarbonate, 1 ml formalin (35%), and 25 mg eosin per 100 ml of water. Total sperm number was determined using counting chambers. The cells were counted with the help of a light microscope (magnification, 200x).

Epididymal Sperm Motility Evaluation

The fluid obtained from the cauda epididymis with a pipette was diluted to 2 ml with Tris buffer solution. A slide was placed on phase-contrast microscope, and an aliquot of this solution was placed on the slide and percent motility was evaluated visually at a magnification of 400 times. Motility estimations were performed from 3 different fields in each sample. The mean of the 3 estimations was used as the final motility score. Samples for motility evaluation were kept at 37°C.

Epididymal Sperm Morphology Evaluation

To determine the percentage of morphologically abnormal spermatozoa in the cauda epididymis, the slides stained with eosin-nigrosin (1.67% eosin, 10% nigrosin and 0.1 M sodium citrate) were prepared. The slides were then viewed under a light microscope at 400x magnification. Two-hundred spermatozoa were examined on each slide, and the head and tail and total abnormality rates of spermatozoa were expressed as percent.

Hystological Analysis

The right testes from the rats were placed in 10% Bouin solution for 24 h for fixation and further pathologic examination. After fixation, the sections were subjected to boutine histologic tissue preparation and dehydrated and embedded in paraffin. Paraffin blocks were sliced to 5-m thickness with a microtome and the slices were subjected to Periodic Acid Schiff-Hematoxylin (PAS-H) staining and were then examined under a light microscope (Nikon ECLIPSE 80i, Nikon, Tokyo, Japan). For each sample, 100 randomly selected seminiferous tubule diameters were measured. In addition, for each section, 100 randomly selected seminiferous tubules were evaluated using the Johnsen classification [20].

Statistical Analyses

Statistical analyses were performed with SPSS for Windows 7 version 9.0 (SPSS, 1993) Results of the parameters were analyzed by One-wayANOVA procedure). Differences between means were tested by Tukey's least significant difference when a difference between groups was significant. Data are presented as means and SEM. NC.

RESULTS

Epididymal Sperm Characteristics

When we evaluated the spermatologic results between the groups, it was determined that sperm motility and concentration of the control group were significantly higher than in the radiation group (P<0.05; *Table 1*). But, vitamin C treatment did not prevent these decreases in motility and concentrations depend on irradiation *(Table 1)*. In addition, the total morphologic defects and tail defects were similar in the control group and all the others groups, but it was determined that radiation and vitamin C-treated group had a higher ratio of spermatozoon head defect than control group (P<0.05; *Table 1*).

Genital Organs Weight

Ionizing radiation no caused significant alterations in prostat and seminal vesicles weight of the rats. In contrast, testicular irradiation resulted in significant decreases in testes and epididymis weights at 55 days post-irradiation *(Table 2)*. Vitamin C treatment did not prevent these decreases in testes and epididymis weights depend on irradiation *(Table 2)*.

Morphometric Parameters

The tubular diameter and the Johnsen's biopsy score were measured and a summary of these results are presented in *Table 2*. As compared with control testes, all values of morphometric parameters were statistically significantly reduced in the irradiated testes at post-irradiation time periods. Vitamin C treatment significantly increased the diameter of seminiferous tubules at 55 days post-irradiation compared to radiation group. But, there was not difference between vitamin C plus radiation group and control group for the Johnsen's biopsy score.

Table 1. *Effects of Vitamin C treatment on sperm characteristics in radiation treated rats*

Tablo 1. *Radyasyon uygulanmış ratlar da Vitamin C'nin spermatolojik özellikler üzerine etkileri*

Measurements	Treatments		
	Control	Radiation	Vitamin C
Motility (%)	73.6±4.32[a]	55.7±5.50[b]	38.6±4.46[b]
Concentration (x10[6]/ml)	46.9±4.45[a]	22.7±4.32[b]	18.9±1.77[b]
Tail Defects (%)	24.1±1.54	24.4±1.54	22.1±1.45
Head Defects (%)	3.4±0.84[b]	8.0±0.61[a]	7.1±0.85[a]
Total Morphologic Defects (%)	27.6±1.41	32.4±1.87	29.3±0.77

The values given for continuous variables are Mean±Standard error
[a,b,c] Means within line with different superscripts differ significantly (P<0.05)

Table 2. *Effects of Vitamin C treatment on genital organ weight and morphometric parameters in radiation treated rats*
Tablo 2. *Radyasyon uygulanmış ratlar da Vitamin C'nin genital organ ağırlıkları ve morfometrik parametreler üzerine etkileri*

Measurements	Treatments		
	Control	Radiation	Vitamin C
Left Testes (g)	1.36±0.02[a]	0.91±0.04[b]	0.95±0.03[b]
Left Epididymis (g)	0.56±0.0[a]	0.43±0.01[b]	0.47±0.04[ab]
Prostate (g)	0.73±0.04	0.77±0.08	0.84±0.03
Seminal vesicles (g)	1.30±0.07	1.31±0.16	1.50±0.08
Johnsen's Biopsy score (/10)	9.8±0.03[a]	8.4±0.12[b]	8.5±0.11[b]
Seminiferous Tubules Dia. (µm)	355.7±5.39[a]	298.5±4.47[c]	326.9±2.74[b]

The values given for continuous variables are Mean±Standard error [a,b,c] Means within line with different superscripts differ significantly (P<0.05)

Light Microscopic Findings

On histopathological examination, control rat testes showed normal morphology and spermatogenesis, containing abundant amounts of spermatids and sperm in the lumen *(Fig 1a, d)*. In contrast to control, the arrangement of the cells was disturbed in the seminiferous tubules of gamma irradiated rats. Germinal epithelial cells were separated from each other and the tubular basement membrane. There was desquamation of germinal cells and consequent appearance of irregular spaces in the epithelium and spermatogenic cells were also decreased. The numbers of spermatozoa in the lumen were significantly low *(Fig 1b, e)*. Vitamin C treatment improved the radiation-induced histopathological changes in rat testes. The tubular diameter in the vitamin C-treated rats was higher compared to the radiotherapy group and the disturbance in the arrangement of the cells was slight in this group *(Fig 1c, f)*.

DISCUSSION

This study was undertaken to investigate the radio-protective effect of vitamin C on gamma-radiation-induced damage to testes and epididimal sperpatozoa. We have determined the extent of changes in rat testes structure and epididymal sperms parameters following 60Co γ-Radiation. Radiation treatment no caused significant alterations in prostat and seminal vesicles weight of the rats. In contrast, testicular irradiation resulted in significant decreases in testes and epididmis weights at 55 days post-irradiation *(Table 2)*. Various tissues and organ systems of an individual differ in their response to radiation and as a rule; systems with proliferating cells are the most sensitive. The testes which generates male germ cells is known to be a radiosensitive organ in the body. The loss of testes weight following irradiation has been reported by several workers [21,22]. The loss in testes weight might be in part

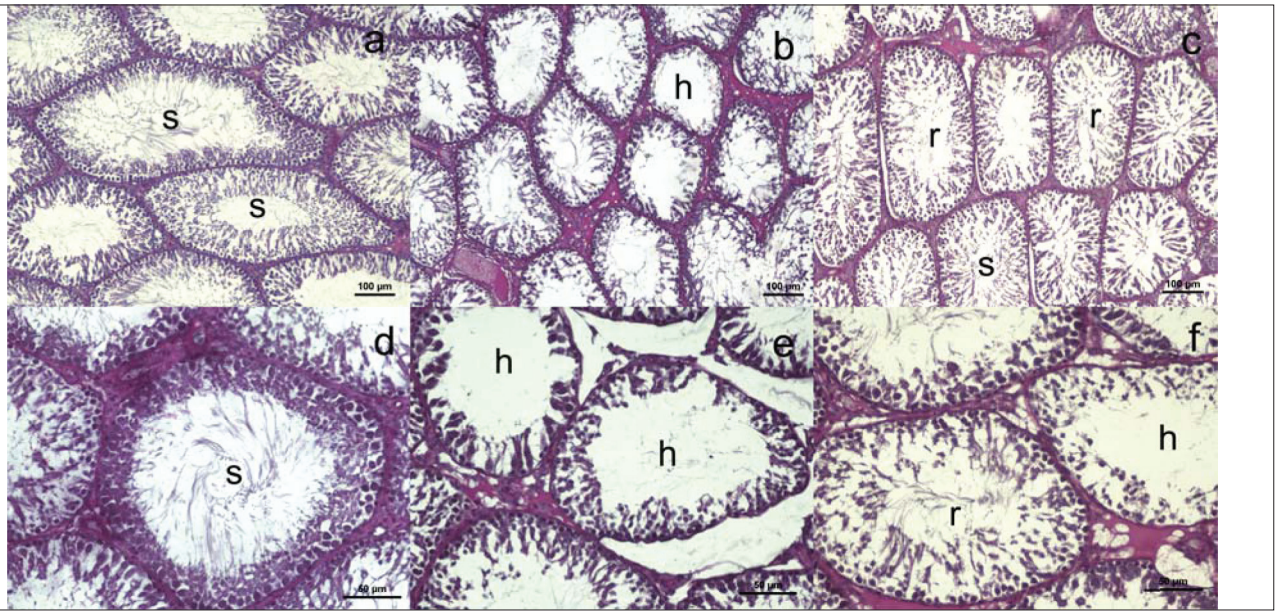

Fig 1. Testes histological sections: Normal spermatogenesis and seminiferous tubular organisation (s) in control group (a and d). In addition to tubular atrophy, hypospermatogenesis (h) and tubular degeneration is observed in 2 Gy Radiotherapy induced group (b and e). Although degenerated tubules and hypospermatogenesis, normal spermatogenesis (s) and regenerative tubules (r) are observed in radiotherapy and vitamin C induced group (c and f). In comparison with Radiotherapy induced group, tubular atrophy is decreased in Radiotherapy and Vitamin C dietary group

Şekil 1. Testes histolojik kesitleri: Kontrol grubunda spermatogenezis ve semimifer tübül organizasyonu normal gözlendi (şekil a ve d). 2 Gy Radyasyon uygulaması yapılan grupta hipospermatogenezis ve tübüler dejenerasyonun (h) yanı sıra tübüler atrofiye göze çarptı (şekil b ve e). Radyasyon ve vitamini c uygulanan grupta dejenere olmuş ve hipospermatogenezisli (h) tübüllerin yanı sıra rejeneratif profil gösteren (r) ve normal spermatogenezis gözlenen seminifer tübüller (s) gösterilmiştir (c and f). Vitamin C diyeti uygulanan grupta Radyasyon grubuna oranla tübüler atrofiyenin azalmış olduğu göze çarpmaktadır

due to loss of the body weight caused by radiation. The testes weight loss is also associated with cellular damage in the testes. Radiation inflicts lethal damage to spermatogonia, which adversely influences spermatogenesis and subsequently the sperm counts. In our study, we determined that sperm concentration and motility of the control group were significantly higher than in the radiation group (P<.05; *Table 1*). The sperm morphology is a characteristic of the genotype of the spermatogenic cells. Radiation is known to induce detrimental genotypic changes in the spermatogenic cells that affect the phenotype of the sperm [23]. Similarly, we found that radiation group had a higher ratio of spermatozoon head defect than control group (P<0.05; *Table 1*). In addition to sperm parameters, both seminiferous tubule diameter and Johnsen's biopsy score are good predictors of fertility status [20]. In this study, as compared with control group testes, the tubular diameter and the Johnsen's biopsy score were significantly reduced in the irradiated testes. In histological examination under a light microscope, control rat testes showed normal morphology and spermatogenesis, containing abundant amounts of spermatids and sperm in the lumen *(Fig 1a, d)*. In contrast to control, the arrangement of the cells was disturbed in the seminiferous tubules of irradiated rats. There was desquamation of germinal cells and consequent appearance of irregular spaces in the epithelium and spermatogenic cells were also decreased. The numbers of spermatozoa in the lumen were significantly low *(Fig 1b, e)*. This finding is in agreement with the previous reports documenting the degenerative effects of irradiation on spermatogenesis in bovine, mouse and rat [24,25].

The bone marrow and other tissues were often used to test the radioprotective action of different compounds, while there is no sufficient information concerning the modulating effect of vitamin C in testes and sperm of irradiated rat. The results of our present study indicate that orally administered vitamin C did not show protective effect against genetic damage (sperm motility, concentration and morphological defect) induced *in vitro* by exposure to gamma radiation. But, vitamin C treatment significantly increased only the diameter of seminiferous tubules at 55 days post-irradiation compared to radiation group. All these results suggested that vitamin C may be limited protective effects under different experimental condition. Previous study indicated that vitamin C at low concentration could protect DNA from radiation-induced damage in mouse bone marrow cells, but at high concentration enhanced the radiation-induced effect [26]. Alike, it was reported that vitamin C alone (0.01 µmol, 1 µmol) did not reduce radiation induced apoptosis in human lymphoblastic cell line, when given before 3 Gy gamma irradiation, but it showed radioprotective effect only at 0.01 µmol concentration after irradiation [27]. The reasons for the lack of radioprotection by dietary vitamin C are unclear. But, all of these findings suggest that presence or absence of radioprotective effect of vitamin C in examinations depend on concentration of vitamin C in biological environment, time of administration, radiation dose rate and type of radiation (low or high). In our experiment, the vitamin C dissolved in water, were administered orally as, one day before irradiation, so the vitamin C was present in the tissue with nontoxic and appropriate concentrations before production of free radicals by irradiation. Lipid peroxidation takes place after irradiation or free radical attack [28]. Vitamin C is an antioxidant molecule and prevents lipid peroxidation in plasma and inside the cell [29,30]. Thus, the histological findings in rat treated with vitamin C in comparison with the radiation group suggest that vitamin C exert its radioprotective effect on the diameter of seminiferous tubules *(Table 2)*. Therefor, also it was shown that vitamin C could have a slight radioprotective effect on rat testes.

In conclusion, present results confirm that the sperm paremeter values and genital organ weights significantly decreased in the irradiated rat testes compared with the control testes. Treatment with vitamin C to irradiation decreases the germ cell apoptosis, suggesting that vitamin C can protect testes from radiation injury. The present results suggest that vitamin C may be beneficial to spermatogenesis and infertility following testicular irradiation by decreasing germ cell apoptosis. However, further investigations and clinical studies are required to elucidate the exact mechanism of antiapoptotic effects of vitamin C in testes.

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RESEARCH ARTICLE

Effects of Dietary Boric Acid and Ascorbic Acid Supplementation on Performance, Some Blood and Bone Parameters in Broilers [1]

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Summary

This study was carried out to determine the effects of boric acid (17.5% boron) and ascorbic acid supplementation on performance, selected blood and bone parameters, carcass yield and malondialdehid (MDA) levels of broilers. The experiment lasted in 42 days. Totally 240 one-day old male Ross 308 broilers were housed at a density of 15 chickhens in each of 16 experimental plots using a completely randomized block experimental design. Diets were based on maize and soybean meal. Experimental groups divided into one control (CON) and 3 experimental groups. The experimental diets supplemented with 200 ppm ascorbic acid (AA) for the first group, 175 ppm boric acid (BA) for the second group and 200 ppm ascorbic acid plus 175 ppm boric acid combination (AABA) for the third group. At the end of experiment period there were statistically significant differences ($P<0.05$) between control and the treatment groups about body weight, body weight gain and feed conversion ratio for first 3 weeks of the experimental period, but there were no statistically different for feed intake. Carcass yield increased with additives especially with boron supplementation ($P<0.05$). Total protein, cholesterol, trigliseride concentration and ALT activity were not affected by addition of feed additives but AST activity increased in BA group while it was reducing in AA and AABA groups. There were also statistically significant differences for left tibia P levels ($P<0.05$) and plasma, liver MDA levels ($P<0.001$). There were no statistically significant differences between control and the treatment groups for tibia ash, Ca levels and some strength parameters. As a result; it can be concluded that boric acid and ascorbic acid supplementation did have positive effect on performance in 0-21 days of the experimental period and also on MDA levels about lipid peroxidation activity, bone mineralization and hot carcass yield studied in the experiment. It is inferred that it will be usefull if these feed additives are studied again at different levels and under different conditions.

Keywords: *Ascorbic acid, Boric acid, Broiler, MDA, Performance, Tibia*

Broyler Rasyonlarına İlave Edilen Borik Asit ve Askorbik Asidin Performans, Bazı Kan ve Kemik Parametreleri Üzerine Etkileri

Özet

Bu çalışma broyler rasyonlarına borik asit ve askorbik asit ilavesinin performans, bazı kan ve kemik parametreleri, karkas kalitesi ve MDA düzeyleri üzerine etkilerini belirlemek amacıyla gerçekleştirilmiştir. Araştırma 42 gün sürdürülmüştür. Araştırmada 16 bölmenin her birinde 15 adet günlük yaşta erkek 240 adet broyler civciv kullanılmıştır. Rasyonun temelini soya ve mısır oluşturmuştur. Bir kontrol ve 3 deneme grubu oluşturulmuş olup, kontrol grubuna herhangi bir ilave yapılmamıştır. Deneme grupları yemlerine ise sırasıyla 200 ppm askorbik asit, 175 ppm borik asit ve 200 ppm askorbik asit + 175 ppm borik asit ilavesi gerçekleştirilmiştir. Araştırma sonuçlarına göre; ilk 3 haftada kontrol grubu ve deneme grupları arasında canlı ağırlık, canlı ağırlık artışı ve yemden yararlanma oranı bakımından istatistiksel farklılıklar görülmüştür ($P<0.05$). Ancak yem tüketimleri arasında bir fark bulunamamıştır. Karkas randımanı özellikle bor ilaveli grupta artış göstermiştir ($P<0.05$). Toplam protein, kolesterol, trigliserit konsantrasyonları ve ALT aktivitesi etkilenmezken, AST aktivitesi borik asit ilaveli grupta artmıştır. Tibia P düzeyleri ile karaciğer ve plazma MDA düzeyleri arasında da istatistik açısından önemli farklılıklar elde edilmiştir ($P<0.001$). Kemik kül, Ca ve bazı mukavemet parametreleri bakımından da guplar arasında bir fark görülmemiştir. Sonuç olarak; borik asit ve askorbik asit takviyesi 0-21 günlerde performans üzerine ve aynı zamanda lipit peroksidasyonu bakımından MDA düzeyine, kemik mineralizasyonuna ve sıcak karkas randımanına olumlu etkilerde bulunmuştur. Karaciğer ve but kası bor düzeyleri bakımından da bor ilaveli gruplarda beklenen artışın söz konusu olduğu dikkati çekmiştir. Bu iki yem katkısının farklı dozlarda ve farklı koşullarda yapılacak çalışmalar sayesinde hayvanlar üzerinde irdelenmesinin faydalı olacağı sonucuna varılmıştır.

Anahtar sözcükler: *Askorbik asit, Borik asit, Broyler, MDA, Performans, Tibia*

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INTRODUCTION

The using of boron compounds in livestock sector are essential for country economy [1]. Boron was found in nature with its compound, especially as boric acids and borates [2,3]. In recent years, there are many investigations about using boron compounds in animal nutrition. Altough high levels of boron has fatal effect, in defficiency of boron, it is seen that inadequate growth and abnormal bone devolepment [4]. However present day it isn't certain that this element is essential for human and animals. Elliot and Edwards [5] have studied with boron (0, 20, 40, 80 ppm) in broilers and they reported that boron increased body weight and did not alter plasma mineral levels. In same study they have used 0, 5, 10 and 20 ppm boron and boron increased bone ash level. When boron levels increased in diets, body weight, tibia strength, liver and muscle boron concentration also increased [6]. Boric acid complexes are found in body structure. For instance; carbohydrates (glucose and polisaccarides), nucleotides (adenosine monophosphate and niacineamide adenindinükleotide), vitamins (ascorbic acid, pyridoxine, ryboflavine) [7]. So boron was used with ascorbic acid in current study and it was aimed to constitude an organoboron.

Ascorbic acid is a chemical name of vitamin C. It has plenty of isomers and vitamin C is equal to L-ascorbic acid. Because only this isomer (L-ascorbic acid) has a biological activitiy [8]. At general conditions ascorbic acid is synthesised sufficiently from kidney for metabolism in poultry [9]. Some metal ions have an important role for fragmentation of ascorbic acid and ascorbic acid composes a chelat with some metal ions [8]. It forms a bond with inorganic elements. When boron binds to an organic material, it is called organoboron. A large number of organoboron compounds are known and many are useful in organic synthesis [10]. According to Sahin et al.[11] 250 mg L-ascorbic acid increased body weight gain, feed intake, feed efficiency, hot and cold carcass weight, decreased serum cholesterol levels. Ascorbic acid (200 ppm) also increased performance parameters and MDA (malondialdehit) levels and decreased SOD (superoxide dismutase) levels [12].

The objective of this research was to evaluate the effects of boric acid and ascorbic acid supplementation on performance, carcass traits some blood and bone parameters and MDA level in broilers.

MATERIAL and METHODS

Animals, Experimental Design and Diets

In this study totally 240 one-day old male broiler chicks housed at a density of 15 chickens in each of 16 experimental plots. Experimental groups divided into one control and 3 treatment groups. The experimental diets supplemented with 200 ppm ascorbic acid for the first group, 175 ppm boric acid for the second group and 200 ppm ascorbic acid plus 175 ppm boric acid combination for the third group. Experiment period lasted in 42 days. Diets were based on maize, full fat soybean and soybean meal. Feed and water intake were offered *ad libitum*. Nutrient analysis in raw metarial and mixed feeds were determined according to AOAC [13] procedures *(Table 1)*. The formula recommended by TSE (Turkish Standards Institute) [14] was used to calculate the levels of metabolisable energy. Feed boron levels were measured by Thermo X-SERIES2 ICP-MS. This trial has been approved by Ankara University Animal Experiments Local Ethics Committee with 2009-38-174 number.

Performance and Biochemical Measurements

During the experimental period, the performance of the broilers was evaluated by weekly recording body weight (BW), body weight gains (BWG), feed intake (FI) and feed conversion ratio (FCR). FCR was calculated as the amount of feed consumed per unit of body weight gain. Mortality was recorded daily.

At the end of the experiments in each subgroup 3 broiler chicks were chosen randomly in each subgroup and slaughtered by cervical dislocation to determine the hot carcass weights and yields and also the absolute and relative weights of some visceral organs (liver, heart, spleen, gizzard, abdominal fat, bursa of Fabricius). Blood samples were obtained from each bird to the tubes with anticoagulant and without anticoagulant while animals were slaughtered. These samples were allowed to clot at room temperature for 6 h and then they were centrifuged at 3.000 rpm for 10 min at room temperature. Sera and plasma were carefully harvested and sera were stored at -20°C, plasmas were stored at -80°C until analysis. Serum total protein, cholesterol, tyrigliceride levels were detected with a commercial kit (Teco Diagnostic) by autoanalyser. AST and ALT levels were also detected with a commercial kit (Erba Mannheim XL System Packs) by autoanalyser. Boron levels in serum were determined by using of ion chromatography (ICS) (Dionex 3000, USA) [15].

Plasma and also liver tissue MDA concentration was measured with high plessure liquid cromotography (HPLC, Shimadzu, Tokyo, Japan) [16], at 250 nm Interstil 5µ C-18 (15 x 4.6 mm) column was used for this analysis.

Liver and thigh muscle boron level were measured by using Thermo X-SERIES2 ICP-MS.

At the end of the experiments 3 broiler chicks in each subgroup were slaughtered humanly and their left and right tibias were dissected. The meat on the bones were removed physically and fat, using an ether solvent. The left tibias were then dried at 105°C for 2 h. The bones were

Table 1. *Ingredients and chemical composition of mixed feeds used in the experiment*
Tablo 1. *Araştırmada kullanılan deneme karma yemlerinin bileşimi ve kimyasal kompozisyonu*

Diet (%)	Starter Diet 0-14 days	Grower Diet 15-28 days	Finisher Diet 29-42 days
Corn	50.50	55.00	57.60
Full fat soybean	12.50	11.00	15.00
Soybean meal	29.00	26.00	19.00
Meat bone meal	3.00	2.00	3.00
Vegetable oil	1.50	3.00	3.00
Dicalcium phosphate	1.00	0.70	0.75
Limestone	1.00	1.00	0.50
DL-Methionine	0.20	0.25	0.20
L-Lysine HCL	0.10	0.05	-
L-Threonine	0.20	0.05	0.05
Vitamin-Mineral Premix[1]	0.30	0.25	0.20
Common Salt	0.30	0.30	0.30
Filling metarial (clinoptilolite)	0.40	0.40	0.40
Calculated Composition			
Metabolic energy, (MJ/kg)	12.64	13.21	13.48
Crude protein, %	23.10	20.90	20.30
Analysed Composition			
Metabolic energy, kcal/kg	12.65	13.26	13.43
Crude protein, %	23.00	21.0	20.34
Boron, ppm	23.50	21.17	18.58

[1] ***Vitamin-Mineral Premix:*** *Supplies per kg Vit. A 13.500.000 IU/kg, Vit. D_3 3.000.000 IU/kg, Vit. E 50.000 mg/kg, Vit. K_3 5.000 mg/kg, Vit. B_1 3.000 mg/kg, Vit. B_2 6.000 mg/kg, Vit. B_6 4.000 mg/kg, Vit. B_{12} 30 mg/kg, pantotenik acid 10.000 mg/kg, folic acid 1.000 mg/kg, niasin 40.000 mg/kg, biotin 50 mg/kg, BHT 10.000 mg/kg, manganese 80.000 mg/kg, iron 60.000 mg/kg, zinc 60.000 mg/kg, cupper 5.000 mg/kg, iode 1.000 mg/kg, cobalt 200 mg/kg, selenium 200 mg/kg*

ashed overnight in a furnance 610°C to determine ash [13]. Bone samples were digested in closed teflon vessels using microwave heating (BERGHOF, MWS-2, Germany), whereupon the boron content was determined through Thermo X-SERIES2 ICP-MS and Ca and P content was determined through spektrometer (Shimadzu, Tokyo, Japan, UV-1208). The right tibias were weighted and measured length. Bone weight/length index were indicated that tibia weights ratio to tibia lengths [17]. Robustness index was calculated with the formule (Robustness index= cubic surd of bone length/bone weight). Some fracture tests were measured by Zwick-Roel Z020 with 155213/2002 series number in material test apparatus with Lloyd TG 18 type A26129204 series number three point bending equipment [18].

Statistical analysis

Statistical analysis were done using SPSS programme (SPSS Inc., Chicago, IL, USA). One-way ANOVA was performed to examine differences among groups. The significance of mean differences between groups was tested by Duncan [19]. Values were given as mean±standard error. Level of significance was taken as $P<0.05$ [20].

RESULTS

The effects of supplemental dietary boric acid and ascorbic acid on body weight of broilers are shown in *Table 2*, body weight gain, feed intake and feed conversion ratio are shown in *Table 3*. Body weight ($P<0.001$) and body weight gain ($P<0.01$) increased and feed efficiency improved ($P<0.05$) greatly during the first three weeks in broilers fed supplemental diets compared with the broilers fed to control diet. Supplemental boric acid and ascorbic acid significantly increased hot carcass yield ($P<0.05$); however these effect was not shown carcass and some internal organ weights ($P>0.05$) *(Table 4)*. The effects of boric acid and ascorbic acid supplementation on serum total protein, cholesterol, triglyceride concentrations, AST and ALT avtivities are shown in *Table 5*. Seperately or as a combination, supplemental boric acid and ascorbic acid did not effect total protein, cholesterol, triglyceride concentrations and ALT activity ($P>0.05$). While ascorbic acid reduced AST activity, boric acid increased ($P<0.05$) that activity. Bone qualities are shown in *Table 6*. Bone phosphorus (P) ($P<0.05$) and boron contents ($P<0.001$)

Table 2. *Effects of boric acid and ascorbic acid on body weight, g (x ± Sx)*
Tablo 2. *Borik asit ve askorbik asidin ortalama canlı ağırlık üzerine etkileri*

Age (week)	n	Control	n	Ascorbic acid	n	Boric acid	n	Ascorbic acid + Boric acid	P
1	59	150.20^{b}±1.74	60	162.23^{a}±2.79	60	157.77^{a}±2.04	60	160.90^{a}±1.71	0.000***
2	59	402.85^{c}±6.30	59	451.75^{a}±6.26	58	431.35^{b}±5.33	60	439.10^{ab}±6.01	0.000***
3	59	804.07^{b}±14.40	59	873.64^{a}±12.45	58	854.40^{a}±9.74	60	869.67^{a}±11.96	0.000***

a, b, c; *Mean values within a row with no common superscript differ significantly, *** P<0.001*

Table 3. *Effects of boric acid and ascorbic acid on weight gain, feed intake and feed conversion ratio (x ± Sx)*
Tablo 3. *Borik asit ve askorbik asidin ortalama canlı ağırlık artışı, yem tüketimi ve yem değerlendirme sayısı üzerine etkileri*

Parameters	Control	Ascorbic acid	Boric acid	Ascorbic acid + Boric acid	P
Body weight gain, g					
0-21 days	760.18±15.06^{b}	830.19±12.64^{a}	810.57±5.84^{a}	825.88±12.44^{a}	0.005**
21-42 days	1589.10±44.09	1507.10±15.98	1559.65±52.88	1508.42±41.09	0.438
0-42 days	2349.28±35.57	2337.39±28.35	2370.22±53.53	2334.30±51.42	0.934
Feed intake, g					
0-21 days	1355.25±19.99	1354.41±17.58	1370.51±12.42	1330.28±20.82	0.492
21-42 days	3168.94±54.43	3133.77±72.63	3107.54±45.63	3035.58±56.94	0.451
0-42 days	4524.18±74.13	4488.19±70.17	4478.05±50.84	4365.87±76.64	0.430
Feed conversion ratio, g feed intake/g body weight gain					
0-21 days	1.79±0.06^{a}	1.63±0.03^{b}	1.69±0.02^{ab}	1.61±0.03^{b}	0.015*
21-42 days	2.00±0.02	2.08±0.04	2.00±0.04	2.01±0.03	0.277
0-42 days	1.93±0.00	1.92±0.02	1.89±0.02	1.87±0.02	0.162

a, b, c; *Mean values within a row with no common superscript differ significantly, ** P<0.01, * P<0.05, n=4*

Table 4. *Effects of boric acid and ascorbic acid on carcass qualities (x ± Sx)*
Tablo 4. *Borik asit ve askorbik asidin karkas sonuçları üzerine etkileri*

Parameters	Control	Ascorbic acid	Boric acid	Ascorbic acid + Boric acid	P
Slaughter weight, g	2433.75±8.70	2483.33±19.21	2440.83±19.84	2442.92±18.17	0.257
Hot carcass weight, g	1773.33±17.99	1812.08±12.74	1812.50±14.38	1790.42±20.48	0.292
Hot carcass yield, %	72.85±0.31^{b}	72.98±0.31^{b}	74.27±0.38^{a}	73.28±0.45^{ab}	0.037*
Liver weight, g	46.83±1.71	47.17±1.32	44.92±1.42	47.50±2.46	0.739
Liver yield, g/100 g BW	1.92±0.07	1.90±0.05	1.84±0.05	1.94±0.10	0.735
Heart weight, g	12.83±0.72	12.50±0.74	12.58±0.54	12.25±0.49	0.933
Heart yield, g/100 g BW	0.53±0.03	0.50±0.03	0.52±0.02	0.50±0.02	0.895
bursa Fabricius weight, g	3.50±0.29	3.36±0.43	3.55±0.39	2.92±0.40	0.625
bursa Fabricius yield, g/100 g BW	0.14±0.01	0.14±0.02	0.15±0.02	0.12±0.02	0.626
Gizzard weight, g	43.42±2.07	45.92±2.05	44.00±0.89	42.25±1.57	0.500
Gizzard yield, g/100 g BW	1.79±0.09	1.85±0.08	1.80±0.04	1.73±0.06	0.689
Abdominal fat weight, g	29.46±3.15	25.83±3.76	30.00±2.12	29.58±3.04	0.751
Abdominal fat yield, g/100 g BW	1.21±0.13	1.04±0.15	1.23±0.09	1.21±0.12	0.662
Spleen weight, g	3.83±0.39	3.25±0.31	2.92±0.19	2.92±0.29	0.118
Spleen yield, g/100 g BW	0.16±0.02	0.13±0.01	0.12±0.01	0.12±0.01	0.118

a, b; *Mean values within a row with no common superscript differ significantly, * P<0.05, n=12,* ***BW:*** *Body weight*

were significantly increased with supplemental boric acid and ascorbic acid and tibia vertical diameter was increased with each supplemental treatment group compared with the control group. However; crude ash, calcium (Ca) level and some bone strength parameters were not affected from these addititon (P>0.05). There

Table 5. *Effects of boric acid and ascorbic acid on some blood parameters (x ± Sx)*
Tablo 5. *Borik asit ve askorbik asidin bazı kan parametreleri üzerine etkileri*

Parameters	Control	Ascorbic acid	Boric acid	Ascorbic acid+Boric acid	P
Total protein, g/dl	2.98±0.23	3.06±0.18	2.75±0.08	3.23±0.21	0.348
Cholesterol, mg/dl	127.80±3.23	124.43±5.75	128.16±3.59	126.10±5.23	0.981
Triglyceride, mg/dl	44.55±3.57	42.17±2.00	47.11±2.20	44.88±1.96	0.594
AST, IU/L	420.88±30.82^{ab}	361.15±15.02^{b}	442.39±24.92^{a}	354.60±19.15^{b}	0.028*
ALT, IU/L	26.41±1.67	26.21±2.71	25.79±4.10	21.58±1.99	0.570

a, b; *Mean values within a row with no common superscript differ significantly, * P<0.05, n=8*

Table 6. *Effects of boric acid and ascorbic acid on some bone parameters (x ± Sx)*
Tablo 6. *Borik asit ve askorbik asidin bazı kemik parametreleri üzerine etkileri*

Parameters	Control	Ascorbic acid	Boric acid	Ascorbic acid + Boric acid	P
Crude ash, % [1]	55.83±0.32	55.93±0.36	56.56±0.32	56.61±0.52	0.352
Ca, % [2]	17.36±1.66	19.57±1.69	18.06±1.50	18.82±1.20	0.760
P, % [2]	8.30±0.61^{b}	9.33±0.33^{ab}	9.39±0.34^{ab}	10.40±0.39^{a}	0.016*
Ca/P [2]	2.28±0.37	2.12±0.19	1.95±0.18	1.83±0.13	0.544
B, ppm [1]	1.03±0.01^{d}	2.05±0.001^{c}	10.76±0.46^{a}	9.73±0.15^{b}	0.000***
Weight, g [1]	13.31±0.47	12.79±0.37	13.07±0.45	12.83±0.20	0.797
Length, cm [1]	9.98±0.04	9.88±0.06	10.09±0.12	9.99±0.13	0.483
Weight / Length index, mg/mm [1]	133.27±4.31	129.30±3.27	129.46±3.87	128.21±2.37	0.759
Robustness index [1]	4.22±0.04	4.23±0.03	4.29±0.05	4.27±0.03	0.551
Horizontal diameter, mm [1]	9.46±0.16	9.48±0.20	8.93±0.19	9.23±0.20	0.142
Vertical diameter, mm [1]	8.24±0.16^{a}	7.83±0.17^{ab}	7.45±0.12^{b}	7.99±0.22^{a}	0.017*
Fracture energy, mJ [1]	946.59±69.64	883.95±76.35	889.67±69.62	812.50±56.23	0.589
Fracture force, N [1]	276.27±15.04	242.30±15.81	245.88±9.32	277.38±18.39	0.204
Fracture stress (rectangle), MPa [1]	84.75±4.69	97.40±6.91	109.94±8.55	93.39±6.29	0.080
Fracture stress (ellipse), MPa [1]	56.06±3.59	62.00±4.40	69.98±5.45	59.45±4.00	0.160

a, b, c, d; *Mean values within a row with no common superscript differ significantly, *** P<0.001, * P<0.05, [1] n=12, [2] n=10*

Table 7. *Effects of boric acid and ascorbic acid on liver and plasma MDA (Malondialdehit) levels (x ± Sx)*
Tablo 7. *Borik asit ve askorbik asidin karaciğer ve plazma MDA düzeyi üzerine etkileri*

Parameters	Control	Ascorbic acid	Boric acid	Ascorbic acid + Boric acid	P
Liver, µg/g	0.59±0.03^{a}	0.43±0.02^{c}	0.65±0.02^{a}	0.51±0.02^{b}	0.000***
Plasma, µg/ml	0.23±0.02^{a}	0.10±0.01^{c}	0.18±0.02^{b}	0.11±0.01^{c}	0.000***

a, b, c; *Mean values within a row with no common superscript differ significantly, *** P<0.001, n=12*

Table 8. *Effect of boric acid and ascorbic acid on liver and thigh muscle boron concentration (x ± Sx)*
Tablo 8. *Borik asit ve askorbik asidin karaciğer ve but kası bor konsantrasyonu üzerine etkisi*

Parameters	Control	Ascorbic acid	Boric acid	Ascorbic acid + Boric acid	P
Liver, ppm	0.27±0.10^{bc}	0.17±0.08^{c}	0.92±0.17^{a}	0.70±0.23^{ab}	0.004**
Thigh muscle, ppm	2.44±0.05^{c}	1.93±0.09^{d}	4.53±0.10^{a}	3.14±0.27^{b}	0.000***

a, b, c, d; *Mean values within a row with no common superscript differ significantly, *** P<0.001, ** P<0.01, n=12*

were statistically significant differences between control and the treatment groups for liver and plasma MDA level *(Table 7)* and liver and thigh muscle boron content *(Table 8)*. Plasma MDA concentration decreased in all experimental groups compared with the control group (P<0.001), but liver MDA concentration increased only with boric acid supplementation group. Dietary boric acid supplementation increased boron concentration in liver (P<0.01) and also thigh muscle (P<0.001) compared with other groups.

DISCUSSION

In the present study, supplementation of boric acid and ascorbic acid resulted in a significant increase in body weight, body weight gain, and feed efficiency during the first 21 days of the experimental period as well as hot carcass yield in broilers. Fassani et al.[21] reported that the addition of boron (30, 60, 90, 120 and 150 ppm) and also the study [6] which was evaluated addition of 5 ppm boron resulted with increasing body weight and body weight gain in 0-21 days. Yıldız et al.[22] suggested that boron addition to diets had no negative effects about performance in 42 days and also did not affect the carcass weight. Sahin et al.[11] found that supplemental ascorbic acid (250 ppm) increased carcass yield in broilers. On the contrary, Sahin et al.[12] observed that ascorbic acid addition significantly decreased the carcass weight and yield in quails, because they studied reared under heat stress conditions.

Results of the present study about serum parameters (total protein, cholesterol, triglyceride and ALT) are in agreement with findings of severel researchers [22,24-26]. Kurtoglu et al.[27] reported that supplemental boric acid did not significantly affect the serum cholesterol concentration in broilers. Eklin et al.[28] have also shown that the addition of boron did not have any affect of cholesterol levels of animals in their trial. On the other hand Eren et al.[29] reported that the amount of serum cholesterol levels of quails decreased as the levels of B (10, 60, 120, 240 mg/kg) increased. Eren and Uyanik [30] also pointed out for laying hen's cholesterol levels were decreased by increasing levels of B (0, 5, 10, 50, 100, 200 or 400 mg/kg) addition to diets. Ascorbic acid supplementation of the present study, in terms of serum parameters data, achieved in results similar to those of boric acid. Gursu et al.[31] stated that dietary ascorbic acid did not change the serum cholosterol, trigyliceride concentration and ALT activity. Similarly, Erdogan et al.[32] reported that these parameters were not affected by ascorbic acid supplementation but AST and ALT activities were increased by addition of ascorbic acid.

In the present experiment, tibia P and boron contents increased whereas vertical diameter unit decreased with both dietary boric acid and ascorbic acid supplementation. Similar to results of the present study, Mizrak et al.[24] found that boric acid supplementation (30 ppm) increased bone P content. Mizrak et al.[33] have also reported that dietary supplementation of boric acid (5 and 25 ppm) increased bone P level. Results of the present study are in agreement with findings of severel researchers [34,35], with respect to dietary boric acid supplementation for bone boron content of poultry. In the present study, boric acid and ascorbic acid supplementation resulted in any effect for bone crude ash, Ca level and some parameters (weight, length, robustness index, fracture energy, force and stress). These findings showed positive correlation with a study [36] which evaluated addition of B (60, 120, 180, 240 and 300 ppm) to broiler diets. Konca et al.[37] have also shown that ascorbic acid supplementation did not change these bone parameters.

Surprisingly, in the present study, supplementation of boric acid decreased plasma MDA concentration similar to ascorbic acid. On the basis of research literature, it could be said that this is the first study to evaluate the effect of boric acid supplementation on plasma concentration of MDA. But there are a lot of study [11,23,38-41] which were investigated the effects of ascorbic acid supplementation to the diets on MDA level. They found that ascorbic acid decreased MDA concentration as an indicator of lipid peroxidation. In this study, the presence of boric acid in the diet resulted in increases of liver and thigh muscle boron concentrations. These findings are in agreement with findings of severel researchers [34,42,43].

The result of the current study indicate that boric acid and ascorbic acid, as dietary supplements improved body weight, body weight gain, feed efficiency and hot carcass yield. Tibia P and tibia, liver and thigh muscle boron content increased with addition of boric acid. However plasma MDA concentrations were decreased. In coclusion, the findings of the current study offer that boric acid and ascorbic acid indicated that the positive effects on performance, blood and bone parameters.

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RESEARCH ARTICLE

Financial Effects of HPAI H5N1 Cases on Backyard Poultry in the Kızılırmak Delta [1]

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Summary

This study was carried out to investigate the risk factors relating Highly Pathogen Avian Influenza H5N1 and to evaluate consumer demand and related economic losses for poultry raised in the Kızılırmak Delta. Data were obtained from 361 householders in the Kızılırmak Delta and surrounding rural areas of the delta where a Highly Pathogen Avian Influenza outbreak occurred in 2008. The total cost of the disease outbreak in the area was estimated as 501.768 TL for the 3.116 enterprises. Based on market prices for 2011, the compensation cost for the disease was estimated at 276 TL per enterprise. The time of the Highly Pathogen Avian Influenza outbreaks had happenned, correlation between the consumption of eggs and poultry families were significant at 0.05 level. In conclusion, the present study contributed to the determination of production losses due to the disease Highly Pathogen Avian Influenza, disease-related control and protection measures, estimated payments and direct economic effects.

Keywords: *HPAI, Poultry, Production loss, Risk Assessment, Kızılırmak Delta*

HPAI H5N1 Vakalarının Kızılırmak Deltasındaki Köy Tavukçuluğuna Finansal Etkileri

Özet

Bu çalışma Highly Pathogen Avian Influenza H5N1 bağlı olarak Kızılırmak Deltasının risk faktörlerinin değerlendirilmesi, kümes hayvanlarında meydana gelen ekonomik kayıpların ve tüketici talebinin incelenmesi amacıyla yapılmıştır. Çalışma verileri 2008 yılında Highly Pathogen Avian Influenza vakası görülen Kızılırmak Delta'sı civarındaki 361 haneye ilişkin verilerdir. Hastalığın alandaki toplam maliyeti 2011 yılı piyasa fiyatlarıyla 3.116 hane için 501.768 TL olarak tahmin edilmiştir. Her bir kanatlı işletmesi için hastalığın tanzim maliyeti ise 276 TL olarak hesaplanmıştır. Highly Pathogen Avian Influenza'nın görülmesi durumunda ailelerin birey sayısı ve yumurta ve kanatlı eti tüketimi arasındaki korelasyon 0.05 düzeyinde önemli bulunmuştur. Bu çalışma neticesinde, Highly Pathogen Avian Influenza'ya bağlı üretim kayıpları tahmin edilmiş ve koruma kontrol ve hastalık tanzim maliyetlerinin belirlenmesi konularında karar merkezlerine gereken destek sağlanmıştır.

Anahtar sözcükler: *HPAI, Kanatlı, Üretim kaybı, Risk değerlendirme, Kızılırmak Deltası*

INTRODUCTION

Influenza viruses in poultry and mammals such as humans, pigs, horses, cats and dogs have caused major economic losses through trade disruption but also involve animal welfare issues [1-3]. The highly pathogenic avian influenza (HPAI) A, H5N1virus infects birds and humans. It's contagious among birds, and can be fatal, especially in domestic poultry.

The first cases of the HPAI were reported in a pandemic in 1959 [4]. Since 2012, HPAI has been continuing a threat, in date of December 2012 was reported in 12 country by the World Organization for Animal Health (OIE) [5]. According to OIE, as HPAI H5N1 viruses evolve, other mammals may be infected with the virus. A total of 257 human cases of Avian Influenza were reported between 2008 and 2012 by WHO [6]. Moreover, 43% of those cases resulted in death. Several studies have reported that wild waterfowl are susceptible [2,7-9]. As the vast majority of these bird species are migratory, areas along their the migration routes are

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at risk from the HPAI H5N1 virus.

The Kızılırmak Delta, located in the central Black Sea Region of northern Turkey, is one of the most important resting and breeding areas for migratory birds. This Natural Protected Area (RAMSAR) listed wetland covers an area of 56.000 ha and contains critical habitat for globally endangered bird species [10,11]. Therefore, the delta area presents a risk in terms of Avian Influenza during every migration period. According to OIE reports, 23% of cases of the disease in the period 2005-2006 in Turkey were detected in Samsun Province, as were 2 of the 6 disease cases in 2008 [12].

The number of poultry, including commercial enterprises, in Samsun province was 2.952.982 in 2008 [13]. The number of villages in the province is 966 so the average number of poultry per village was 3.057. The estimated production losses due to the disease are difficult to estimate, as there are difficulties in determining the direct costs from the disease due to data collection problems. Nevertheless, data collection is vital for these kinds of cost analyses and the development of models for control programs.

Hence, the aim of this study was to investigate the risk factors related to HPAI H5N1 and also to evaluate consumer demand and related economic losses for poultry raised in the Kızılırmak Delta.

MATERIAL and METHODS

Data were obtained from survey questionnaires conducted face to face with householders in the Kızılırmak Delta and surrounding rural areas of the delta where a HPAI outbreak occurred in 2008.

Research Area

An outbreaks had happened the Yörükler town in Samsun, The case started January 26 2008, and it was evaluated reverse transcription - polymerase chain reaction (RT-PCR) and found as positive. The disease came to an end on 25 February 2008 [12]. This town is one of the nearest settlements to the Kızılırmak Delta and is also the nearest town to Samsun. In the present study, a total of 23 settlements, including Yörükler town, and 22 associated villages and districts, were investigated. The settlements in the area are indicated by the coordinates shown in *Fig. 1*. A total of 3.116 households, from the official records of the local administrators, was included in the study.

Sample Size

Determination of effective sample size was used this formulation.

$n = N.t^2.p.q/d^2.(N-1) + t^2.p.q$

N: the number of individual target group

n: the number of individual sampled

p: examined the frequency of the incident (the probability of occurrence)

q: nothing on examined the frequency of the incident (nothing of the probability of occurrence)

t: A certain level of significance, according to the statement of the theoretical value of t ($\alpha = 0.05$)

d: accepted that ± incidence according to the frequency of sampling error

This non-homogeneous structure with the formula of 95% confidence interval for the universe, with a sampling error of ± 0.05% of the required sample size n = 343,

Fig 1. Location of the Kızılırmak Delta and settlements

Şekil 1. Kızılırmak Deltası ve yerleşim alanları

respectively. In this context, 361 households selected randomly administered questionnaires.

Calculating value of poultry by species, Turkish Statistical Data were accepted [14].

The equation, Production Loss (Turkish Lira = TL) = A x B x C, where A is the number of households, B is the number of poultry per household, and C is the chicken price, was used to calculate poultry production losses.

Scope of Study Area and Sampling

Determining the sample size in the study area and to ensure the participation of breeders willing to work; primarily the front of the village headmen were interviewed. Three hundred and sixty one households selected by random sampling method specified in the study area were surveyed. Field work was completed in 22 days. Seventeen questions were asked in the survey. Some of them are the total number of individuals in the family, as the total number of birds and species, number of eggs and chickens purchased for consumption on families, on the fate of the assets of avian influenza in poultry were asked to hand in a time of. It is also investigated the risk area of the disease, according to an emergency national action plan [15].

In the collection of survey data, it was important to ensure that the questions were simple and clear due to the low level of education of people involved in the present study. In addition, some restrictions and limitations were assumed in the data, i) all poultry meat consumed was considered fresh whole chicken, ii) consumed eggs were considered chicken eggs and iii) every family unit was considered as a commercial farm. Accordingly, the questionnaire in the survey determined family consumption habits, farm structure and responses to the presence of the disease.

The data set obtained from the surveys was analysed with the SPSS. Evaluating of the data descriptive statistical methods (number, percentage, mean, standard deviation, maximum and minimum) were used. Kolmogorov-Smirnov test was applied to the normal distribution of the study variables. Normally distribution were not detected ($P<0.05$). Spearman's Correlation test was used for the analysis of the nonparametric methods. The findings of the 95% confidence interval was evaluated at the level of significance of 5%.

RESULTS

Cases of HPAI reported by OIE were examined in the present study. A total of 23 locations *(Fig. 1)* were investigated. According to records officially obtained from local authorities, there were 3.116 households in the study area and 361 of them were sampled. The average number of households per village was 135 and average family size was 5. The present study in the Kızılırmak Delta area were evaluated in terms of risk. For this purpose, it is used interviews with local mayors, field observations and data from the literature *(Table 1)*.

Descriptive statistics of backyard poultry enterprises were studied *(Table 2)*.

The most common species of poultry is chicken and less common type of poultry is other in backyard poultry enterprises *(Table 2)*.

The average number of poultry per household enter-

Table 1. *Risk assessment of the Kızılırmak Delta*
Tablo 1. *Kızılırmak Deltası'nın risk değerlendirilmesi*

Risk Criteria	**Risk Assessment**
Migration routes	Yes [10,11]
Natural Parks and Lakes	Yes [10,11]
Wildlife and Hunting	Possible [10,11]
Stagnant water contaminated with bird droppings and streams	Yes (field observation)
Village poultry animals	Yes (field observation)
Movements of infected birds	Possible (field observation)
Live birds market	No (field observation)
Human movements of infected areas	Possible (field observation)
Infected live poultry or poultry products imports legally or illegally	No [15]
To be significant risk criteria are not included in the emergency action plan	Risk assessment
The transportation of feed, pharmaceuticals, food additives, tools and equipment and materials	Yes (field observation)
Animal movements	Yes (field observation)
Tourism	Yes (field observation)
Poultry products processing unit., outlets and consumption unit of product markets	Yes (field observation)

prise was 23, comprising 89.0% chickens, 2.4% goose, 7.8% ducks, 0.5% turkeys and 0.3% other poultry breeds *(Table 3)*.

In the event of the occurrence of the disease HPAI, poultry production by small sized farming enterprises is eliminated. For that reason, farmers who normally meet their poultry needs out of their own resources are forced to purchase their needs. In considering HPAI outbreaks, the annual consumption of poultry by farmers is relevant to the determination of economic losses. Therefore, in the present study, demand for poultry products by farmers during the period of the HPAI outbreak was determined. Accordingly, the demand for eggs by households ranged from 210.9 to 1621.8 and average number of poultry carcasses purchased per household was 12.29 (*Table 4* and *Table 5*).

Table 2. *Descriptive statistics of the number of households with poultry*
Tablo 2. *Kanatlı yetiştiriciliği yapan hanelere ilişkin tanımlayıcı istatistikler*

Type of Poultry	N	Minimum	Maximum	Mean	Std. Deviation
Chicken	339	1	120	21.72	15.193
Turkey	16	1	6	2.75	1.571
Duck	112	2	20	5.74	3.541
Goose	23	2	100	8.87	20.017
Other	5	2	9	5.40	3.362
Total	341	1	135	24.52	17.135

Non-parametric tests Spearman's correlation test was used to study data to investigate the relationship between the demand for eggs with the number of individual households. The correlation coefficient "r" was -0.026. There is a weak negative relationship between the number of family size and purchase of eggs. It was used to study data to investigate the relationship between the demand for chicken with the number of individual households. The correlation coefficient "r" was -0.048. Relationship is still negative but stronger the demand of eggs.

Family's consumption of eggs's and chicken meat's the correlation coefficient is -0.110 and, 131, respectively. Correlations are significant at 0.05. It's explained that people react to they eat eggs less but eat chicken meat more when the outbreak had happened.

Payment of compensation was carried out after legislated process of culling was completed in the affected area. However, during the outbreak of the disease, 38% of poultry farmers reported that they hadn't received their compensation due to the lack of information about the process, and 42% of farmers reported that they continued breeding poultry. Furthermore, 20% of the farmers reported that they had slaughtered their poultry and consumed them during this period. After factoring in the information above, the total cost of the disease outbreak in the area was calculated at 501.768 TL for the 3.116 enterprises. Based on market prices for 2011, the compensation cost for the disease was estimated at 276 TL per enterprise *(Table 6)*.

Table 3. *The number of species of poultry, poultry prices and the financial value of backyard poultry*
Tablo 3. *Türler itibariyle kanatlı sayıları, kanatlı fiyatları ve köy tavukçuluğunun finansal değeri*

Type	The Number of Examined Poultry	The Percentage of Examined Poultry (%)	Unit Price (TL)	The Total Price of the Examined Poultry (TL)	Total Poultry Number	Total Price (TL)*
Chicken	7363	89.0	12	88.356	63554	762.648
Turkey	44	0.5	34	1.496	380	12.920
Duck	643	7.8	12	7.716	5550	66.600
Goose	204	2.4	34	6.936	1761	59.874
Other	27	0.3	15	405	233	3.495
Total	8281	100		104.909	71478	905.537

** Financial value of 3116 household backyard poultry (TL)*

Table 4. *The number of purchased eggs for 361 housholds*
Tablo 4. *361 hanenin satın aldığı yumurta sayıları*

Number of Purchased Eggs	Household Number	Lower Limit of the Annual Number of Purchased Eggs (Calculation-Number)	Upper Limit of the Annual Number of Purchased Eggs (Calculation-Number)
None	199	-	-
1-10	70	3.640	36.400
11-20	42	24.024	480.480
21-30	43	46.956	67.080
31 and above *	7	1.519	1.519
Total	361	76.139	585.479

Table 5. *The number of purchased chickens for 361 housholds*
Tablo 5. *361 hanenin satın aldığı kanatlı sayısı*

The Monthly Number of Purchased Chicken	Household Number	The Annual Number of Puchased Chicken
None	130	-
1 pieces	132	1584
2 pieces	59	1416
3 piece and above	40	1440
Total	361	4440

very important in the control and in the preparation of management protocols. However, the lack of quantitative data for village-type poultry production makes it difficult to determine appropriate measures. That is one reason why the present study was undertaken.

Estimating poultry numbers in the affected area is important for implementing control measures for the disease and providing compensation. Based on the number of commercial and village poultry farms and the number of affected villages, the average number of poultry per village was 3.057 and mean number of households for the 23 villages was 135. Therefore, according of the results of the present study, the reported number of poultry in the study area may be lower than determined in the previous studies. Underestimating of poultry number in those case can lead to the failure of intervention programs.

Table 6.*The financial value of the estimated production losses*
Tablo 6. *Üretim kayıplarının tahmini finansal değeri*

Effects	N	Production Loss (TL) for 341 Households	N (Calculation)	Production Loss (TL) for 3116 Households
I have culled my poultry	131	36.156	1197	330.372
I have slaughtered my poultry and eaten	68	18.768	621	171.396
I continued to produce poultry	142		1297	
Total	341	54.924	3116	501.7680

DISCUSSION

The Kızılırmak Delta area is protected by RAMSAR site status [10,11]. The delta is also important for the livelihood of persons who live there. Poultry and other livestock such as dairy animals are two of the main sources of agricultural income for households. Farmers in the area use low technology in poultry production due to small scale of their enterprises. Rushton [16] reported that poultry production in rural areas was carried out on the principle of low input-low output and noted that these systems are extremely inefficient in terms of investment and disease control; poultry management and breeding requires huge investments for modern production systems that maximise productivity and minimise disease risk. Furthermore, low technology poultry farms constitute a risk to modern enterprises [16].

The average poultry size were found 23 in this study. By comparison, flock size in backyard poultry enterprises in France ranged from 15 to 20 [17] and in Africa ranged from 10 to 20 [3,18].

HPAI is a disease that requires the implementation of varying degrees of biosecurity measures across all components of the poultry sector and those measures are interpreted in economic terms [19]. In that context, poultryhousing and care were poor in the study area. Overall, housing and biosecurity for HPAI management should be considered together. Therefore, as far as compensation for HPAI is concerned, its investment in the redesign of poultry housing for improved biosecurity in the outbreak area could be considered more economic. Knowledge of the financial costs of animal diseases is

In the present study, the total cost of the disease outbreak in the area was calculated at TL 501.768 for the 3.116 enterprises. Based on market prices for 2011, the compensation cost for the disease was estimated at TL 276 per enterprise.

The study investigated the output of eggs and chicken meat consumption trends. Correlations are significant at 0.05. Negative correlation coefficient of egg consumption in households due to the disease. Positive correlation coefficient of chicken meat consumption in households due to the disease. Increasing consumption of eggs in the absence of disease, and the disease is called reduced. Depending on the cutting poultry in this period so much, poultry meat consumption seems to be increased.

Considering of the criteria of animal welfare for poultry, concept of poultry production in rural farming type is gradually spread in European Union [20]. However, Turkey has an adventage in this context, as 35% of the total population of Turkey lives in rural areas, village enterprises play a very important role in poultry production. Therefore, in the management of epidemic diseases, particularly avian influenza, the re-establishment of poultry farming and Broiler sector after disease outbreaks is of great importance [21].

Turkey's poultry sector affected the HPAI outbreaks in

the period of 2005-2006. Several studies have reported on economic consequens of the disease and disease control applications [22-24]. In these studies, the emphasis is on the importance of the sector and reveals that the chicken farming is necessiate in the countryside. On the other hand, the lack of information in terms of human health should be solved [25].

The present study contributed to the determination of production losses due to the disease HPAI, disease-related control and protection measures, estimated payments and direct economic effects. If the need arises for vaccination, correctly estimating the number of affected poultry is essential for the determination of the vaccine costs. Furthermore, knowledge of the disease process and its management is vital in the poultry sector in the context of food security.

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RESEARCH ARTICLE

Anadolu Mandalarının Değişik Metotlara Göre Tahmin Edilen Süt Verimleri Üzerine Bazı Çevresel Faktörlerin Etkilerinin Belirlenmesi

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Özet

Bu çalışma, Tokat ili ve ilçelerinde yetiştirilen Anadolu mandalarının farklı metotlar ile belirlenen süt verimlerine ait düzeyler ve bunlar üzerindeki bazı çevre faktörlerinin etkilerinin belirlenmesi amacıyla yapılmıştır. Çalışmada etkisi ölçülebilir çevre faktörleri olarak bölge, malaklama yaşı ve malaklama mevsimin etkileri üzerinde durulmuştur. Verilerin analizlerinde, çevresel faktörlerin etki paylarının belirlenmesinde minimum kareler metodu ve bunların karşılaştırılmasında DUNCAN çoklu karşılaştırma testi kullanılmıştır. Anadolu mandalarının Hollanda, İsveç, Vogel, Trapez I ve Trapez II metotlarına göre tespit edilen laktasyon süt verimi sırası ile 734.0±16.0 kg, 735.4±16.0 kg, 761.4±16.4 kg, 657.7±13.7 kg ve 654.7±13.5 kg olarak saptanmıştır. Ayrıca, laktasyon süresi ve günlük ortalama süt verimi sırası ile 146.55±1.79 gün, 5.21±0.096 kg olarak belirlenmiştir. Araştırma sonucunda, tahmin edilen süt verimleri ve laktasyon süresi üzerine bölge, malaklama yaşı ve malaklama mevsiminin etkisinin önemli olduğu bulunmuştur (P<0.01).

Anahtar sözcükler: *Anadolu mandası, Süt verimi, Çevre faktörleri*

Some Environmental Factors Effect on Milk Yield Estimated with Different Methods in Anatolian Buffaloes

Summary

The objective of this study was to determine the effects of some environmental factors on milk yield levels that were estimated with different methods in Anatolian Buffaloes raised in Tokat Province in Turkey. The effects of region, calving age and calving season were analysed in terms of measurable environmental factors. The data was statistically analyzed by means of the least square method for the determination of the effects of environmental factors and by DUNCAN multiple range test. In this study lactation milk yield estimated by Holland, Sweden, Vogel, Trapezoid I and Trapezoid II methods were 734.0±16.0 kg, 735.4±16.0 kg, 761.4±16.4 kg, 657.7±13.7 kg and 654.7±13.5 kg, respectively. Also, lactation length and daily milk yield were determined 146.55±1.79 days, 5.21±0.096 kg, respectively. The effects of regions, calving age and calving season on lactation milk yields and lactation length were found as statistically significant (P<0.01).

Keywords: *Anatolian buffaloes, Milk yield, Environmental factors*

GİRİŞ

Çevre koşullarına adaptasyon kabiliyeti yüksek, hastalıklara karşı dayanıklı, kanaatkâr bir tür olan mandanın et, süt ve deri gibi çeşitli verimlerinden insanlar uzun yıllar yararlanmaktadır. Son yirmi yıllık süreçte Türkiye'de manda varlığında ciddi oranda (%73.33) azalma olmuştur [1,2]. Genellikle ekstansif yetiştiricilik için uygun bir mera hayvanı olan manda, kaba yemler içerisinde kalitesi düşük, selüloz oranı yüksek olan ucuz yemleri tüketerek, hayvansal ürüne dönüştürür [3,4]. Türkiye sahip olduğu ekolojik şartlar itibarı ile manda yetiştiriciliği için uygun bir ülkedir. Bataklık ve sazlık alanlarda, ucuz kaba yemlerin bulunduğu bölgelerde manda yetiştiriciliği oldukça ekonomiktir. Türkiye'de manda İstanbul, Afyon, Samsun, Tokat, Sinop, Çorum ve Amasya illerinde yaygın olarak yetiştirilmektedir. Manda yetiştiriciliği dar gelirli ailelerin gelir kaynaklarından bir tanesidir [3-5]. Günümüzde Türkiye'de yetiştirilen mandalar, nehir mandalarının bir alt grubu olan Akdeniz mandalarından köken almakta ve Anadolu

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mandası olarak adlandırılmaktadırlar [6]. Anadolu mandalarının laktasyon süresinin ortalama 232 gün (112-449 gün) olduğu; laktasyon süt verimlerinin; ırk, bakım-besleme, yaş, laktasyon ve kuruda kalma süresi gibi çeşitli faktörlere bağlı olmak üzere, 925 kg olduğu bildirilmiştir [4]. Mandalarda genel olarak en yüksek süt veriminin 6-7 yaş arasında, yani 3. laktasyonda gerçekleştiği bildirilmektedir [7,8]. Özenç ve ark.[9] mandaların laktasyon süt verimlerinin 350-1580 kg arasında değiştiğini ortalama 943.2 kg olduğunu belirlemişlerdir. Afyon Mandacılık Araştırma Enstitüsünde yetiştirilen mandaların 1. laktasyon süt verimlerinin 227-1443 kg arasında değiştiği, ortalama 813 kg olduğu bildirilmiştir [7]. Mandaların laktasyon süt verimlerinin Avrupa'da 1.200 kg, Türkiye'de ise 600-800 kg arasında değiştiği Kreul ve Sarıcan [10] tarafından bildirilmiştir. Laktasyon süt verimi ile yakından ilgili olan laktasyon süresi ortalaması Afyon mandacılık araştırma enstitüsünde yerli mandalarda 220 gün, melezlerde 225 gün olarak saptanmıştır [11]. İlaslan ve ark.[12] mandalarda ortalama laktasyon süresi uzunluğunu 224 gün olarak belirlemiştir.

Bu araştırma, Tokat ilinde yetiştirici koşullarında yetiştirilen Anadolu mandalarının Hollanda, İsveç, Vogel, Trapez I ve Trapez II metotları ile belirlenen süt verimleri ve bu verimler üzerine bazı çevre faktörlerinin etkilerinin belirlenmesi amacıyla yapılmıştır.

MATERYAL ve METOT

Araştırma materyalini Tokat ili ve ilçelerinde 89 farklı işletmede yetiştirilen sağmal Anadolu mandalarına ait 452 verim kaydı oluşturmuştur. Araştırmada, Tarımsal Araştırmalar ve Politikalar Genel Müdürlüğü tarafından desteklenen Halk Elinde Manda Islahı Ülkesel projesi kapsamında çalıştırılan teknik elemanlar tarafından 2011-2012 yıllarında süt kontrol günlerinde tespit edilen veriler değerlendirilmiştir. Yetiştiriciler özellikle mer'a döneminde mandalara genellikle ek yemleme uygulamamakta, ancak kış aylarında elde mevcut yemlere göre (saman, kuru yonca otu, silaj vb) ek yemleme yapmaktadırlar. Bölgede mevsim şartlarının otlatma için uygun olduğu günlerde sabah sağımından sonra meraya çıkartılmaktadır. Bölgedeki yetiştiricilerin tümü sağımı el ile yapmaktadırlar. En az ilk 4 kontrol verimi bilinen mandalara ait laktasyon süt verimleri İsveç, Hollanda, Vogel, Trapez I, Trapez II metotları kullanılarak hesaplanmıştır [13-15]. İncelenen özelliklere etki eden çevresel faktörlerin belirlenmesinde SPSS 17.0 [16] paket programı, önemli bulunan faktörlerin alt gruplarının karşılaştırılmasında Duncan [17] çoklu karşılaştırma testi kullanılmıştır. Bu çalışmada kullanılan verilerin bölgelere göre dağılımları *Tablo 1*'de özetlenmiştir.

Tablo 1. *Verilerin bölgelere göre dağılımı*
Table 1. *The regional distribution of the data*

Bölgeler	N	İşletme Sayısı
Erbaa	118	25
Merkez	72	14
Pazar	96	22
Turhal	166	28
Toplam	452	89

Süt Veriminin Belirlenmesi

Mandaların süt kontrolleri paslanmaz süt ölçeği kullanılarak birer aylık aralıklar ile tespit edilmiştir. Litre olarak belirlenen süt verimleri, kontrol günlerinde alınan süt örneklerinin yoğunluğu (1029 g/cm^3) kullanılarak kilograma dönüştürülmüştür. Tespit edilen aylık süt verimleri kayıt altına alınmıştır. Laktasyon süt verimleri Hollanda, İsveç, Voegel ve Trapez I ve Trapez II yöntemlerinden yararlanılarak tespit edilmiştir. Laktasyon süt verimlerinin tahmin edilmesi için sırasıyla; 30 (X_1), 60 (X_2), 90 (X_3), 120 (X_4), 150 (X_5), 180 (X_6), 210 (X_7), 240 (X_8) günlük kısmi laktasyon verimlerinden yararlanılmıştır.

Laktasyon süresi, günlük ortalama süt verimi ve laktasyon süt verimleri aşağıda verilen eşitlikler yardımı ile hesaplanmıştır.

Günlük Ortalama Süt Verimi

$$GOSV = (\sum_{i=1}^{n} k_i / n)$$

Hollanda Metodu

Belirli aralıklar ile yapılan kontrol sağımlarında belirlenen süt verimleri toplanmıştır. Kontrol gününde tespit edilen verimlerin ortalaması, laktasyon süresince günlük ortalama süt verimi olarak kabul edilmiş ve bulunan günlük ortalama süt verimi laktasyon süresi (LS) ile çarpılarak laktasyon süt verimi hesaplanmıştır. Ancak burada laktasyon süresi sabit bir değer olarak alınmış veya tahmin edilmiştir. Laktasyon süresinin tahmininde aşağıdaki eşitlik kullanılmıştır.

$$LS = na - (a/2 - A)$$

Belirlenen laktasyon süresi ile aşağıdaki eşitlik yardımıyla laktasyon süt verimi tahmin edilmiştir [18,19];

$$SV_H = (\sum_{i=1}^{n} k_i / n)L$$

İsveç Metodu

Bu metotta belirli kontrol aralıkları tespit edilip, kontrol gününün, kontrol periyodunun tam ortasına isabet ettiği varsayılmıştır. Kontrol günlerinde tespit edilen süt verimleri toplanmış ve dönem içerisindeki gün sayısı ile çarpılarak o periyot için süt verimi belirlenmiştir. Her periyot için ayrı ayrı yapılan kontroller toplanarak laktasyon süt verimi

hesaplanmıştır. Bu yöntemle laktasyon süt verimi aşağıdaki eşitlik yardımı ile belirlenmiştir [18,19];

$$SV_i = a\sum_{i=1}^{n} k_i + (a/2 - A)k_1$$

a_i: Dönem uzunluğu (gün), A: doğum ile ilk kontrol arası süre (gün), k_1: 1. kontrolde saptanan verim (kg), k_i : i. kontrolde saptanan verim (kg),

Vogel Metodu

Bu metotta tüm kontrol günlerinde saptanan süt verimleri toplanıp, kontrol günlerinde tespit edilen verimlerin toplamı, kontrol aralığında geçen süre ile çarpılarak laktasyon süt verimi hesaplanmıştır. Wogel metodu ile laktasyon süt veriminin belirlenmesinde aşağıdaki eşitlik kullanılmıştır [18,19].

$$SV_V = a\sum_{i=1}^{n} k_i$$

Trapez Metotları

İki kontrol günü arasındaki süre, bir kontrol aralığı olarak ele alınmıştır. Her kontrol aralığının sonundaki ve başındaki kontrol günlerinde belirlenen süt verimlerinin ortalaması alınmış ve o kontrol aralığı için ortalama süt verimi bulunmuştur. Bulunan bu değer kontrol günüdeki süt verimi ile çarpılarak o kontrol aralığına ait süt verimi tespit edilmiştir. Kontrol dönemlerinde elde edilen verimlerin toplamı ile laktasyon süt verimi saptanmıştır. İlk kontrol gününe kadar olan süt verimi ise; ilk kontrolde elde edilen verim ile doğum ile ilk kontrol arasındaki süre ile çapılarak belirlenmiştir. Aynı şekilde son kontrol süt verimi ise son kontrol ve kuruya çıktığı tarih arasındaki süre ile çarpılarak tespit edilmiştir. Bulunan bu verimler toplam süt verimine ilave edilmiştir [18,19].

- Trapez I Metodu

$$SV_T = k_1 A + (\sum_{i=1}^{n} k_1 + k_{i+1}/2)a_i$$

- Trapez II Metodu

Bu metotta, Trapez I'den farklı olarak son kontrol verimi son kontrol ve kuruya çıktığı tarih arasındaki süre ile çarpılarak belirlenen süt verimi toplam süt verimine eklenmemiştir.

$$SV_T = k_1 A + (\sum_{i=1}^{n} k_1/2)a_i$$

Laktasyon süresi, günlük ortalama süt verimi ve laktasyon süt verimi üzerine çeşitli çevre faktörlerinin etkisi aşağıdaki model kullanılarak incelenmiştir.

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

Y_{ijkl}: i. yaş, j. mevsim, k. bölgede yetiştirilen mandanın üzerinde durulan özelliğine ait gözlem değeri,

μ: popülasyon ortalaması,

a_i: malaklama yaşının etkisi (3, 4, 5, 6, 7, 8, 9≤),

b_j: malaklama mevsiminin etkisi (Kış, İlkbahar, Yaz, Sonbahar),

c_k: bölgelerin etkisi (Tokat Merkez, Erbaa, Turhal, Pazar),

e_{ijkl} : tesadüfi çevre faktörlerinin etkisi (hata, $\delta^2 e$),

BULGULAR

Süt Verim Özellikleri

Bu çalışmada Anadolu mandalarının farklı metotlar ile tespit edilen süt verimleri, laktasyon süresi ve günlük ortalama süt verimi üzerine bazı çevre faktörlerinin etkisi incelenmiştir. En yüksek süt verimi Vogel yöntemi ile 761.4±16.4 kg olarak belirlenmiş, Vogel yöntemini sırası ile Hollanda (735.4±16.0 kg), İsveç (734.0±16.0 kg), Trapez I (657.7±13.7 kg) ve Trapez II (654.7±13.5 kg) metotları izlemiştir *(Tablo 2, Tablo 3)*.

Mandaların yetiştirildiği bölgelerin süt verimi üzerine etkisinin önemli (P<0.05) olduğu belirlenmiştir. Anadolu mandalarında en yüksek süt verimi ve en uzun laktayon süresi Turhal ve Pazar bölgelerinde elde edilirken, bu bölgeleri merkez ilçe ve Erbaa bölgesi izlemiştir. Hollanda, İsveç, Vogel, Trapez I ve Trapez II yöntemleri ile tespit edilen süt verimlerine ait en küçük kareler ortalamaları *Tablo 2* ve *Tablo 3*'te özetlenmiştir.

TARTIŞMA ve SONUÇ

Farklı yöntemlere göre belirlenen süt verimleri üzerine malaklama yaşı, malaklama mevsimi ve bölge etkilerinin önemli olduğu saptanmıştır (P<0.05). Anadolu mandalarının laktasyon süt verimlerinin; ırk, bakım besleme, yaş, laktasyon ve kuruda kalma süresi gibi çeşitli faktörlere bağlı olmak üzere değiştiği ve ortalama 925 kg olduğu bildirilmiştir [4]. Özenç ve ark.[9] tarafından mandaların laktasyon süt verimlerinin 350-1.580 kg arasında değiştiği ve ortalama 943.2 kg olduğu bildirilmiştir. Ayrıca, Afyon Mandacılık Araştırma Enstitüsünde yapılan bir çalışmada [7] mandaların 1. laktasyon süt verimlerinin 227-1.443 kg arasında değiştiği ve ortalama 813 kg olduğu tespit edilmiştir. Diğer taraftan Türkiye'de yetiştirilen mandaların laktasyon süt verimlerinin 600-800 kg arasında değiştiği Kreul ve Sarıcan [10] tarafından bildirilmiştir. Pakistan'da yapılan bir araştırmada [20], Nili Ravi ırkı mandaların laktasyon süresi ve laktasyon süt verimi 273.3±52.8 gün ve 1831.6±530.9 L olarak belirlenmiştir. Şekerden [21] tarafından yapılan bir çalışmada Anadolu

Tablo 2. *İsveç, Hollanda, Vogel, Trapez I ve Trapez II yöntemlerine göre belirlenen laktasyon verimleri ile ilgili en küçük kareler ortalaması (X±Sx)*
Table 2. *Least squares means for lactation milk yields estimated by Holland, Sweden, Vogel, Trapezoid I methods (X±Sx)*

Faktörler	N	İsveç X±Sx	Holanda X±Sx	Vogel X±Sx	Trapez I X±Sx
Genel	452	734.0±16.0	735.4±16.0	761.4±16.4	657.7±13.7
Bölgeler		**	**	**	**
Erbaa	118	649.0±36.68 ^{b}	649.4±36.56 ^{b}	672.4±37.45 ^{a}	577.2±31.39 ^{b}
Merkez	72	736.7±43.74 ^{ab}	738.0±43.60 ^{ab}	763.5±44.67 ^{ab}	650.1±37.44 ^{ab}
Pazar	96	754.7±40.47 ^{a}	755.4±40.34 ^{a}	778.9±41.33 ^{a}	678.6±34.64 ^{a}
Turhal	166	754.6±35.56 ^{a}	753.6±35.45 ^{a}	783.1±36.31 ^{a}	672.2±30.44 ^{a}
Malaklama Yaşı (Yıl)		**	**	**	**
3	63	698.8±46.18 ^{ab}	700.0±46.04 ^{ab}	722.9±47.16 ^{ab}	620.5±39.53 ^{abc}
4	61	656.1±48.49 ^{b}	657.2±48.34 ^{b}	680.3±49.52 ^{ab}	581.6±41.50 ^{bc}
5	56	619.2±48.38 ^{b}	619.0±48.23 ^{b}	640.2±49.41 ^{b}	555.8±41.41 ^{c}
6	61	816.1±46.89 ^{a}	815.3±46.74 ^{a}	843.8±47.88 ^{b}	717.3±40.13 ^{a}
7	52	736.7±51.47 ^{ab}	736.3±51.32 ^{ab}	763.3±52.57 ^{ab}	665.4±44.06 ^{ab}
8	48	741.4±51.30 ^{ab}	742.1±51.14 ^{ab}	767.8±52.39 ^{ab}	664.4±43.91 ^{ab}
≥9	111	797.8±37.31 ^{a}	798.8±37.20 ^{a}	827.3±38.10 ^{a}	706.9±31.94 ^{a}
Malaklama Mevsimi		**	**	**	**
Kış	136	789.8±29.14 ^{ab}	791.5±29.05 ^{ab}	816.8±29.76 ^{ab}	697.9±24.94 ^{a}
İlkbahar	258	721.7±21.72 ^{b}	723.7±21.65 ^{b}	749.0±22.18 ^{b}	650.6±18.59 ^{a}
Yaz	44	491.6±49.99 ^{c}	491.8±46.84 ^{c}	515.1±51.05 ^{c}	463.9±42.79 ^{b}
Sonbahar	14	891.9±87.02 ^{a}	889.5±86.75 ^{a}	916.9±88.86 ^{a}	765.8±74.48 ^{a}

*** P<0.05, * P>0.05, ^{a,c} aynı sütunda farklı harfler ile gösterilen ortalamalar arasındaki farklılıklar istatistiki olarak önemlidir*

Tablo 3*. Laktasyon süresi (LS), Trapez II yöntemlerine göre belirlenen laktasyon verimleri ile ilgili en küçük kareler ortalaması (X±Sx)*
Table 3. *Least squares means for lactation milk yields estimated by Trapezoid II methods, lactation length and daily milk yield (X±Sx)*

Faktörler	N	LS (gün) X±Sx	Trapez II X±Sx	GOSV (kg) X±Sx
Genel	452	146.55±1.79	654.7±13.5	5.2110±0.096
Bölgeler		**	**	**
Erbaa	118	141.7±3.753 ^{c}	572.7±30.98 ^{a}	4.60±0.219 ^{b}
Merkez	72	151.0±4.475 ^{b}	647.0±36.94 ^{ab}	5.09±0.261 ^{b}
Pazar	96	165.5±4.141 ^{a}	671.5±34.18 ^{a}	4.70±0.242 ^{b}
Turhal	166	134.9±3.639 ^{c}	667.9±30.03 ^{a}	5.88±0.212 ^{a}
Malaklama Yaşı (Yıl)		**	**	**
3	63	156.0±4.725 ^{c}	615.3±39.00 ^{abc}	4.587±0.2761 ^{bc}
4	61	149.3±4.962 ^{ab}	575.5±40.95 ^{bc}	4.615±0.2899 ^{bc}
5	56	147.3±4.951 ^{ab}	551.2±40.86 ^{c}	4.359±0.2893 ^{c}
6	61	148.9±4.798 ^{ab}	711.6±39.60 ^{a}	5.699±0.2804 ^{a}
7	52	142.3±5.267 ^{b}	660.6±43.48 ^{ab}	5.411±0.3078 ^{a}
8	48	146.8±5.250 ^{ab}	660.1±43.33 ^{ab}	5.137±0.3068 ^{ab}
≥9	111	147.4±3.818 ^{ab}	704.2±31.51 ^{a}	5.696±0.2231 ^{a}
Malaklama Mevsimi		**	**	*
Kış	136	160.7±2.982 ^{ab}	692.3±24.61 ^{a}	5.062±0.1742
İlkbahar	258	148.0±2.222 ^{b}	648.6±18.34 ^{a}	5.072±0.1299
Yaz	44	110.3±5.116 ^{c}	463.0±42.22 ^{b}	4.672±0.2989
Sonbahar	14	174.1±8.904 ^{a}	755.4±73.495 ^{a}	5.482±0.5203

*** P<0.05, * P>0.05 ^{a,c} aynı sütunda farklı harfler ile gösterilen ortalamalar arasındaki farklılıklar istatistiki olarak önemlidir*

mandalarının laktasyon süt verimlerinin 1300±39.27 L olduğu belirlenmiştir.

Araştırmada Anadolu mandalarının günlük ortalama süt verimi 5.21±0.096 kg olarak tespit edilmiştir. Araştırma bulgusu Gongaze ve Lorenzo [22] (5.210 kg); Penchev ve ark.'nın [23] bulguları ile (5.61±0.043 kg) uyumlu bulunmuştur. Jorge ve ark.[24] Murrah ırkı mandalarda günlük ortalama süt verimini 4.07±1.3 kg olarak belirlemiştir. Diğer taraftan Bansal ve ark.[25] mandalarda günlük ortalama süt verimini 7.88±2.56 kg olarak saptamıştır. Anadolu mandalarının günlük süt verimleri ise 5.7±0.306 L olarak bildirilmiştir [21]. Şekerden ve ark.[26] Anadolu mandalarının günlük ortalama süt verimlerinin ise 2.2 ile 3.5 L arasında değiştiğini bildirmişlerdir. Araştırma bulgusu Şekerden ve ark.'nın [26] Hatay İli Kırıkhan ilçesi Ilıkpınar Köyü'nde yetiştirilen mandalar için belirlediği değerlerden yüksek bulunmuştur.

Bu çalışmada, ortalama laktasyon süresi 146.55±1.79 gün olarak tespit edilmiştir *(Tablo 3)*. Ayrıca, Anadolu mandalarında laktasyon süresi uzunluğunun 112-449 gün arasında değiştiği bildirilmiştir [4]. Laktasyon süt verimi ile ilgili olan laktasyon süresi ortalaması Afyon Mandacılık Araştırma Enstitüsü'nde yerli mandalarda 220 gün, melezlerde 225 gün olarak saptanmıştır [11]. İlaslan ve ark.[12] mandalarda ortalama laktasyon süresini 224 gün olarak belirlemiştir. Bu araştırmada en uzun laktasyon süresi 3 yaşında yani 1. Laktasyonda olan mandalarda elde edilmiştir. Benzer şekilde Nili Ravi ırkı mandalarda yapılan bir çalışmada da [27], en uzun laktasyon süresi 1. laktasyonunda olan mandalarda tespit edilmiştir.

Bu çalışmada, laktasyon süresinin uzun olduğu dönemlerde elde edilen süt verimlerinin, laktasyon süresinin kısa olduğu dönemlere göre yüksek olduğu görülmektedir *(Tablo 2, Tablo 3)*. Süt verimleri ve laktasyon süresi arasındaki korelasyonların yüksek ve önemli olduğu belirlenmiştir. Nitekim laktasyon süresi ve süt verimi arasındaki korelasyonların mandalarda ve sığırlarda yüksek ve önemli olduğu bir çok araştırıcı tarafından bildirilmiştir [28-30].

Çevre Faktörleri

Kış mevsiminde malaklayan mandaların süt verimlerinin yaz mevsiminde malaklayanlardan, sonbaharda malaklayanların ise, ilkbaharda malaklayanlardan daha fazla süt verimine sahip oldukları görülmektedir *(Tablo 2)*. Kış aylarında malaklayan mandaların süt verimlerinin diğer mevsimlerde malaklayanlardan yüksek olmasında, mandaların mevsimler itibarı ile oluşan kritik hava sıcaklıklarından etkilenmelerinin, içeride yemlenmelerinin ve daha uzun süre sağılmalarının etkisinin olduğu düşünülebilir. Nitekim, yörede üretilen manda sütü gerek çiğ süt olarak gerekse de çeşitli süt ürünlerine dönüştürülmek sureti ile pazarlanmaktadır. Bu yüzden kış aylarında sürekli olarak entansif koşullarda yetiştirilen mandaların bakım beslenmesine özen gösterilmektedir. Ayrıca, kış mevsiminde malaklayan mandaların, yaz mevsiminde malaklayanlardan, sonbahar mevsiminde malaklayanların ise ilkbahar mevsiminde malaklayanlardan daha uzun laktasyon süresine sahip oldukları görülmektedir *(Tablo 3)*. Bu durum kış mevsiminde ve sonbahar mevsiminde süt verimlerinin yüksek olmasında etkili olmuştur.

Yaz mevsiminde doğuran mandaların süt verimlerinin diğer mevsimlere göre düşük olmasında, yaz mevsimindeki hava sıcaklığının diğer mevsimlerde gözlenen sıcaklıktan daha fazla olmasının, bu dönemde meraların vejetasyonunun, yem temininde karşılaşılan güçlüklerin, sıcaklık stresinin etkisinin olduğu söylenebilir. Ayrıca, yaz mevsimi için belirlenen laktasyon süresi uzunluğu da, süt veriminin diğer mevsimlerden düşük olmasında rol oynamış olabilir. Nitekim bu çalışmada, yaz mevsiminde doğuran mandalar kış mevsiminde doğuranlara göre, sonbahar mevsiminde doğuran mandaların ise, ilkbahar mevsiminde doğum yapanlara göre daha uzun laktasyon süresine sahip oldukları görülmektedir *(Tablo 3)*. Bu çalışmada, olduğu gibi bir çok araştırmada da [8,26,31-34] malaklama mevsiminin süt verimi üzerine etkisinin önemli olduğunu saptamıştır. Benzer şekilde Pakistan'da Nili Ravi ırkı mandalarda yapılan çalışmalarda da [20,35,36] malaklama mevsiminin süt verimi ve laktasyon süresi üzerine etkisinin önemli olduğu ($P<0.05$), kış mevsiminde malaklayan mandaların laktasyon uzunluğu ve süt verimlerinin yaz mevsiminde malaklayanlardan, ilkbahar mevsiminde malaklayan mandaların laktasyon uzunluğu ve süt verimlerinin ise sonbahar mevsiminde malaklayanlardan fazla olduğu belirtilmiştir. Benzer şekilde bataklık ve nehir mandalarının verimlerini değerlendiren Thevamanoharan [37] malaklama mevsiminin süt verimi ve laktasyon süresi üzerine etkisini önemli ($P<0.05$) bulmuştur. Ahmad ve Shafiq [38] tarafından Nili Ravi ırkı mandalar üzerinde yapılan bir çalışmada da mandalarda en yüksek süt verimi kış mevsiminde (2.400 kg) en düşük verim ise yaz mevsiminde elde edilmiştir. Araştırma bulgusu ve bu bildirişlerin aksine Nili Ravi ve Murrah ırkı bazı manda ırkları üzerinde yapılan çalışmalarda [39-42] malaklama mevsiminin süt verimi üzerine etkisinin önemsiz ($P>0.05$) olduğu bildirilmiştir.

Yem temininde sıkıntılar ve mevsimsel stres faktörleri mandaların süt verimlerinin mevsimler arasında farklılık göstermesine neden olmuş olabilir. Ancak bu faktörlerin etkisi yönetim, bakım ve besleme koşullarını iyileştirmek sureti ile giderilebilir.

Araştırma sonunda süt verimi üzerine malaklama yaşının etkisinin önemli olduğu belirlenmiştir ($P<0.05$). İlk laktasyonunda olan yani üç yaşında olan mandaların diğer laktasyonlardakilere göre daha uzun süre sağıldıkları görülmektedir *(Tablo 3)*. Benzer şekilde Khan ve Chaudhry [27], birinci laktasyonunda olan Nili Ravi ırkı mandaların daha uzun süre sağıldığını bildirmiştir. En yüksek süt verimi 6 yaşlı mandalarda ve 3. laktasyonunda olan mandalarda elde edilmiştir. Bu durum Anadolu mandalarının süt verim seviyesine, 6-7 yaş arasında, yani 3. laktasyonda

gerçekleştiğini bildiren İzgi ve Asker [11] ve Metin'in [8] bildirişleri ile uyum içerisinde bulunmuştur. Afraz ve ark.[20] ise Nili Ravi ırkı mandalarda en yüksek süt veriminin 4. laktasyonda olduğunu tespit etmişlerdir. Benzer şekilde, yapılan bir çok çalışmada da [31,32,34,43] süt verimi üzerine malaklama yaşı etkisinin önemli olduğu saptanmıştır.

Bu çalışmada laktasyon süresinin malaklama yaşından etkilenmediği (P>0.05) tespit edilmiştir. Araştırma bulgusunun aksine Khan ve Chaudhry [27] malaklama yaşının laktasyon süresi üzerine etkisinin önemli olduğunu tespit etmiştir. Benzer şekilde Umrikar ve Deshpande [44] de malaklama yaşının laktasyon süresi üzerine etkisinin önemli olduğunu bildirmiştir. Araştırmada malaklama mevsiminin laktasyon süresi üzerine etkisinin önemli (P<0.05) olduğu belirlenmiştir. Benzer şekilde, Murrah ırkı mandalarda Umrikar ve Deshpande [44], Nili Ravi ırkı mandalarda Hussain ve ark.[36], yaptıkları çalışmalarda söz konusu etkinin önemli (P<0.05) olduğunu belirlemişlerdir. Araştırma bulgu ve bu bildirişlerin aksine, mandalar üzerinde yapılan bazı çalışmalarda [20,27,41,45] söz konusu etkinin önemsiz (P>0.05) olduğu bildirilmiştir. Mandaların yetiştirildikleri işletmelerde damızlık seçimi yanında, bakım, besleme ve sürü idaresini iyileştirici tedbirlerin alınmasının verim bakımından işletme mevcut durumunun iyileştirilmesine katkı sağlayacağı düşünülmektedir.

Genel olarak araştırmada incelenen çevresel faktörlerden bölge, mevsim ve yaşın süt verimleri üzerine etkilerinin önemli olduğu belirlenmiştir. Anadolu mandalarının laktasyon süt verimleri Vogel yöntemi ile 761.4±16.4 kg, Hollanda 735.4±16.0 kg, İsveç yöntemi ile 734.0±16.0 kg, Trapez I yöntemi ile 657.7±13.7 kg ve Trapez II yöntemi ile 654.7±13.5 kg olarak belirlenmiştir. Laktasyon süt verimlerinin ve laktasyon süresinin yurt dışında tespit edilen değerlerden düşük olduğu belirlenmiştir.

Bu çalışmada Vogel yöntemi (761 kg) ile Trapez I ve Trapez II yöntemleri arasındaki farklılık sırası ile 105 ve 107 kg olarak tespit edilmiş Vogel yönteminin (761 kg) Trapez I ve Trapez II yöntemlerinden istatistiki olarak önemli farklılığa sahip olduğu belirlenmiştir (P<0.05). Hollanda ve İsveç metotları ile tahmin edilen süt verimleri sırası ile 734.0±16.0 kg ve 735.4±16.0 kg olarak tespit edilmiş, bu metotlar ile en yüksek süt veriminin tahmin edildiği Vogel metodu arasındaki fark istatistiki olarak önemli bulunmuştur (P<0.05) Laktasyon süt verimleri Hollanda ve İsveç metotları ile 734.0 ±16.0 kg ve 735.4±16.0 kg, Trapez I ve Trapez II metotları ile 657.7±13.7 ve 654.7±13.5 kg olarak tahmin edilmiş, Hollanda ve İsveç metotları ile tahmin edilen süt verimlerinin Trapez I ve Trapez II metotları ile tahmin edilen süt verimlerinden istatistiki olarak farklı olduğu (P>0.05) tespit edilmiştir. Vogel, Hollanda ve İsveç metotları arasında süt verimlerinin tahmininde en fazla 26 kg düzeyinde bir farklılık olduğu belirlenmiştir. Süt verimi tahmin metotlarından Hollanda, İsveç, Vogel ve Trapez metotlarının incelendiği bu çalışmada, laktasyon süt verimi için gerek genel ve gerekse alt guruplar arasında istatistiki olarak önemli bir farklılığın bulunması nedeniyle, bu çalışmada laktasyon süt verimlerinin tahmininde İsveç, Hollanda ve Vogel yöntemlerinden bir tanesi kullanılabilir.

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RESEARCH ARTICLE

The Effect of *Saccharomyces cerevisiae* on the Morphological and Histochemical Characteristics of the Duodenal Mucosa in the Rabbit [1]

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Summary

The aim of this study was to determine the effect of *Saccharomyces cerevisiae* (SC) on the morphological and histochemical properties of duodenum and duodenal submucosal glands of rabbits. Twenty 5-6 weeks old male New Zealand White Rabbits were obtained from the experimental animal laboratory of Uludag University, Bursa. The rabbits were divided randomly into two groups for 90 day. The first group (control group) received the basal diet, the second group (SC group) received basal diet supplemented with *Saccharomyces cerevisiae* at a level of 3 g/kg of feed. Duodenal tissue were taken at the end of the experiment from duodenum of animals and fixed in 10% neutral buffered formalin and embedded in paraffin. Sections were stained for localizing and characterizing glycoproteins (GPs) and morphometric measurements. In this study, the total mucosa, villus heights and gland depth of the duodenum were found to be longer than those of the control group in the SC group. However, duodenal crypt depth was greater in the duodenum of control groups, but no significant difference between the groups. The Goblet cells showed similar reaction in the both groups. Brunner glands were similar stained with AB pH 1, pH 2.5 and PAS/AB pH 1 in the both groups. However, they showed stronger positive reaction with PAS and PAS/AB pH 2.5 staining in the SC group compared with the control. In conclusion, the addition of SC to the diet of rabbits increased the total mucosa, villus height, and gland depth. However, the addition of SC also little affected the histochemical features of the duodenum by increased the secretion neutral and acidic mucins in the Brunner's glands. Therefore, it may be proposed that higher doses of *Saccharomyces cerevisiae* may be used for digestive health.

Keywords: *Histology, Histochemistry, Rabbit, Saccharomyces cerevisiae, Duodenum*

Saccharomyces cerevisiae'nin Tavşan Duodenumunun Morfolojik ve Histokimyasal Özellikleri Üzerine Etkisi

Özet

Çalışma, tavşanların duodenum ve submukozal bezlerinin morfolojik ve histokimyasal özellikleri üzerine *Saccharomyces cerevisiae*'nin (SC) etkisini belirlemek amacıyla planlanmıştır. Çalışmada Uludağ Üniversitesi deney hayvanları uygulama ve araştırma merkezinden temin edilen 20 adet, 5-6 haftalık, erkek Yeni Zelanda Beyaz Tavşanı kullanıldı. Hayvanlar rastgele iki gruba ayrılarak 90 gün boyunca bakım ve beslemesi yapıldı. I. grup (kontrol grubu) bazal diet ile beslenirken, II. grup (SC grubu) 3 g/kg dozda *Saccharomyces cerevisiae* ilave edilen bazal dietle beslendi. Deneyin sonunda hayvanların duodenumlarından örnekler alındı, %10 nötral formol ile tespit edildi ve parafine gömüldü. Kesitler, glikoproteinlerin (GP) lokalizasyonu, karakterizasyonu ve morfolojik ölçümler için boyandı. Deney sonrasında, SC grubunda duodenumun total mukoza, villus yüksekliği ve bez derinliği kontrol grubuna göre daha fazlaydı. Duodenumun kript derinliği kontrol grubunda daha fazlaydı fakat gruplar arasında istatistiksel bir farklılık gözlenmedi. Her iki grupta Goblet hücreleri benzer reaksiyon gösterdi. Brunner bezleri AB pH 1, pH 2.5 ve PAS/AB pH 1 boyamaları bakımından iki grupta benzer reaksiyon gösterirken, PAS ve PAS/AB pH 2.5 boyamaları kontrol grubuna göre SC grubunda güçlü pozitif reaksiyon gösterdi. Sonuç olarak, tavşan diyetine ilave edilen SC duodenumun total mukoza, villus yüksekliği ve bez derinliğini arttırdı. Bununla birlikte SC, Brunner bezlerinde asidik ve nötral musinlerin sekresyonunu arttırarak duodenumun histokimyasal özelliğini çok az etkiledi. *Saccharomyces cerevisiae*'in daha yüksek dozlarının sindirim sağlığı için kullanılabileceği düşünülmektedir.

Anahtar sözcükler: *Histoloji, Histokimya, Tavşan, Saccharomyces cerevisiae, Duodenum*

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INTRODUCTION

Probiotics are preparation of live microorganisms (like *Lactobacillus acidophilus, Streptococcus faecium* and *Saccharomyces cerevisiae*), which have beneficial effects on the health of the human or animal when administered adequately [1]. Several commercial formulations of *Saccharomyces cerevisiae* (SC) or its derivatives are used as prebiotics or probiotics in animal diets or feed additives. There have been numerous studies in humans and animals on the ability of probiotics to change the types and numbers of gut microflora [2-4]. Probiotics inhibit the growth of pathogenic microorganisms and provide digestive enzymes, a desirable effect for the host, and as a result changes in the intestinal microflora, antibiotic production, and synthesis of lactic acid leading to lowering of the intestinal pH, adhesion or colonization to intestinal mucosa and prevention of ammonium synthesis [5,6]. However, probiotic Saccharomyces spp may also help to reestablish a normal gut function after long term antibiotic therapy [7]. *Saccharomyces* spp have protective effects, and specific activities, against various enteric pathogens [8].

Currently there are only two probiotics approved for rabbits in the EU. One of them is bacterial, *Bacillus cereus* var. toyoi, the other is yeast, *Saccharomyces cerevisiae* strain NCYC Sc47 [9]. Studies with probiotics in rabbits are less than in other monogastric farm species. Because the rabbits have a high prolific nature, rapid growth rate, feed efficiency and economic management, they have been used as material in the present study.

However, there are no conclusive data on the effects in the duodenum when live yeast is used as a dietary supplement. Therefore, the objective of the present study was to assess the effects of a dietary supplement of *Saccharomyces cerevisiae* (live yeast culture) on the morphometric characteristics and histochemical activity of the duodenum in the rabbits.

MATERIAL and METHODS

Animals and Feeding

Twenty, five-six weeks old male New Zealand White rabbits with a mean body weight of 1.000 g were included in this study. The rabbits were housed individually in metal cages, feed and water were offered *ad libitum* to the rabbits throughout the 90-day trial. After adaptation, rabbits were equally divided in two groups. The first group of animals (basal diet group) was fed with a standard feed. Basal diet (pelleted) was formulated to contain 2.500 kcal ME/kg metabolizable energy, 16% crude protein and was designed to meet maintenance requirements according to the National Research Council (NRC). The second group (SC diet group) was fed with *Saccharomyces cerevisiae* live yeast culture (Yea Sacc, Altech, Nicholasville: $1x10^9$ CFU g^{-1}) added at concentration 3.0 g/kg into the basal diet *(Table 1)*. The experimental protocols were approved by the Animal Care and Use Committee of Uludag University and are in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (2010 09/01).

Histology and Morphometric Analysis of the Duodenum

At the end of the experimental period the rabbits were slaughtered and duodenum samples approximately 3-4 cm below the pylorus were taken out. Samples were fixed in 10% neutral buffered formalin. The routine histological methods were applied to the samples and embedded in paraffin. Five µm thick sections were cut from paraffin blocks, mounted on slides, and dried overnight. After dewaxing and rehydration, sections were stained by the Crossman's triple stain for morphometric examination and duodenal mucosa morphology. Moreover, histochemical techniques were used to distinguish the duodenal glycoproteins (GPs).

The villus height, the depth of the crypts, glands and

Table 1. *Composition of the basal diet fed to rabbit*[a]
Tablo 1. *Tavşanların bazal diyet kompozisyonu*[a]

Ingredients	Usage Rate, %
Barley	30.00
Corn	17.61
Rice bran	10.00
Corn bran	3.60
Alfalfa meal	25.00
Soybean meal	10.83
Marble dust	1.40
Dcp	0.28
Salt	0.80
Methionin	0.09
Anticoccidial	0.03
Vitaminpremix[b]	0.25
Antioccidial	0.03
Calculated analysis (% DM)	
Dry matter %	88.89
Crude fiber %[c]	10.95
Crude protein %[c]	16.00
Ether extracts %[c]	3.52
Ash	7.68

[a] *Yeasacc containing $1x10^9$ CFU of Saccharomyces cerevisiae was added to the basal diet at 3.0 g/kg to provide dietary treatments,*
[b] ***Premix:*** *Vit A 4.800.000 IU, Vit D 800.000 IU, Vit E 14.000 mg, Biotin 18 mg, CH-CL 50.000 mg, Folic acid 400 mg, Niacin 8.000 mg, Pant.Acid 4.000 mg, Riboflavin 2.800 mg, Thiamin 1.200 mg, Pyridoxine 2.000 mg, Vit K 1.600 mg, Zinc 24.000 mg, Iron 2.000 mg, Iodine 400 mg, Manganese 32.000 mg, Selenium 60 mg, Copper 24.000 mg*
[c] *Based on % Dry Matter*

total mucosa were measured and micrographs were taken with Nikon 80i microscope. The villus height was measured from the villus tip to villus-crypt junction level for randomly 5 villi per section. Crypt depth was measured from the villus-crypt junction to the lower limit of the crypt was estimated for 5 corresponding crypts per section [10,11]. The thickness of Brunner's glands was measured from the lower limit of the crypt to the tunica muscularis. Total mucosa thickness was measured from top of the villus to the lower limit of the crypt. *Fig. 1* illustrates the measurements that were made.

Histochemistry

Sections were stained with histochemical procedures for glycoproteins (GPs) identification;

1. PAS (Periodic Acid-Schiff's reagent) to demonstrate neutral mucosubstance [12].

2. AB pH 2.5 (Alcian Blue 8GX pH 2.5) to demonstrate acidic GCs with carboxylated and sulphated esters [13].

3. AB pH 1.0 (Alcian Blue 8GX pH 1.0) to demonstrate GPs with O-sulfate esters [13].

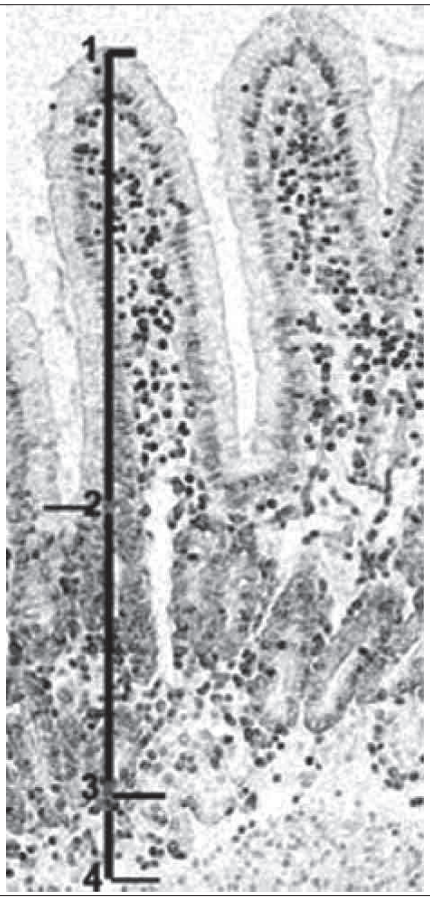

Fig 1. Morphological measurements in the duodenum, (1-2) villus height, (2-3) depth of crypt, (3-4) thickness of Brunner's glands, (1-3) thickness of total mucosa

Şekil 1. Duodenumda morfolojik ölçümler, (1-2) villus yüksekliği, (2-3) kript derinliği, (3-4) Brunner bez kalınlığı, (1-3) total mukoza kalınlığı

4. AB pH 2.5/PAS (Alcian Blue 8GX pH 2.5/Periodic Acid-Schiff staining) to demonstrate neutral and/or acid rich GCs [14].

5. AB pH 1.0/PAS to demonstrate GPs with O-sulfate esters, periodate-reactive vicinal diols, and presence of GPs with O-sulfate esters together with periodate-reactive vicinal diols [14].

All the slides were coded so that the investigator was blinded to staining for each slide and graded them according to the following scale: - no staining, + slight, ++ medium, +++ strong.

Statistical Analysis

Statistical analysis of results was performed by Mann Whitney U test (SPSS 16.0). Values are presented as means±SE. Group differences were declared significant at $P<0.05$.

RESULTS

Morphology results of the total mucosa, villus height, crypt depth and gland depth are presented in *Table 2*. In this study, the total mucosa, villus heights and gland depth of the duodenum were found to be longer than those of the control group in the SC group, but there was no statistically significant ($P>0.05$) difference between groups. However, duodenal crypt depth was decreased in rabbits fed with SC compared with control rabbits but not statistically significant ($P>0.05$).

The implementation of different histochemical techniques to demonstrate the presence of GPs in the goblet cells showed a similar pattern of distribution at both groups *(Table 3)*. The Goblet cells showed a strong positive reaction with the PAS *(Fig. 2)*, and PAS/AB (pH 2.5, pH 1) staining, while no reaction with the AB staining at different pHs in both control group and SC group.

AB pH 1 technique, which allowed the identification of GPs with O-sulfate esters, showed a slightly positive reaction at Brunner Glands of both diet groups. However SC group's neutral mucosubstance in Brunner glands were

Table 2. *Total mucosa, villus height, crypt depth and gland depth of duodenum in the groups (mean ± SE)*

Tablo 2. *Gruplarda duodenumun total mukoza, villus yüksekliği, kript derinliği ve bez derinlikleri (mean±SE)*

Regions of Duodenum	Group	
	Control	SC
Total Mucosa (µm)	362.09±43.15	433.99±47.60
Villus Height (µm)	280.30±28.94	357.90±39.77
Crypt Depth (µm)	81.80±14.40	80.81±7.20
Gland Depth (µm)	198.75±18.73	225.57±17.16

Table 3. *Histochemical staining score values of glycoproteins in the goblet cells and Brunner gland cells of duodenum in the groups*

Tablo 3. *Grupların duodenumunda goblet hücreleri ve Brunner bezi hücrelerinde glikoproteinlerin histokimyasal boyanma skorları*

Procedures	Goblet Cells		Brunner Glands	
	Control	SC	Control	SC
PAS	+3	+3	+1	+2
AB pH 2.5	0	0	+3	+3
AB pH 1	0	0	+1	+1
AB pH 2.5/PAS	+3	+3	+2	+3
AB pH 1/PAS	+3	+3	+2	+2

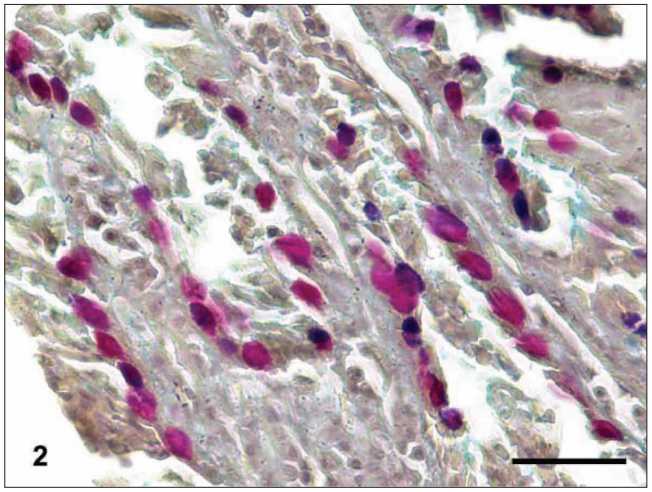

Fig 2. Histochemical characterization of the duodenum; Goblet cells reacted positively with PAS in the control group. PAS staining, Bar: 50 µm

Şekil 2. Duodenumun histokimyasal karakterizasyonu; Kontrol grubunun Goblet hücrelerinde PAS pozitif reaksiyon. PAS boyama, Bar: 50 µm

moderately stained by PAS, control groups' were showed slightly positive reaction *(Fig. 3 A,B)*. Also Brunner glands at both diet groups were strongly stained blue with AB pH 2.5 which allowed the identification of GPs with carboxyl groups. They were stained strongly positive at SC group on the other hand moderately stained at control group with PAS/AB pH 2.5; to demonstrate GPs with carboxyl groups and GPs with O-sulfate esters *(Fig. 4 A,B)*. Both diet groups were stained moderate red by PAS/AB pH 1.

DISCUSSION

This study contains the histological and histochemical changes of duodenum after feeding rabbits with SC. In our study, we observed that total mucosa height was higher in the SC group compared with those in the control groups. This result was related with increasing villus height. However, the difference was not statistically significant. It is assumed that an increased villus height is paralleled by an increased digestive and absorptive function of the intestine due to increased absorptive surface area [15].

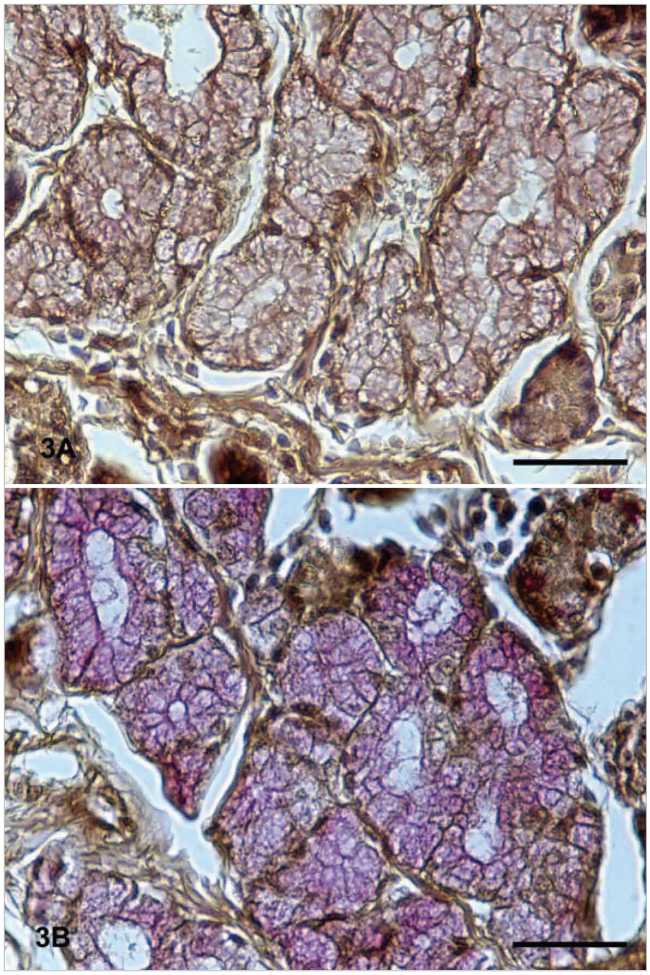

Fig 3. Histochemical characterization of the duodenum, PAS staining, **A-** Brunner's glands stained slightly PAS positive in control group, **B-** Brunner's glands stained moderately PAS positive in SC group, Bar: 50 µm

Şekil 3. Duodenumun histokimyasal karakterizasyonu, PAS boyama, **A-** Kontrol grubunun, Brunner bezlerinde zayıf PAS pozitif reaksiyon, **B-** SC grubunun, Brunner bezlerinde orta şiddette PAS pozitif reaksiyon, Bar: 50 µm

According to Buts et al.[16] *Saccharomyces* have a positive effect on the villus height. Likewise, Baum et al.[17] also found that villus length was greater in the small intestine of piglets fed yeast than controls. In addition, it was indicated that longer villi are correlated with activation of cell mitosis [18]. Hence, our results confirm this hypothesis that these yeasts could stimulate the development of the intestinal villi by an increasing cell proliferation.

In the present study, a greater villus and shorter crypts were observed in SC fed rabbits. Santin et al.[19] reported very similar results in that SC added at 0.2% of broiler diets that a reduction in crypt depth and an increase in villus height. Likewise, Bradley et al.[20] reported that crypt depth in the ileal mucosa was reduced when the broiler diet was supplemented with SC. In this study the Brunner's glands in the duodenum of the group feeding with SC was found to be higher than those in the control group but not

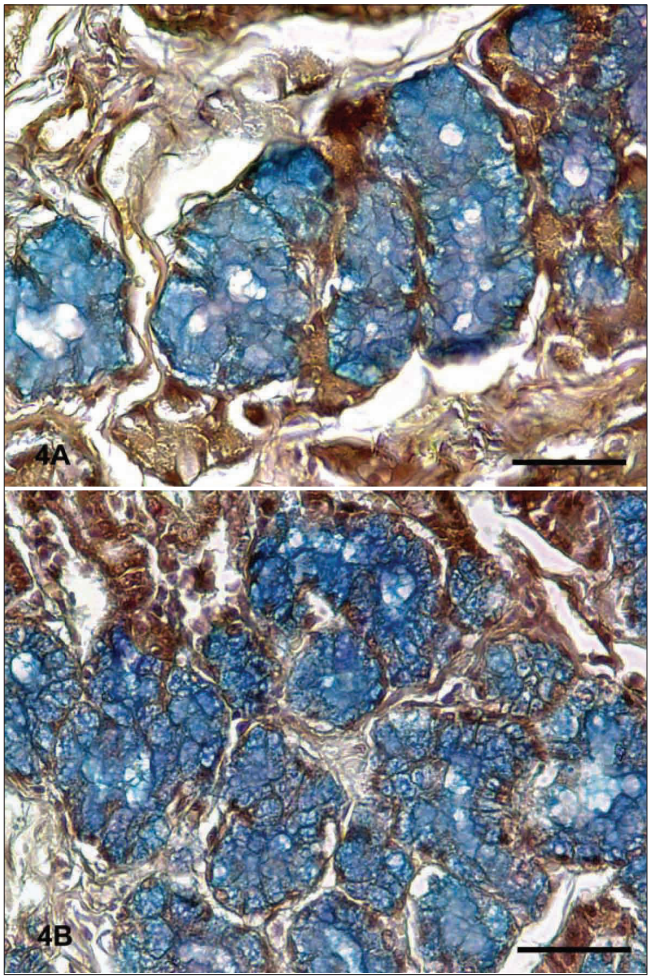

Fig 4. Histochemical characterization of the duodenum, AB pH 2.5/PAS staining, **A-** Brunner's glands stained moderately AB/PAS positive in control group, **B-** Brunner's glands stained strongly AB/PAS positive in SC group, Bar: 50 µm

Şekil 4. Duodenumun histokimyasal karakterizasyonu, AB pH 2.5/PAS boyama, **A-** Kontrol grubunun, Brunner bezlerinde orta şiddette AB/PAS pozitif reaksiyon, **B-** SC grubunun Brunner bezlerinde şiddetli AB/PAS pozitif reaksiyon, Bar: 50 µm

statistically significant. This study showed that the gland depth may be increased by SC's inducing the enlargement of the Brunner's glands.

Also, we demonstrated the presence of GPs with different histochemical techniques in the duodenal glands of the groups. In mammals, GPs layer of gastrointestinal tract protects the epithelial cells and mucosa from proteolytic enzymes invasion of enteric bacteria, bacterial and environmental toxins, and some dietary components [21,22]. This glycoprotein compounds also known as mucins which secreted by goblet cells [23]. Various authors have suggested that goblet cells contain neutral or acidic mucin glycoproteins or the combination of both types of mucin [24-26]. In classic carbonhydrate histochemistry, positive PAS reaction indicates the presence of neutral carbonhydrate, while positive Alcian Blue reactions at pH 1.0 and 2.5 indicate the presence of acidic sulphated and acidic carboxylated residues respectively. Mucin synthesis and secretion are influenced by the diet [27]. However, in the present study, staining properties with PAS, Alcian blue (pH 1.0 and 2.5) and PAS/AB (pH 1.0 and 2.5) of goblet cells not showed marked differences between two groups. There was no effect of the SC on mucins, which secreted by goblet cells. This result may be due to the use of low dose of SC. In the present study, the Brunner's glands were stained strongly positive with PAS and PAS/AB pH 2.5 in the SC treatment group than those of the control group. But, they were stained sligthly with AB pH 1 in both diet groups. Our results showed that neutral and acidic mucins were enhanced by feed supplemented with SC in the Brunner's glands. The results suggest that SC have to be protecting against enteric pathogens. Ozpinar et al.[28] also reported, SC protects from invading pathogens by mucosal immunity.

In conclusion, the addition of SC to the diet of rabbits affected the morphology of the duodenum by increasing the total mucosa, villus height, and the gland depth with inducing enlargement of the Brunner's glands. However, the addition of SC also little affected the histochemical features of the duodenum by increasing the secretion of neutral and acidic mucins in the Brunner's glands. We think that the effects may be generally related to the dose of SC. It may be proposed that higher doses of yeast may be used for digestive health. In addition, further studies are necessary to obtain definitive evidence on the effects of yeast supplementation on digestive system.

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RESEARCH ARTICLE

Molecular Epidemiology of *Mycoplasma synoviae* Infection in Commercial Layers

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Summary

Mycoplasma synoviae (*M. synoviae*) infection is a cause of great economic loss in commercial egg layer hens. The aims of this study were to investigate the prevalence of *M. synoviae* and to compare the characteristics of *M. synoviae* infected and free flocks of commercial layers. A total of 400 tracheal swabs and 400 blood serum samples were collected from 20 different layer flocks. Random Amplified Polymorfic DNA (RAPD) analysis was used for the molecular typing of *M. synoviae* isolates to determine the source of infection. *M. synoviae* was isolated from 89 tracheal swabs collected from 5 out of 20 flocks. The genetic similarity between field strains ranged from 53% to 100% as determined by RAPD analysis. All *M. synoviae* strains separated into 2 main-clusters in UPGMA dendogram. These results revealed that the 5 infected flocks were contaminated from 2 different sources. The egg production of positive flocks was statistically lower ($P<0.05$) than that of the pathogen free flocks. Infection was more frequent in multi-age farms and on sites with several houses. The mortality of infected flocks was higher than uninfected flocks, but this difference was not statistically significant. The mean weight of eggs and the average live weight of hens were similar in free and infected flocks. In conclusion, *M. synoviae* infection significantly decreased egg production in layer hens and was more frequent in multiage farms.

Keywords: *Mycoplasma synoviae, Layer, Epidemiology, RAPD*

Ticari Yumurtacı Tavuklarda *Mycoplasma synoviae* Enfeksiyonunun Moleküler Epidemiyolojisi

Özet

Mycoplasma synoviae (*M. synoviae*) yumurtacı tavuk sektöründe büyük ekonomik kayıplara neden olmaktadır. Bu çalışmada, ticari yumurtacı tavuk kümeslerinde *M. synoviae*'nın prevalansını araştırmak ve enfekte ve sağlıklı kümeslerin özelliklerini karşılaştırmak amaçlanmıştır. Toplam 400 trakeal sıvap ve 400 kan serum örneği 20 farklı kümesten toplandı. Enfeksiyonun kaynağını belirlemek için *M. synoviae* suşlarının moleküler tiplendirmesi Random Amplified Polymorfic DNA (RAPD) analizi ile yapıldı. Trakeal sıvap örneklerinin 89'undan *M. synoviae* izole edildi ve bu pozitif sıvap örnekleri 5 farklı kümesten toplanmıştı. Sahadan izole edilen suşlar arasındaki genetik benzerlik oranları RAPD analizi ile %53 ile %100 arasında bulundu. UPGMA dendogramında izolatlar 2 ayrı ana kümede yer aldılar ve bu sonuç gösterdi ki 5 farklı enfekte kümes 2 ayrı kaynaktan kontamine olmuştur. Enfekte kümeslerin yumurta verimi sağlıklı kümeslerden daha düşük (istatistiki olarak anlamlı) olarak bulundu. Enfeksiyon, farklı yaşlardaki tavuk kümeslerinin aynı bahçede olduğu çiftliklerde daha sık tespit edildi. Enfekte kümeslerin mortalite oranları sağlıklı kümeslere oranla daha yüksek (istatistiki olarak anlamsız) bulundu. Ortalama yumurta ağırlığı ve tavukların canlı ağırlığı ise enfekte ve sağlıklı sürülerde benzerdi. Sonuç olarak, *M. synoviae* enfeksiyonu yumurtacı tavuklarda yumurta verimini azaltmakta ve farklı yaşlardaki tavuk kümeslerinin aynı bahçede olduğu çiftliklerde daha sık görülmektedir.

Anahtar sözcükler: *Mycoplasma synoviae, Yumurtacı tavuk, Epidemiyoloji, RAPD*

INTRODUCTION

Mycoplasma synoviae (*M. synoviae*) mainly causes chronic subclinical upper respiratory disease, infectious synovitis, and airsacculitis in chickens and turkeys. It has been acknowledged as the cause of great economic loss in commercial poultry industry due to poor growth and decreased egg production. The infection occurs world-

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wide in commercial egg layers, broilers, and turkeys. The disease may be transmitted either vertically through the eggs, or horizontally, often by direct contact between contaminated materials and susceptible animals [1].

The diagnosis of *M. synoviae* infection has traditionally been carried out by serological procedures and isolation of the causative organism [1,2]. The polymerase chain reaction (PCR) is an alternative to bacteriological isolation for diagnosis of avian mycoplasmosis. It is a rapid, highly sensitive, very specific and inexpensive technique to detect *M. synoviae* DNA [3-7]. *M. synoviae* PCR assays have been based on the 16S rRNA gene [3,4,6] and *VlhA* haemagglutinin gene [5,7,8].

Molecular typing of bacteria is an important tool for determining the source of an infection, for recognizing outbreaks, monitoring vaccination programs, and determining the relationships amongst strains isolated from neighboring flocks. Random amplified polymorphic DNA (RAPD) assay was first described in 1990 as a means of genotyping microorganisms [9] and has been employed for the molecular typing of *M. synoviae* isolates [10,11].

There are few studies that have attempted to characterise *M. synoviae* infected flocks and it has been reported that *M. synoviae* infection does not statistically effect egg production [11,12], egg quality [13] or the mortality [11] of the birds. However, there is no information concerning the effect of Turkish strains of *M. synoviae* on egg production, egg quality or mortality of hens.

The aims of this study were to investigate the prevalence of *M. synoviae* and to compare the characteristics of *M. synoviae* infected and free flocks in commercial egg layers. The characteristics determined were egg production, egg weight, live weights and mortality rates of the hens. Additionally, an RAPD based method was used to type the *M. synoviae* isolates to detect possible routes of contaminations.

MATERIAL and METHODS

Study Sample

Tracheal swabs and blood serum samples were collected from 20 different commercial egg laying flocks in the Konya region of Turkey during the later six months of 2010. As the farms of the main egg production companies in Turkey are located in the Konya region and contain more than 8 millions laying hens in 2010, it is an important city for poultry sector of Turkey. Twenty tracheal swabs were randomly collected from each flock and were transported in Frey's medium to the laboratory for *M. synoviae* isolation. A flock was considered *M. synoviae* positive if at least one tracheal swab yielded *M. synoviae*. Additionally, 20 blood serum samples were collected from the same layer flocks for serological investigation. Sera were kept in aliquots at -20°C until analysed. The study protocol was approved by Selcuk University Veterinary Faculty Ethical Committee (2009/49).

Egg production, egg weight, live weight of hens, mortality rate of flocks, and farm characteristics was investigated by questionnaire forms obtained from the farm veterinarians. Flocks were defined as hens of same age group (55-60 weeks) and same origin. All of the 20 flocks visited in this study belonged to different farms located in different parts of the region.

Culture and Identification

Specimens were cultured using the method described by Frey et al.[14]. All tracheal swabs were placed in 2 ml Frey's broth for transport and used for mycoplasma culture. All broths were incubated at 37°C under an atmosphere with 10% CO_2 and subcultured onto Frey's agar until a color change was observed. Isolates were identified according to a *M. synoviae* specific Multiplex PCR assay [4].

DNA Extraction

M. synoviae sample and reference (*M. synoviae* WVU 1853) strains were grown in Frey's broth. DNA was extracted using the protocol provided in Promega Wizard Genomic DNA purification Kit (Cat No: A1120). DNA concentration was determined spectrophotometrically (Eppendorf, Model 6131, Germany) by absorbance readings at 260 and 280 nm. The samples were stored at -20°C until used as templates for amplification.

Identification of M. Synoviae by Multiplex PCR Assay

M. synoviae and *M. gallisepticum* specific primer pairs were used in PCR, as described by Wang et al.[4]. The sequence of forward primer was 5'- GAA GCA AAT AGT GAT ATC A -3' and reverse primer was 5'- GTC GTC TCG AAG TTA ACA A -3' for *M. synoviae*. The sequence of *M. gallisepticum* specific forward and reverse primer pairs were 5'-GGA TCC CAT CTC GAC CAG GAG AAA A-3' and 5'-CTT TCA ATC AGT GAG TAA CTG ATG A-3', respectively. PCR was performed as previously described [4].

RAPD Genotyping

Three *M. synoviae* clones per infected flock were analyzed by RAPD analysis. This analysis was performed as described by Geary et al.[15]. The 1254 primer (5'-CCG CAGCCAA-3') coupled with the 1281 primer (5'-AACGC GCAAC-3') were used in assay and obtained from IDT Technologies, USA.

Computer Analysis of RAPD Patterns

All fragments were compared on the same gel. Each band was treated on gel and was scored as 1 (when present) or 0 (when absent). These data matrices were recorded to the software program of PubMLST data analysis *(http://*

pubmlst.org/), and then obtained Unweighted Pair Group Method with Arithmetic Means (UPGMA) dendogram. Additionally, the genetic diversity for the bacteria was calculated according to Nei and Li [16]. Similarity index between pairs of strains were calculated with the following formula: F=2NAB/NA+NB, where NA and NB are the number of fragments in strains A and B, respectively, and NAB is the number of fragments in strains A that match fragments in strain B.

Hunter and Gaston's Index

The discriminatory power of RAPD assay was evaluated by the Gaston and Hunter's index D [17] which is calculated according to the following equation:

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{s} n_j (n_j - 1)$$

Where *N* is the total number of strains, s is the total number of types described, and *nj* is the number of strains belonging to the j-th type. An index higher than 0.90 indicates that the typing system is discriminates and can be used as epidemiological typing system.

The reproducibility of the PCR products was tested by interassay analysis of five isolates randomly chosen. The same five isolates were tested for five consecutive days during the interassay analysis.

Rapid Serum Agglutination Test (RSAT)

M. synoviae RSAT antigen was provided from Pendik Veterinary Control and Research Institute, Turkey. RSAT was performed according to procedures described by Kleven and Ferguson-Noel [1]. Briefly, an equal volume (30 *µ*l) of the antigen and serum was mixed on a clean glass slide and shaken gently. Formation of agglutination was accepted as a positive result.

Statistical Test

Chi-square test was used to compare the distributions of *M. synoviae* infected or free flocks according to flock characteristics. A significance level of 5% was used. The statistical analyses were performed using SPSS software version 12.

RESULTS

M. synoviae was isolated from 89 tracheal swabs which were collected from 5 (25%) out of 20 flocks. Three hundred and eleven of the tracheal swabs from remaning 15 (75%) flocks were bacteriologically negative. A total of 89 *M. synoviae* strains gave 207 bp *M. synoviae* specific bands but *M. gallisepticum* was not detected by Multiplex PCR.

Of the 400 sera examinated in this study, 90 (22.5%) were found to be positive for *M. synoviae* antibodies by RSAT. These 90 positive sera were collected from the 5 flocks that were bacteriologically positive. The 5 *M. synoviae* positive flocks were examined for other viral and bacterial diseases, but no disease was detected in these flocks.

The mean size of flocks was 26.375 hens. Three out of 20 flocks were kept in a one-house farm, 6 flocks in two-house farms, 3 flocks in three-house farms and 8 flocks in more than 3 house farms. Ten of 20 farms harboured hens of multiple ages. The five infected flocks belonged to five different farms which harboured hens of multiple ages.

The mortalities of *M. synoviae* positive or negative flocks were compared and while the mortality of the infected flocks was higher (2.80%) than that of the uninfected flocks (2.62%), this difference was not statistically significant.

The number of eggs per hen produced by the positive flocks (222.5) was statistically lower than the number produced in free flocks (242.4, $P<0.05$). The age of the infected flocks was 56-58 weeks. The mean weight of eggs and the average live weight of the hens were similar in the free and infected flocks at 63.8/62.9 g and 1803.4/1805 g, respectively *(Table 1)*. These differences were not statistically significant.

For each positive flock, three different *M. synoviae*

Table 1. *Characteristics of M. synoviae infected and free flocks*
Tablo 1. *M. synoviae ile enfekte ve sağlıklı sürülere ait özellikler*

Flocks	n	Positive Swap	Positive Sera	Mortality (%)	Eggs Production	Mean Weight of Eggs (g)	Average Live Weight of Hens (g)	RAPD Profiles
Infected flock 1	20	20	20	3.20	218.7	62.3	1825	A´
Infected flock 2	20	16	16	3.04	228.9	61.8	1850.2	A´
Infected flock 3	20	20	20	2.52	212.3	62.8	1792.6	A´
Infected flock 4	20	18	19	2.40	223.3	64.1	1772.2	B´
Infected flock 5	20	15	15	2.81	229.3	63.5	1785	B´
Free flocks (n:15)	300	0	0	2.62	242.4	63.8	1803.4	-

n: *number of sampled hens (**n:** örneklenen tavuk sayısı)*

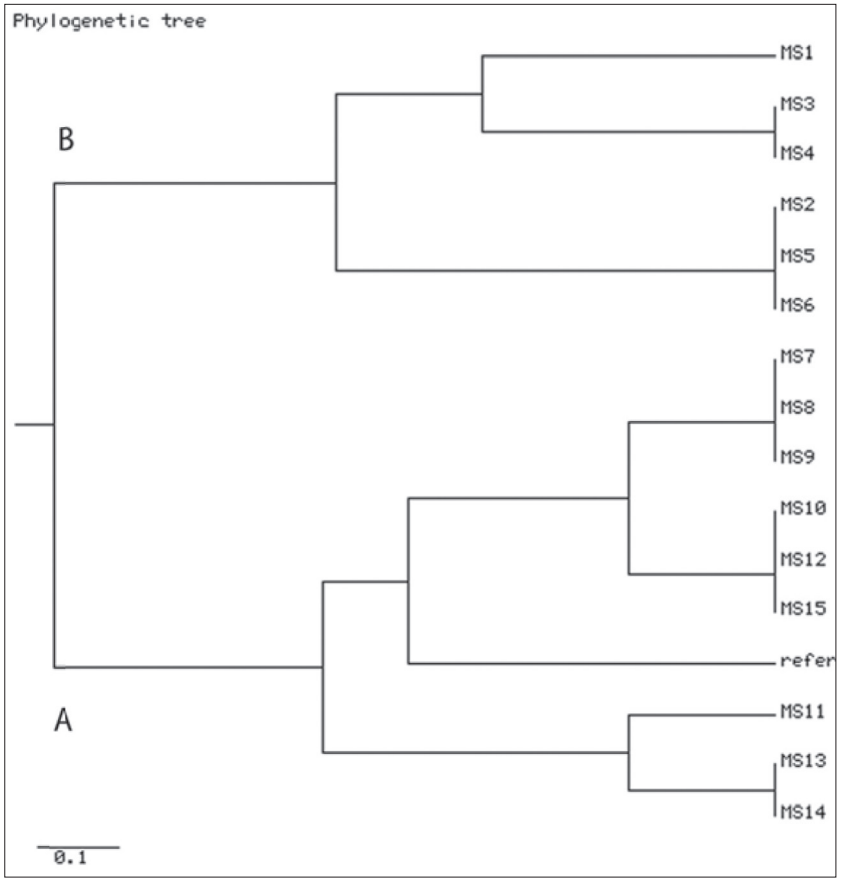

Fig 1. The UPGMA dendogram. Refer, *M. synoviae* reference strain (WVU 1853); MS1-MS6, *M. synoviae* field strains in B cluster; MS7-MS15 *M. synoviae* field strains in A cluster

Şekil 1. UPGMA dendogramı. Refer, *M. synoviae* referans suş (WVU 1853); MS1-MS6, B kümesinde bulunan *M. synoviae* saha izolatları; MS7-MS15 A kümesinde bulunan *M. synoviae* saha izolatları

clones could be typed by RAPD and typeability of assay was found 100%. The reproducibility and discriminatory power index (Gaston and Hunter's index) for RAPD were 100% and 0.90, respectively. Using the RAPD assay 7 different patterns were obtained from the 15 *M. synoviae* clones. The genetic similarity between strains was calculated to range from 53% to 100%, according to Nei and Li [16]. The UPGMA dendogram is shown in *Fig. 1* and all *M. synoviae* clones were placed in 2 main separate clusters (A and B profiles). The reference strain (*M. synoviae* WVU 1853) and 9 of the field isolates made up the A´ cluster while the other 6 field clones formed cluster B´. The source of *M. synoviae* field isolates which were separated in cluster A´ and B´ are shown on *Table 1*.

DISCUSSION

In the present study *M. synoviae* and specific antibodies were detected by culture and serological examination, respectively, in 5 (25%) of the 20 flocks examined. However, this is in sharp contrast to the incidence of *M. synoviae* infection in commercial flocks in Australia, France and Brazil [6,11,13] where the reported infection rates are 69%, 68% and 72.7%, respectively.

Serological tests are widely used to identify infected flocks, but cross-reaction with *M. gallisepticum* and other nonspecific reactions may occur. Reactors are confirmed as positive by isolation and identification of the organism [1]. In this study, 90 of 400 serum samples were found to be *M. synoviae* positive by RSAT, which was also confirmed by culture. This result indicates that RSAT can be used for the diagnosis of subclinical *M. synoviae* infection. Indeed RSAT is recommended as a screening test for poultry flocks by the Turkish Ministry of Agriculture.

In this study, the characteristics of *M. synoviae* infected and free flocks of commercial egg layers were compared. The characteristics examined were egg production, egg weight, live weight of the hens and their mortality rate. Few studies have investigated the effect of *M. synoviae* infection on egg laying and found no significant association between egg production and naturally infection with *M. synoviae* [11,12,18]. In contrast, we found that the number of eggs per hen was statistically lower in *M. synoviae* infected flocks. The mean weight of eggs and the mean live weight of hens were found similar in free and infected flocks. These differences were also not statistically significant.

The mortality rate of *M. synoviae* positive or negative flocks was compared and it was found that although the mortality of the infected flocks was marginally higher than that of the uninfected flocks the difference was not statistically significant. Similar results have been observed by other researchers [11,12]. Mohammed et al.[12] reported that mortality can be as high as 10% following infection

with *M. synoviae*. In the present work, the mortality rate of infected flocks was 2.80%.

Hagan et al.[19] reported that *M. synoviae* infection was more frequent on sites where the hens were kept in several houses. In the present study, all of infected flocks were found in farms those the birds were kept in 5 to 6 house supporting that this may be a risk factor for *M. synoviae* infection. It has also been reported that infection was more frequent in multiage farms [11]. In our study, all of the infected flocks contained hens with varied widely in age.

The diversity of *M. synoviae* field strains was investigated by RAPD genotyping method to determine if a there was genetic links between the isolates obtained from the different egg laying flocks. The reproducibility, typeability, and discriminatory power index for the RAPD assay was 100%, 100% and 0.90, respectively. All the *M. synoviae* strains analysed in this study could be put into 2 separate clusters comprising 8 sub-groups *(Fig. 1)*. The genetic similarity between strains was found to vary from 53% to 100%. Nine (60%) out of 15 *M. synoviae* clones from 3 different infected flocks belonged to the same cluster *(Fig. 1, A´)*. This group also included the reference strain (*M. synoviae* WVU 1853). The 6 remaning clones belonged to the other cluster *(Fig. 1, B´)*. These results supported that the 5 infected flocks were contaminated from 2 different sources.

In conclusion, *M. synoviae* infection significantly decreased egg production in layer hens and it was more frequent in multiage farms and on sites with several houses. RAPD profiles showed that infected flocks were contaminated from 2 different sources. This genotyping method appears valuable tool for epidemiological studies on various *M. synoviae* isolates.

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RESEARCH ARTICLE

The Effects of Organic or Inorganic Zinc and Microbial Phytase, Alone or in Combination, on the Performance, Biochemical Parameters and Nutrient Utilization of Broilers Fed A Diet Low in Available Phosphorus [1]

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Summary

This study examined the effects of zinc (Zn) from different sources and microbial phytase on the broiler performance, biochemical parameters and digestibility of nutrients when they were added to broiler diets containing low available phosphorus. A total of 875, 1-day-old male broilers of the Ross 308 strain were randomly separated into two control groups (positive and negative) and five treatment groups each containing 125 birds; each group was divided into 5 replicates of 25 birds. The positive control (PC) group was fed a diet containing adequate concentration (0.45 %) of available phosphorus due to mineral premix (except zinc) and feeds. The negative control (NC) group was fed a basal diet including low concentration (0.30%) of available phosphorus due to mineral premix (except zinc) and feeds. The basal diet was supplemented with 0.30% phosphorus and 500 FTU phytase (PH); 0.30% phosphorus and organic zinc (OZ; 75 mg/kg of Zn from Zn-proteinate); 0.30% phosphorus and inorganic zinc (IZ; 75 mg/kg of Zn from $ZnSO_4$); 0.30% phosphorus, organic zinc and 500 FTU phytase (OZ + PH); and 0.30% phosphorus, inorganic zinc and 500 FTU phytase (IZ + PH) in the treatment groups 1, 2, 3, 4 and 5, respectively. The lowest value for mean body weight was in the negative control group on a diet containing low available phosphorus. The use of supplementation with organic and inorganic zinc alone or in combination with microbial phytase significantly ($P<0.05$) increased the digestibility of Zn in the male broilers. Supplementation of those diets with OZ + PH or IZ + PH was very effective for increasing the body weight, body weight gain and the feed conversion ratio. In conclusion, the effects on broilers of diets with low phosphorus levels may be overcome by the addition of inorganic or organic zinc compounds in combination with microbial phytase.

Keywords: *Broiler, Performance, Phytase, Phosphorus, Zinc*

Düşük Fosforlu Diyetlere Organik ve İnorganik Çinko İle Mikrobiyal Fitaz İlavesinin Broylerlerde Performans, Biyokimyasal Parametreler ve Besin Madde Kullanımı Üzerine Etkisi

Özet

Bu araştırma, düşük fosforlu diyetlere organik ve inorganik çinko ile mikrobiyal fitaz ilavesinin broilerlerde performans, biyokimyasal parametreler ve besin madde kullanımı üzerine etkisini araştırmak amacıyla yapılmıştır. Araştırmada hayvan materyali olarak toplam 875 adet günlük civciv denemeye alınmıştır. Her grupta 125 adet hayvan olacak şekilde, iki kontrol (pozitif ve negatif) ve beş deneme grubu oluşturulmuştur. Her grup 5 alt gruptan, her alt grupta ise 25 civcivden oluşturulmuştur. Pozitif kotrol grubu, çinko içermeyen mineral ön karması ve yeterli miktarda yararlanılabilir fosfor (%0.45) içeren rasyonla beslenmiştir. Negatif kontrol grubu ise, çinko içermeyen mineral ön karması ve düşük fosfor (%0.30) içeren temel bir rasyonla beslenmiştir. Temel rasyona %0.30 fosfor ve 500 FTU fitaz ilave edilmiştir (PH). Deneme grupları 1, 2, 3, 4 ve 5 ise sırasıyla %0.30 fosfor ve organik çinko (OZ; 75 mg/kg Zn-proteinat); 0.30% fosfor ve inorganik çinko (IZ; 75 mg/kg $ZnSO_4$); 0.30% fosfor, organik çinko ve 500 FTU fitaz (OZ + PH); ve 0.30% fosfor, inorganik çinko ve 500 FTU fitaz (IZ + PH) oluşturmuştur. Düşük fosfor içeren negatif kontrol grubunda en düşük canlı ağırlık belirlenmiştir. Organik çinko ve inoganik çinko ile mikrobiyal fitaz kombinasyonunda çinkonun sindirilebilirliği artmıştır. Rasyonlarına OZ + PH ve IZ + PH ilave edilen gruplarda canlı ağırlık ve canlı ağırlık artışı artarken, yemden yararlanma oranı olumlu yönde etkilenmiştir. Sonuç olarak, düşük fosforlu diyetlerle beslenen broylerlerde organik ve inorganik çinkonun mikrobiyal fitaz ile kombinasyonlarının yeterli olacağı kanısına varılmıştır.

Anahtar sözcükler: *Broyler, Performans, Fitaz, Fosfor, Çinko*

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INTRODUCTION

An important consideration for phosphorus in most feedstuffs used in broiler diets is that in the phytate form it is unavailable [1]. Phytic acid is a polyanionic molecule with six phosphate groups. Phytic acid forms insoluble complexes with the divalent cations of zinc, calcium, magnesium and iron in weak acidic to neutral pH conditions. It reduces their availability in chickens [2]. The reduction in Zn availability due to binding by phytate decreases the growth rate of chickens [3]. Conversely, Lonnerdahl *et al.*[4] demonstrated that dephytinization of soybean meal increased Zn availability to chickens.

Zinc is an essential trace mineral for broiler growth. It plays a role in the immune system, reproduction, maintaining correct insulin levels, thyroid function and enzyme systems. Zinc also plays an important role in DNA, RNA and protein production. Zinc must be added to most poultry diets to meet requirements because of the poor availability of Zn in plant feed ingredients caused by the binding of Zn by phytate [5-7]. The NRC [8] estimated the Zn requirement for broiler chickens to be 40 mg kg^{-1} in the diet. Burrell *et al.*[9] reported improved performance when broilers consumed diets formulated to contain 110 mg Zn kg^{-1}. Furthermore, it is common practice in the U.S. broiler industry to formulate diets that contain 100-120 mg supplemental Zn kg^{-1} [9].

Phytase is an enzyme that hydrolyzes phytate to inositol and inorganic phosphate. That enzyme is at a low level in the chicken gastro-intestinal tract. Hydrolizing phytate also liberates Zn and thus, adding microbial phytase to diets increases Zn availability to chicks [10,11]. Animal nutritionists have long regarded phytate as both indigestible and an anti-nutritional factor in non-ruminant animals [12]. An organically complexed mineral is linked to protein/peptide/amino acids and has a higher bioavailability than those in inorganic salts [13] and lower manure loading [14]. The lack of phytase activity within the digestive tract results in phytate phosphorus and other minerals that are bound to it to be poorly digested. The phytase enzyme can be added to the diet of broilers to hydrolyze phytate within the digestive tract, resulting in phytate phosphorus and bound minerals being available for use by the animal and decreasing the need for inorganic phosphorus supplementation [15]. In addition to, replacing inorganic P with exogenous phytase has some additional benefits - it is more environmentally helpful due to reduced P excretion, and in the long run, may be more cost effective. Exogenous microbial phytase is a common ingredient added to broiler diets to improve availability of phytate P and thus reduce the P outflow into the environment in animal waste [16].

Cabahug *et al.*[17] reported that there were remarkably little differences in responses to 400 or 800 FTU kg^{-1} phytase over a wide range of parameters in broilers. Furthermore, the addition of seven levels of phytase activity (0-1000 FTU kg^{-1}) to broiler diets containing 7.5 and 3.0 g total P kg^{-1} were investigated by Ravindran *et al.*[18]. While increasing phytase from 750 to 1000 FTU kg^{-1} slightly benefited amino acid digestibility; on the contrary, weight gain, feed efficiency and apparent metabolisable energy responses to phytase reached a plateau at 750 FTU kg^{-1}.

Therefore, in recent years, organic sources for trace minerals have been used increasingly in poultry diets [19-22]. However, only a few publications in the poultry field have reported the effects of Zn when it is supplied from organic sources. The supplementation of diets with microbial phytase increases the availability of phytate P in chicks [10,11]. Likewise, supplementation of phytase was more effective in a diet with a low concentration of available phosphorus [23]. Therefore, it would be highly desirable to supplement the low-AP diet with phytase with a high efficiency in releasing phytate P. Therefore, the objectives of the present study were to analyse the effects of the interaction between Zn source and microbial phytase on performance, some blood parameters and digestibility of nutrients in broilers.

MATERIAL and METHODS

The study was approved by the Local Ethics Committee on Animal Experiments of Abant Izzet Baysal University (AİBÜ, 01.07.2009, HADYEK/23).

Poultry, Diets and Design of Experiment

The present study was performed at the research farm of the Mudurnu Sureyya Astarcı Vocational School of Higher Education of the Abant Izzet Baysal University in Bolu, Turkey. The poultry were housed in an environmentally controlled room with 35 floor pens of 2 m × 2 m for 42 d. The initial room temperature of 31°C was gradually reduced to 21°C at 42 d. A commercial standard lighting regimen (23L:1D, 1 to 42 d) was provided by incandescent lights with intensities of 30 lx during days 1 to 7,15 lx during days 8 to 28, and 5 lx for the remaining period. Eight hundred and seventy five 1 day old male broilers of the Ross 308 strain were obtained from a commercial hatchery (Pak Tavuk Gıda ve San. AS, Bolu, Turkey). Organic Zn (Bioplex, Alltech, Inc., Nicholasville, KY) and inorganic Zn (zinc sulphate-$ZnS0_4.7H0_2$) and microbial phytase (Karyzyme® P 500, Kartal Kimya Inc, Istanbul, Turkey) were obtained from commercial suppliers. Broiler chicks were randomly allocated to PC and NC groups and five treatment groups each containing 125 birds; each group was then divided into 5 replicate groups. The duration of the experiment was 42 days. All groups were fed broiler starter diets from days 1 to 21 and finishing diets from days 22 to 42. Chicks were given *ad libitum* access to feed and tap water containing no detectable Zn. The levels of all the essential nutrients contained in the basal diet met the requirements suggested by the NRC [8] . The PC group was

fed a diet containing adequate concentration (0.45%) of available phosphorus due to mineral premix (except zinc) and feeds. The NC group was fed a basal diet including low concentration (0.30%) of available phosphorus due to mineral premix (except zinc) and feeds. This level of available phosphorus was selected to maintain the dietary available P of current NRC [8] recommendations and to ensure responses with phytase additions. The basal diet was supplemented with 0.30% phosphorus and 500 FTU phytase; 0.30% phosphorus and organic zinc (75 mg/kg of Zn from Zn-proteinat); 0.30% phosphorus and inorganic zinc (75 mg/kg of Zn from $ZnSO_4$); 0.30% phosphorus, organic zinc and 500 FTU phytase; or 0.30% phosphorus, inorganic zinc and 500 FTU phytase, for the treatment groups 1, 2, 3, 4 and 5, respectively. Dietary treatments included the basic diet or basic diet supplemented with 75 mg/kg of Zn as feed-grade Zn sulfate from conventional inorganic sources or Zn-methionine inorganic Zn compounds. The ingredients and the nutrient composition of the basal diets are shown in *Table 1*.

Determination of Body Weight and Feed Intake

Broilers were weighed during the study period to determine body weight (BW) and body weight gain (BWG) at weekly intervals. Feed consumption (FC) was observed weekly. Feed conversion ratio (FCR) was calculated as feed-to-gain ratio on day 7, 14, 21, 28, 35 and 42 and over days 1 to 42 of the experiment.

Sample Collection and Analysis

For determination of the digestibility of nutrients at 28 days of age, clean stainless steel collection trays were placed under each cage (12 per treatment) and excreta from each group were collected for 72 h [25]. A sub-sample of excreta was collected in polyethylene bags, weighed and dried. Excreta were mixed thoroughly, frozen at –20°C and freeze-dried. The feed and dried excreta samples were ground to pass through a 0.5 mm screen and then mixed thoroughly before analysis. The components of the samples were determined according to the standard

Table 1. *Ingredient and chemical composition (as-fed basis) of the basal diet (%)*
Tablo 1. *Temel rasyonun yem ham maddeleri ve besin madde bileşimi*

Ingredient	Starter (days 1-21)		Grower (days 22-42)	
	PC	NC (Low P)	PC	NC (Low P)
Maize	48.70	48.50	53.00	54.00
Wheat	1.20	2.10	2.00	2.00
Soybean meal (46.50 % CP)	41.20	41.00	34.40	34.00
Soybean oil	5.30	5.10	7.00	6.70
Limestone	1.00	1.55	1.00	1.55
Dicalcium phosphate [1]	1.85	1.00	1.85	1.00
Vitamin premix [2]	0.10	0.10	0.10	0.10
Zn-free mineral premix [3]	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
DL-Methionine	0.15	0.15	0.15	0.15
Total	100.00	100.00	100.00	100.00
Analysed nutrient content				
Dry matter	90.37	90.42	90.65	90.28
Metabolizable energy [4], kcal/kg	3035	3063	3179	3185
Crude protein, %	22.80	23.20	19.86	20.15
Crude fat, %	7.80	7.50	9.82	9.35
Starch, %	29.00	30.05	31.00	32.00
Sugar, %	5.93	5.80	5.90	5.75
Crude fiber, %	3.79	3.84	3.48	3.25
Ash, %	5.94	5.58	5.61	5.34
Ca, %	0.87	0.89	0.92	0.85
$P_{Available}$, %	0.43	0.31	0.47	0.28

[1] Contains 23% Ca and 18.10% available P; [2] Supplied per kilogram of diet: Vitamin A, 15.000 IU; cholecalciferol, 1.500 ICU; vitamin E, 30.0 IU (dl-α-tocopheryl acetate); menadione, 5.0 mg; thiamine, 3.0 mg; riboflavin, 6.0 mg; niacin, 20.0 mg; panthotenic acid, 8.0 mg; pyridoxine, 5.0 mg; folic acid, 1.0 mg; vitamin B_{12}, 15 mcg; [3] Supplied per kilogram of diet: 80 mg of iron as $FeSO_47H_2O$, 6 mg of copper as $CuSO_45H_2O$, 60 mg of manganese as $MnSO_4H_2O$, 0.35 mg of iodine as KIO_3, and 0.15 mg of selenium as sodium selenite; [4] Metabolizable energy was calculated using the equation of Carpenter and Clegg [24]

AOAC methods [26] for crude protein, ether extract and Zn. Digestibility of any given nutrient can be calculated as follows:

Nutrient digestibility (%) = [(Nutrient intake - Nutrient in feces)/Nutrient intake] x 100

At 42 days, 30 broilers from each group (6 chicks from each replicate) were randomly selected and bled from the brachial vein. Blood samples were transferred to vacutainer tubes with no anticoagulant. After sampling, tubes were centrifuged at 3000 rpm for 10 min and then left at 37°C for 30 min. Serum samples were then transferred to 2-ml eppendorf microcentrifuge tubes and stored at -20°C prior to analysis. Serum triglyceride, cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) and glucose levels were determined using an autoanalyser (AMS Vegasys Chemistry Analyzer) and commercial kits (Audit Diagnostics, Ireland). Serum insulin values were determined using a plate reader (Das, Italy) and its commercial kits. Serum, feed and feces levels of Zn were measured with an atomic absorption spectrophotometer (Perkin ElmerAAnalyst 100). The levels of phosphorus in serum were measured with a spectrophotometer (Shimadzu UV-1700 Pharma Spec, Japan) by using commercial kit (RANDOX-Co., UK) at 340 nm absorbance.

Statistical Analysis

Data were processed with analysis of variance (ANOVA) using the Least Square Method of the SAS GLM procedure [27]. The differences among the means of groups were determined by using Duncan's multiple-range test (P<0.05). All results were summarized as mean ± standard error of means.

RESULTS

The effects of Zn supplementation from either an organic or inorganic source and microbial phytase on broiler body weight, body weight gain, feed consumption and feed conversion are presented in *Table 2* and *3*. The means of a number of serum parameters, survival rates and digestibility of diet components are presented in *Table 4, 5* and *6*, respectively.

DISCUSSION

The average live weights obtained in the PC, NC, PH, OZ, IZ, OZ + PH and IZ + PH groups were 2638.2, 2497.5, 2647.4, 2577.5, 2562.1, 2757.0 and 2733.9g, respectively. At the end of the research, birds fed the OZ + PH and IZ + PH diets had higher (P<0.05) body weight and body weight gain than the other groups (*Table 2* and *3*). The present study indicated that phytase supplementation of broiler diets low in phosphorus increased body weight, which was in agreement with previous reports [28,29]. Davies and Nightingale [3] reported that the reduction in Zn availability due to its binding by phytate decreased chicken growth rate. Roberson and Edwards [30] reported that 15 to 30 mg/kg of Zn supplementation increased growth rate in broilers. Burrell *et al.*[9] showed that during a 45-d study, addition of Zn to the basal diet (0, 20, 40, and 80 mg/kg of Zn from $ZnSO_4$) significantly increased body weight gain in broilers reared under thermoneutral (TN) conditions. This is supported by the results of the present study. Better feed conversion efficiencies (P<0.05) were obtained with diets containing OZ + PH and IZ + PH *(Table 3)*. These results match those of Ao *et al.*[31], Broz *et al.*[32] and Sebastian et al.[33]. There were no significant differences (P>0.05) between the control and treatment groups with regard to mean feed intake *(Table 3)*. The results of the present study also indicated that survival rates were not significantly different (P>0.05) between control and treatment groups *(Table 5)*. Burrell *et al.*[9] also reported that addition of Zn to the basal diet did not affect mortality.

In poultry nutrition, it is difficult to exclude phytates as they are the main storage forms of phosphorus in seeds. Diets based on corn and soybean meal generally contain between 2.0 and 2.5 g phytic phosphorus per kilogram.

Table 2. *Body weight mean of groups during experimental period, g (mean ± S.E.M)*
Tablo 2. *Deneme süresince grupların ortalama canlı ağırlıkları, g (ortalama ± S.E.M)*

Weeks	Control		Treatment Groups					P
	PC	NC	PH	OZ	IZ	OZ+PH	IZ+PH	
0	45.0±0.33	45.1±0.29	45.0±0.31	45.8±0.32	44.9±0.30	45.5±0.31	45.1±0.32	NS
1	150.1±1.37b	143.2±1.28c	152.0±1.56ab	148.8±1.53b	147.7±1.24b	154.8±1.66a	154.4±1.57a	*
2	411.4±5.09bc	375.2±5.44e	417.4±6.53abc	403.48±6.65dc	392.0±5.24d	431.2±5.92a	422.1±5.96ab	*
3	797.4±11.60abc	753.3±10.07d	810.2±10.61a	774.8±9.63bcd	769.6±10.16cd	822.7±11.52a	805.6±10.45ab	*
4	1390±18.10b	1305±17.72c	1412±20.66b	1369±18.77b	1365±17.40b	1469±19.09a	1466±17.29a	*
5	1997±24.14bc	1855±23.91d	2005±26.20b	1946±22.20bc	1930±28.87c	2108±22.05a	2092±22.97a	*
6	2638±29.90bc	2498±32.14d	2647±27.44b	2578±22.86bc	2562±27.40cd	2757±27.39a	2734±23.81a	*

NS: *Non significant;* * *P<0.05;* ***a,b,c,d,e:*** *The mean values within the same row with different superscript differ significantly (P<0.05);* ***PC:*** *Positive control,* ***NC:*** *Negative control,* ***PH:*** *Phytase,* ***OZ:*** *Organic zinc,* ***IZ:*** *Inorganic zinc,* ***OZ+PH:*** *Organic zinc + phytase,* ***IZ+PH:*** *Inorganic zinc + phytas*

Table 3. *Means of the body weight gain, feed intake and feed:gain ratio by groups (mean ± S.E.M)*
Tablo 3. *Deneme gruplarının ortalama canlı ağırlık artışı, yem tüketimi ve yemden yararlanma oranı (ortalama± S.E.M)*

Weeks	Control		Treatment Groups					P
	PC	NC	PH	OZ	IZ	OZ+PH	IZ+PH	
Body weight gain (g/bird)								
1	105.1±1.19bc	98.1±1.05d	107.1±1.30 ab	103.1±1.32c	102.8±0.98c	109.3±1.39a	109.3±1.30a	*
2	261.7±4.50b	231.9±4.23d	265.1±5.05ab	254.6±5.33bc	244.1±4.1dc	276.4±4.46a	267.7±4.63ab	*
3	382.5±7.40ab	376.3±5.30ab	391.1±6.84a	370.5±4.10b	377.6±7.04ab	390.4±6.30ab	383.5±7.42ab	*
4	592.3±8.46b	550.00±8.34c	602.0±12.55b	592.1±11.97b	594.2±12.47b	643.1±8.47a	660.7±12.34a	*
5	603.3±11.96ab	544.7±7.73e	589.9±7.80bcd	577.9±12.80cde	561.4±17.6ed	638.5±12.48a	625.3±13.82ab	*
6	634.6±10.80	625.1±11.04	642.5±8.89	627.9±6.15	629.6±13.47	644.9±12.76	642.3±6.93	NS
0-6	2593±29.60bc	2452±31.90d	2602±27.16b	2532±22.63bc	2517±27.16cd	2711±27.11a	2689±23.56a	*
Feed intake (g/bird, as-fed basis)								
1	122.0±2.08	119.7±1.83	124.0±2.75	125.8±2.85	124.4±0.91	125.7±2.13	124.6±2.70	NS
2	325.1±9.49	306.0±2.68	329.0±11.13	330.2±10.35	312.6±4.95	337.1±3.88	324.0±16.08	NS
3	33.3±19.24	548.4±19.80	536.3±36.24	522.3±15.25	530.8±45.25	516.3±16.30	497.28±27.98	NS
4	88.8±19.36	871.1±14.59	896.8±36.10	909.4±67.49	903.6±60.13	925.6±12.80	958.6±75.52	NS
5	95.7±52.52	951.9±30.24	962.1±21.56	970.5±84.35	949.18±145.23	1009.5±86.83	972.52±79.79	NS
6	1136±39.17	1200±48.23	1127±45.05	1158±16.65	1137±103.82	1104±60.77	1096±26.27	NS
0-6	4001±24.89	3997±70.90	3975±59.18	4016±41.61	3958±93.69	4018±35.34	3973±53.81	NS
Feed:Gain ratio								
1	1.16±0.008b	1.22±0.007a	1.16±0.006b	1.22±0.010a	1.21±0.007a	1.15±0.007b	1.14±0.007b	*
2	1.24±0.011c	1.32±0.011a	1.24±0.007c	1.29±0.009b	1.28±0.007b	1.22±0.010dc	1.21±0.007d	*
3	1.38±0.007dc	1.45±0.007a	1.36±0.015d	1.41±0.007b	1.40±0.012bc	1.32±0.011e	1.30±0.007e	*
4	1.50±0.011b	1.58±0.013a	1.49±0.011b	1.53±0.013b	1.52±0.016b	1.43±0.013c	1.45±0.016c	*
5	1.64±0.011cd	1.73±0.016a	1.62±0.012d	1.68±0.015b	1.67±0.007bc	1.58±0.011e	1.56±0.012e	*
6	1.77±0.011cd	1.88±0.014a	1.75±0.007d	1.83±0.011b	1.80±0.016bc	1.70±0.011e	1.68±0.011e	*
0-6	1.54±0.011cd	1.63±0.010a	1.53±0.010d	1.58±0.010b	1.57±0.013bc	1.48±0.011e	1.47±0.009e	*

NS: *Non significant; * P<0.05;* ***a,b,c,d,e:*** *The mean values within the same row with different superscript differ significantly (P<0.05);* ***PC:*** *Positive control,* ***NC:*** *Negative control,* ***PH:*** *Phytase,* ***OZ:*** *Organic zinc,* ***IZ:*** *Inorganic zinc,* ***OZ+PH:*** *Organic zinc + phytase,* ***IZ+PH:*** *Inorganic zinc + phytas*

Table 4. *Means of the some blood serum parameters by groups[$] (mean ± S.E.M)*
Tablo 4. *Grupların ortalama bazı kan parametreleri (ortalama ± S.E.M)*

Parameters	Control		Treatment Groups					P
	PC	NC	PH	OZ	IZ	OZ+PH	IZ+PH	
Triglyceride, mg/dl	323.7±12.6a	224.9±11.3cd	265.4±17.3bc	305.0±18.5ab	236.9±17.2cd	247.1±15.4cd	212.3±8.4d	*
Cholesterol, mg/dl	187.5±2.5a	190.4±3.4a	190.9±2.6a	177.9±1.8b	186.8±2.3a	171.4±1.8c	177.4±1.7bc	*
LDL C, mg/dl	78.2±4.6a	76.6±2.9a	75.8±4.3ab	72.2±9.5ab	70.3±6.2ab	60.2±1.9b	68.6±4.8ab	*
HDL-C, mg/dl	102.0±3.6ab	84.6±5.8b	106.5±7.1a	101.6±9.7ab	102.7±6.0ab	98.5±7.4ab	105.9±3.0a	*
Glucose, mg/dl	217.3±14a	181.3±6.7b	192.2±9.5ab	177.7±7.5b	196.1±8.6ab	187.9±6.4b	200.9±8.3ab	*
Insulin µIU/ml	3.6±0.01a	3.6±0.02a	3.6±0.03a	3.5±0.02ab	3.5±0.01b	3.5±0.01b	3.45±0.01b	*
P, mg/dl	4.46±0.07ab	4.06±0.11d	4.29±0.06bc	4.14±0.10cd	4.01±0.04d	4.54±0.09a	4.62±0.10a	*
Zn, µg/dl	40.5±6.5c	34.6±4.7c	89.07±7.9b	107.2±10.6b	184.7±10.8a	172.2±11.1a	165.9±8.8a	*

** P<0.05;* ***a,b,c,d,e:*** *The mean values within the same row with different superscript differ significantly (P<0.05);* ***PC:*** *Positive control,* ***NC:*** *Negative control,* ***PH:*** *Phytase,* ***OZ:*** *Organic zinc,* ***IZ:*** *Inorganic zinc,* ***OZ+PH:*** *Organic zinc + phytase,* ***IZ+PH:*** *Inorganic zinc + phytas, [$] Blood samples were collected to assess biochemical variables related to lipit and mineral metabolism on d 28 of study*

Table 5. *Means of survival rates by groups (mean ± S.E.M)*
Tablo 5. *Deneme boyunca grupların yaşama gücü (ortalama ± S.E.M)*

Days	Control		Treatment Groups					P
	PC	NC	PH	OZ	IZ	OZ+PH	IZ+PH	
0	100	100	100	100	100	100	100	NS
7	100	100	100	100	100	100	100	NS
14	99.20	99.20	99.20	100	99.20	100	100	NS
21	96.00	99.20	98.40	99.20	100	99.20	99.20	NS
28	99.20	99.20	97.60	99.20	99.20	98.40	98.40	NS
35	99.20	98.40	99.20	100	99.20	100	100	NS
42	97.60	96.00	100	99.20	99.20	99.20	99.20	NS
0-42	98.74	98.86	99.20	99.66	99.54	99.54	99.54	NS

NS: *Non significant,* ***PC:*** *Positive control,* ***NC:*** *Negative control,* ***PH:*** *Phytase,* ***OZ:*** *Organic zinc,* ***IZ:*** *Inorganic zinc,* ***OZ+PH:*** *Organic zinc + phytase,* ***IZ+PH:*** *Inorganic zinc + phytas*

Table 6. *Means of nutrient digestibility in groups, % (mean ± S.E.M)*
Tablo 6. *Gruplarda ortama besin madde sindirilebilirliği, % (ortalama ± S.E.M)*

Parameters	Control		Treatment Groups					P
	PC	NC	PH	OZ	IZ	OZ+PH	IZ+PH	
Dry matter	73.6±1.13ab	75.2±1.51ab	76.7±0.74 a	72.7±1.56b	77.3±1.41a	77.2±1.06a	75.3±0.31ab	*
Crude ash	41.8±3.47ab	44.4±2.03ab	44.3±2.89ab	29.4±1.18c	48.2±3.9a	36.7±2.42bc	39.7±1.49b	*
Crude protein	64.5±2.29c	70.3±2.79abc	70.3±1.18abc	66.0±2.34bc	71.8±1.98ab	74.1±2.23a	67.8±0.72abc	*
Zn	12.9±0.86c	12.6±0.68c	12.6±0.44c	30.4±2.03a	28.6±1.92ab	27.3±2.06ab	25.1±1.64b	*

NS: *Non significant, * P<0.05;* ***a,b,c,d,e:*** *The mean values within the same row with different superscript differ significantly (P<0.05);* ***PC:*** *Positive control,* ***NC:*** *Negative control,* ***PH:*** *Phytase,* ***OZ:*** *Organic zinc,* ***IZ:*** *Inorganic zinc,* ***OZ+PH:*** *Organic zinc + phytase,* ***IZ+PH:*** *Inorganic zinc + phytas*

Zinc content in feed components of plant origin is positively correlated with the phytic phosphorus content, with ~10 mg of Zn to 1 g phytic phosphorus [34]. However, zinc absorption is reduced whenever diets are high in phytate. The present study indicated that the use of organic and inorganic Zn alone or in combination with microbial phytase significantly increased (P<0.05) the digestibility of Zn. Furthermore, because of the poor availability of Zn in plant feed ingredients due to the binding of Zn by phytate, the present study also revealed that the use of organic and inorganic Zn alone or in combination with microbial phytase are necessary in the diet of broilers. Sahin and Kucuk [35] reported that increasing supplemental Zn from 0 mg/kg to 30 mg/kg and 60 mg/kg linearly increased digestibility of dry matter, crude protein and ether extracts. The present study also indicated that better digestibility of dry matter, crude ash and ether extracts were obtained in the group given a diet supplemented with inorganic Zn *(Table 6)*.

In the present study, supplementation with PH, OZ, IZ, OZ + PH and IZ + PH significantly increased (P<0.05) serum concentrations of Zn in broilers *(Table 4)*. The results indicate that phytate, which is present in plant feed ingredients, has a strong negative effect on zinc absorption from composite meals, because the level of zinc in NC and PC is found very low rather than treatment groups. Therefore, the present study proves that OZ + PH and IZ + PH should be added to diets of broilers. That proposal is supported by the results of Ao *et al.*[31] who reported that dietary Zn supplementation linearly increased plasma Zn concentrations in broilers kept under TN conditions. Furthermore, Zhou *et al.*[36] determined that supplementation of broiler diets with phytase increased Zn content in the plasma at 42 d, which also concurs with the results of the present study.

Zinc positively affects feed utilization through participating in the metabolism of carbohydrates, lipids and proteins [37]. However, it must be supplemented to most diets of poultry [5-7]. The digestibility of Zn by male broilers was lower in the microbial phytase, NC and PC than in the organic and inorganic Zn alone or in combination with microbial phytase *(Table 6)*. Spears [38] and Wedekind *et al.*[20] reported greater bioefficacy for organic Zn sources than that observed for inorganic forms, including Zn oxide and Zn sulfate; consequently, organic forms of the trace element have been used with increasing frequency by the feed industry. The negative effects on broilers of diets with low phosphorus levels may be overcome by the addition of inorganic or organic Zn compounds in combination with microbial phytase. Accordingly these

results, the use of OZ + PH and IZ + PH in broiler ration exhibit a similar effect. So, it may be preferred because IZ + PH is economically more proper than OZ + PH.

Zinc is required for normal protein synthesis and metabolism, and it is also a component of insulin, which has a role in carbohydrate metabolism. Zinc is necessary for the proper functioning of many enzymatic systems, and the insulin system is probably the most important one. Because zinc plays so many important metabolic roles in the body, it is an essential element in the diet of poultry. Supplementation of the diet with OZ + PH and IZ + PH decreased cholesterol and insulin levels ($P<0.05$) compared to control groups. Furthermore, supplementation of diets with only organic or inorganic Zn did not increase insulin levels. On the contrary, they decreased insulin levels.

Zinc is also involved in lipid metabolism. The present study indicated that higher levels of serum Zn decreased the serum cholesterol levels of the OZ + PH and IZ + PH groups. Triglyceride values were highest in the PC group and lowest in the IZ + PH group *(Table 5)*. Herzig *et al.*[39] demonstrated that there was a significant decrease of plasma cholesterol when high amounts of Zn were administered to broiler chickens. Aksu *et al*.[40] also reported the decrease of total cholesterol and LDL cholesterol, combined with the increase in HDL cholesterol, in the blood plasma of chickens when the feed mixtures were supplemented with organic complexes of Zn. In contrast, Kucuk *et al.*[41] did not report any significant changes in the concentrations of total cholesterol, triglycerides and glucose when a feed mixture was supplemented with 30 mg of Zn per kg. Furthermore, Lu and Combs [42] reported that inorganic Zn did not affect the serum cholesterol level. Their finding was in agreement with that of the present study. However, Boukaiba *et al.*[43] and Uyanık *et al*.[44] reported that dietary supplementation with inorganic Zn decreased the serum cholesterol level.

The results of the current study indicate that dietary Zn supplementation increases serum Zn levels in male broilers. The low availability of Zn in plant feed ingredients caused by the binding of Zn by phytate means that the use of organic and inorganic Zn alone or in combination with microbial phytase is necessary in their diet. Morever, higher serum Zn levels in the OZ + PH and IZ + PH groups decreased serum cholesterol levels. Supplementation of low phosphorus broiler diets with OZ + PH or IZ + PH was very effective for increasing the body weight, body weight gain and the feed conversion ratio. To summarize, the negative effects on broilers of diets with low phosphorus levels may be overcome by the addition of inorganic or organic Zn compounds in combination with microbial phytase.

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RESEARCH ARTICLE

The Cost-Benefit Analysis of Alternative Brucellosis Control Strategies in Turkey [1]

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[1] *This study was summarized a part of PhD thesis entitled "Estimation of the financial losses resulted from Brucella abortus and Brucella melitensis infections and cost-benefit analysis of alternative brucellosis control strategies in Turkey"*

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Summary

Brucellosis is a zoonotic disease and leads to serious financial losses in infected species. The aim of this study is to determine the most financially rational brucellosis control strategy for Turkey by means of cost-benefit analyses. In this study, four different infection control strategies were designed under three different scenarios named optimistic, expected and pessimistic scenarios. The most financially rational infection control option for Turkey was found to be the second strategy, which is "only young animals, three to six month old female bovine and three to six month old male and female ovine, have been vaccinated and after reaching the target prevalence for each species, vaccinations will be terminated and in the same year test and compulsory slaughter methods will be implemented throughout the country". For the optimistic, expected and pessimistic scenarios according to second strategy the net present value was estimated as -$3.1 million, $29.2 million and $41.9 million respectively, the benefit-cost ratio was estimated 0.86, 2.26 and 2.84 respectively. The results of this study indicated that fighting with brucellosis is financially rational for expected and pessimistic scenarios. However, it should not be forgotten that a financially rational control strategy doesn't means that it is always suitable technically or it is rational in respect to public health.

Keywords: *Brucellosis, Bovine, Control, Cost, Financial, Prevalence*

Türkiye'de Alternatif Bruselloz Kontrol Stratejilerinin Maliyet-Fayda Analizi

Özet

Bruselloz zoonotik bir hastalıktır ve enfekte türlerde ciddi mali kayıplara yol açmaktadır. Bu çalışmanın amacı Türkiye için mali açıdan en rasyonel bruselloz kontrol stratejisinin maliyet-fayda analizleriyle belirlenmesidir. Çalışma kapsamında dört farklı enfeksiyon kontrol stratejisi iyimser, beklenen ve kötümser olmak üzere üç farklı senaryo altında dizayn edilmiştir. Türkiye için mali açıdan en rasyonel enfeksiyon kontrol seçeneğinin ikinci strateji olan "büyükbaş hayvanlar için üç-altı aylık dişilerin, küçükbaş hayvanlar için üç-altı aylık dişi ve erkek genç hayvanların aşılanması ve her bir türde hedef prevalans düzeyine ulaşıldıktan sonra aşılamanın sonlandırılarak aynı yıl test ve zorunlu kesim yöntemlerinin ülke genelinde uygulanması" olduğu belirlenmiştir. İkinci strateji kapsamında iyimser, beklenen ve kötümser senaryolar için net bugünkü değer sırasıyla -$3.1, $29.2 ve $41.9 milyon; fayda-maliyet oranı ise sırasıyla 0.86, 2.26 ve 2.84 olarak tahmin edilmiştir. Bu çalışmanın sonuçları brusellozla mücadelenin beklenen ve kötümser senaryolar için mali açıdan rasyonel olduğunu göstermiştir. Bununla beraber, mali açıdan rasyonel bir kontrol stratejisinin, her zaman için teknik olarak da uygun veya halk sağlığı açısından da rasyonel anlamı taşımayacağı unutulmamalıdır.

Anahtar sözcükler: *Bruselloz, Büyükbaş, Kontrol, Maliyet, Mali, Prevalans*

INTRODUCTION

Brucellosis is a zoonotic disease that causes serious health problems in animals and humans. The disease leads to serious financial losses as it causes reductions in the performance of infected animals and humans, leads to

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yield and labor losses, and entails diagnosis and treatment expenditures. Country-wide total financial losses for 2009 in Turkey caused by brucellosis, respectively in optimistic, expected and pessimistic scenarios, were calculated as $20.066.875, $41.337.446 and $61.711.571 [1].

Disease reports and serosurveys on brucellosis in Turkey indicated that, despite country-wide infection control efforts over many years, the disease is still prevalent [2-7]. In 2010, the numbers of *B.abortus* and *B. melitensis* outbreaks in livestock enterprises for cattle and small ruminants were reported as 412 and 199 respectively; also brucellosis infected human cases were reported as 7703 [8,9]. This situation clearly indicates that the disease causes large country-wide financial losses.

For the assignment of resources needed in the fight against the infection, the submission of proper reasons to public authorities is required. For this reason, it is necessary to perform economical and/or financial analysis to clearly present the costs and benefits of infection control strategies. In this study, alternative infection control strategies were designed under three different scenarios and cost-benefit analyses were performed to determine the most financially rational brucellosis control strategy for Turkey.

MATERIAL and METHODS

Data Collection

The required data was provided by Delphi Expert Opinion Surveys (DEOS) conducted in two rounds with 32 specialist veterinarians, Republic of Turkey Ministry of Food, Agriculture and Livestock (TMFAL) and the TURKVET veterinary-information system. The DEOS were conducted over two sessions in Ankara, Sinop, Igdir, Hatay and Balikesir provinces, which are located in five different geographical regions of Turkey. Data collection started in July 2008 and continued until the end of September 2009.

Designed Alternative Infection Control Strategies

It is indicated that the theoretical base for the control of brucellosis exist over fifty years and comprehensive elimination schemes have been successfully operated in many countries [10]. Considering the technical and financial resources of TMFAL and legal regulations in Turkey, four separate brucellosis control strategies were designed as follows:

According to 1st strategy, all of the young and adult animals have been vaccinated. After reaching/achieving the target prevalence (0.1%) for each species, vaccinations will be terminated and in the same year test and compulsory slaughter methods will be implemented throughout the country. In accordance with test and compulsory slaughter methods, positive detected animals will be sent to slaughterhouse. Payments are only made to positive cattle according to compensation policy of TMFAL and there is no any compensation payment for positive ovine in Turkey.

According to 2nd strategy, only young animals, three to six month old female bovine and three to six month old male and female ovine have been vaccinated. After reaching the target prevalence for each species, 0.1%, vaccinations will be terminated and in the same year test and compulsory slaughter methods will be implemented throughout the country. In accordance with test and slaughter methods, positive detected animals will be sent to slaughterhouse.

According to 3rd strategy, only young animals, three to six month old female bovine and three to six month old male and female ovine, have been vaccinated throughout the country. Test and compulsory slaughter methods have been implemented simultaneously in only infection/outbreak zones. In other words, vaccination, test and compulsory slaughter practices have been combined and started from the first year, without reaching target prevalence. These practices will continue to until reaching the target prevalence. This strategy has put into practice after 2008 by TMFAL.

According to 4th strategy, all of the young and adult female animals have been vaccinated in provinces where 1% or more prevalence is observed and also in infection/outbreak zones. Test and compulsory slaughter methods have been implemented simultaneously in only infection zones. In other words, vaccination, test and compulsory slaughter practices have been combined and started from the first year, without reaching target prevalence. These practices will continue to until reaching the target prevalence. This strategy was being implemented before 2008 by TMFAL.

Cost-Benefit Analysis for Different Control Strategies

In this study, the costs incurred by brucellosis control strategies consist of vaccinations, testing, diagnosis, compulsory slaughter, transport and workforce expenditure in application; while the benefit of these strategies is reduction of disease originated losses by the reduction of the prevalence of brucellosis. The study is taken as reference for the Turkey-wide, brucellosis-originated financial losses [1]. We also taking into account last 10-year average inflation and interest rates for choosing the appropriate discount rate is 10%. Unit values of the some of the important parameters are given in *Table 1*.

Due to the unreliability and/or lack of the some required data, an advanced model could not be generated. In this study, simple mathematical equations were used in order to make a prediction for the future prevalence of

Table 1. *Some of the important parameters considered in financial analysis*
Tablo 1. *Mali analizlerde dikkate alınan bazı önemli parametreler*

Parameters	Value	Source
The total number of bovines in Turkey	10 859 942	TSI[1]
The total number of ovines in Turkey	29 568 152	
The total number of three to six month old female bovine in Turkey	434 398	TSI[1] and TVIS[2]
The total number of three to six month old male and female ovine in Turkey	1 478 407	
The total number of annually vaccinated bovines	434 397	TVIS[2]
The total number of annually vaccinated ovines	1 478 407	
Total number of compulsory slaughter cattle	3840	
The number of outbreaks resulted from *B. abortus,* in 2009	865	
The number of outbreaks resulted from *B. melitensis,* in 2009	131	
The number of villages in Turkey	35 000	TSI[1]
Average number of samples sent from an enterprise in which infection was observed	10	Survey
Average number of visits to livestock enterprises infection is detected, until the eradication of the infection	9	
Average time spent for bureaucratic procedures like data entry (hour)	4	
Average time spent in a laboratory to analyze one sample (hour)	4	
Average time spent in the field for vaccinations (hour)	8	
Target prevalence values for bovine and ovines	0.1%	Assumption
Initial prevalence of brucellosis in bovine	1.43%	TMFAL[3]
Initial prevalence of brucellosis in ovine	1.97%	
Expected immunization rate after the vaccination	75%	VCRI[4]
Discount rate chosen for the financial analysis	10%	TSI[1]
The cost of one dose of vaccine for young cattle	$0.73	TMFAL[3]
The cost of one dose of vaccine for adult cattle	$0.47	
The cost of one dose of vaccine for ovines	$0.14	
Average compensation paid by the state for an infected bovines	$617	
Average compensation paid by the state for an infected ovines	$89	
Average daily workforce costs for veterinarians	$9	Survey and Calculation
Average daily workforce costs for medical technicians	$6	
Average daily workforce costs for drivers	$5	
Average daily workforce costs for laboratory specialist	$11	
Average costs of analysis of suspected samples in a laboratory	$24	
Average cost of medical tools and equipment (Blood collecting tube, injection syringe, cannula, etc.) used in a village	$11	
Average cost of transportation to go to the village	$28	

[1] Turkish Statistical Institute, [2] TURKVET Veterinary Information Systems, [3] The Ministry of Food, Agriculture and Livestock, [4] Veterinary Control and Research Institutes

brucellosis. Prevalence models were used to determine the benefits obtained by applying the strategies. The fundamental factors of these models were based on the calculation of the effects on prevalence of the number of animals immunized through vaccination and brucellosis positive animals are sent to compulsory slaughter.

For every strategy, initial prevalence values were taken as 1.43% for cattle and 1.96% for ovines; these values were taken from the most extensive country-wide sero-survey results conducted by the TMFAL [2]. Sensitivity and specificity of the Complement Fixation Test are taken into account 89.0% and 83.5%, respectively [11]. The period required to reach 0.1% prevalence was used to determine the application period for each strategy. In other words, time period was determined by prevalence values of the different strategies. Detailed explanations related to models were given below;

1st model

For bovines

End of the 1st year value, $P_{B1} = [((B \times P_B) - (V_B \times I_B)) + C_s)]/B$

End of the 2nd year value, $P_{B2} = [((B \times P_{B1}) - (V_B \times I_B)) + (C_s \times (PB_1/PB_0))]/B$

End of the 3rd year value, $P_{B3} = [((B \times P_{B2}) - (V_B \times I_B)) + (C_s \times (PB_2/PB_1))]/B$

For ovines

End of the 1st year value, $P_{O1} = [(O \times PO_0) - (V_O \times I_O)]/O$

End of the 2nd year value, $P_{O2} = [(O \times PO_1) - (V_O \times I_O)]/O$

End of the 3rd year value, $P_{O3} = [(O \times PO_2) - (V_O \times I_O)]/O$

Note: For bovines and ovines, similar formulas were used for following years until the prevalence value reach targeted value.

Where;

B = The total number of bovines in Turkey

O = The total number of ovines in Turkey

V_B = The total number of annually vaccinated bovines

V_O = The total number of annually vaccinated ovines

P_B = Initial prevalence of brucellosis in bovine

P_O = Initial prevalence of brucellosis in ovine

P_{B1} = End of the 1st year prevalence in bovine

P_{O1} = End of the 1st year prevalence in ovine

P_{B2} = End of the 2nd year prevalence in bovine

P_{O2} = End of the 2nd year prevalence in ovine

I_B = Expected immunization rate after the vaccination in bovines

I_O = Expected immunization rate after the vaccination in ovines

C_s = Total number of compulsory slaughter cattle

2nd model: The differences of the second strategy from first one are as follows,

B_{3-6} = The total number of three to six month old female bovine in Turkey

O_{3-6} = The total number of three to six month old male and female ovine in Turkey

There is no another change in the formula.

For bovines and ovines:

End of the 1st year value, $P_{B1} = [((B_{3-6} \times P_B) - (V_B \times I_B)) + C_s)]/B$

End of the 1st year value, $P_{O1} = [(O_{3-6} \times PO_0) - (V_O \times I_O)]/O$

3rd model: The differences of the third strategy from second one are as follows,

In this strategy, vaccination, test and compulsory slaughter practices have been combined and started from the first year, without reaching target prevalence. Therefore, "C_s" should be removed from the total number of infected bovines. In another words, it is contributes to decreasing of prevalence. There is no another change in the formula.

For bovines

End of the 1st year value, $P_{B1} = [((B \times P_B) - (V_B \times I_B)) - C_s)]/B$

4th model: The differences of the fourth strategy from third one are as follows,

B = The total number of young and adult female bovines have been vaccinated in provinces where 1% or more prevalence is observed and also in infection zones.

O = The total number of young and adult ovines have been vaccinated in provinces 1% or more prevalence is observed and also in infection zones.

There is no another change in the formula.

RESULTS

The costs and benefits of the application of four different brucellosis control strategies and the Net Present Value (NPV) and Benefit-Cost Ratio (BCR) were summarized in *Table 2, Table 3,* and *Fig. 1* below.

Table 2. *Costs of the alternative brucellosis control strategies (U.S. Dollar)*
Tablo 2. *Alternatif bruselloz kontrol stratejilerinin maliyetleri (Amerikan Doları)*

Cost Components	1st Strategy	2nd Strategy	3rd Strategy	4th Strategy
I. Total vaccination expenses	6.062.151	324.878	818.822	430.791
I. a. Vaccination of bovines	3.940.398	201.424	327.529	185.240
I. b. Vaccination of ovines	2.121.753	123.454	491.293	245.551
II. Transport and workforce	5.442.500	3.211.250	3.211.250	1.948.549
II. a. Transportation	979.650	995.487	995.487	350.738
II. b. Workforce	4.462.850	2.215.763	2.215.763	1.597.811
III. Test, diagnosis and compulsory slaughtering	2.922.550	2.922.550	4.662.033	4.662.033
III. a. Laboratory analyses of samples	1.519.726	1.519.726	1.538.471	1.538.470
III. b. Medical tools used in screening tests	350.706	350.706	-	-
III. c. Compensation payment	29.225	29.225	2.377.637	2.377.637
III. d. Workforce and bureaucratic procedures	1.022.893	1.022.893	745.925	745.926
Total Costs by the end of projected period	$60.445.805	$38.283.830	$52.152.630	$42.248.238

Table 3. *Results of the financial analysis for the different strategies and scenarios.*
Tablo 3. *Farklı strateji ve senaryolar için mali analizlerin sonuçları*

Financial Appraisal	Scenarios	1st Strategy	2nd Strategy	3rd Strategy	4th Strategy
NPV [1]	Optimistic	- 16.6	- 3.1	- 16.0	- 5.9
	Expected	25.5	29.2	25.0	32.3
	Pessimistic	49.2	41.9	35.8	50.8
BCR [2]	Optimistic	0.63	0.86	0.58	0.80
	Expected	1.56	2.26	1.66	2.06
	Pessimistic	2.08	2.84	1.95	2.67

[1] Net Present Value (million US Dollar, [2] Benefit-Cost Ratio

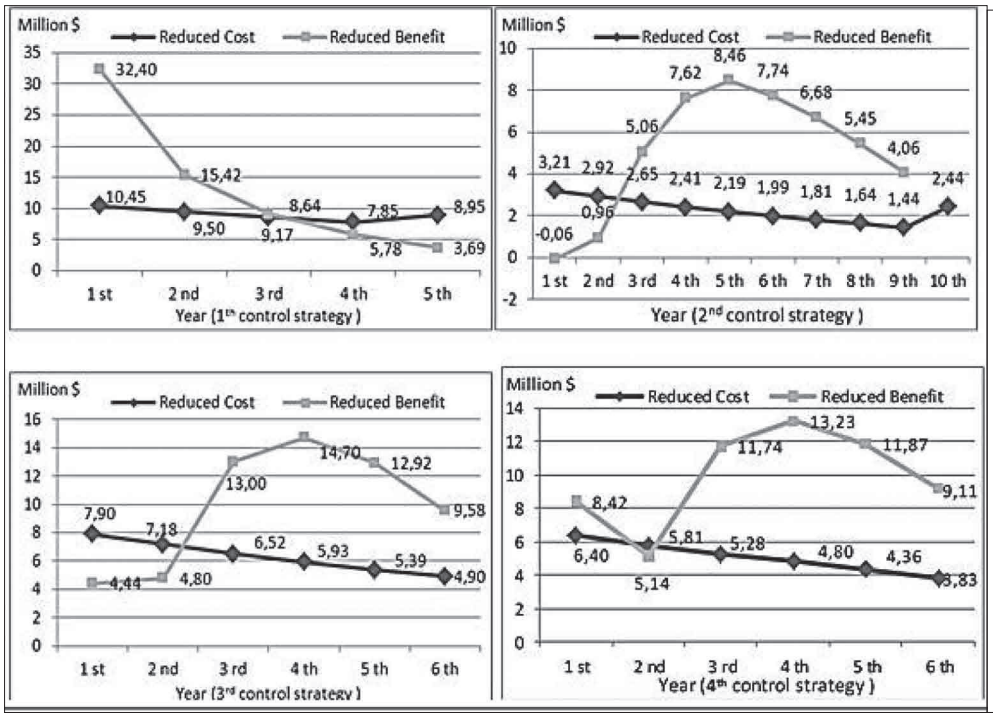

Fig 1. Reduced costs and benefits for different control strategies by years

Şekil 1. Farklı stratejiler için yıllar itibariyle indirgenmiş maliyet ve faydalar

For the first strategy, the highest expenditures are 50% for "country-wide vaccination costs" and 45% for "transport/arrival and workforce originated expenditures". It is presumed that this strategy will be sustained for five years for both bovines and ovines. *Fig. 1* demonstrates that the benefits of the first strategy are high for the first two years, but for the following years, the benefits obtained from this strategy are reduced. Accordingly, for the first four years, expenditures decreased steadily but costs for additional tests and compulsory slaughter in the last year caused a rise in expenditures.

For the second strategy, the highest costs will be 84% for "transport and workforce originated expenditures", and the biggest portion of this consists of workforce costs. Although with the estimated sustained period of ten years for bovines and eight years for ovines this strategy has less expenditure than the first, it is important to notice that it takes more time to reach the target prevalence value. *Fig. 1* demonstrates that the benefits of the second strategy will show a remarkable increase by the third year and peak in fifth year before beginning to decrease steadily. While in the first nine years the expenditures are decreased, in the last year, the costs for additional tests and compulsory slaughter will rise.

For the third strategy, the highest costs will be 54% for "test and compulsory slaughter costs in the disease outbreak zone". It is estimated that the strategy will be suspended for six years for both cattle and small ruminants. *Fig. 1* shows that benefits of the third strategy will show a remarkable increase by the third year and peak in the fourth year, but will then begin to decrease steadily. For expenditures, a steady decrease is estimated over six years.

The highest costs for the fourth strategy will be "test and compulsory slaughter costs in the disease outbreak zone" at 66%. It is estimated that for bovines, it will continue for six years and for ovines it will continue for five years. The benefits of this strategy are similar to the benefits of the third.

Calculated NPV and BCR values for four separate brucellosis control strategies under three different scenarios were demonstrated in *Table 3*. For all strategies in the scope of the expected scenario, BCR was found to be higher than 1 and the highest BCR value observed in the second strategy was 2.26. For both strategies in the scope of optimistic scenario, NPV negative was observed and the highest BCR value all among the strategies is determined as 0.86 for the second strategy. For both strategies in the scope of the pessimistic scenario, the NPV positive was observed and the highest BCR value all among the strategies is determined as 2.84 for the second strategy.

Finally, the second strategy was determined to be the most financially advantageous of all the scenarios in conclusion of the financial analysis.

DISCUSSION

One of the most important factors in determining which strategy to use in the fight against brucellosis is evaluating the strategies not only from technical aspects but the strategies should also be applicable and rational from an economical aspect. Therefore, it was the aim of this study to determine the costs and benefits of different strategies planned for the control of brucellosis in Turkey.

A value of BCR 2.96 which was determined for a vaccination program for 30 years, is close to the value of the expected scenario of the second strategy, and for the pessimistic scenario it is close to second and fourth strategies in of our study. BCR 5.04 which was determined for test and slaughtering strategies is higher than all strategies BCR values in this study. The main reason for this is probably that they did not take the effects on public health and exportation potential into account [12]. BCR 1.13 which is determined for a vaccination based bovine brucellosis control program is close to the ratios in second and fourth strategies in the optimistic scenario in this study [13]. The total net benefits from the application of different bovine brucellosis control programs for 19 years is calculated as $296 million to $768 million [14]. This result is much higher than our findings and the probable reason was the program applied for a longer period and the cattle population in the United States is proportionally much higher than Turkey. A study was conducted for a cattle brucellosis control program based on a test and slaughtering which begins with a brucellosis prevalence value of 11% and continues over 14 years shows 0.59 BCR and negative for NPV [15]. This ratio is much lower than our findings except for the optimistic scenario. The reasons for this difference could be that they did not take the effects on public health into account; and did not make any attempt to reduce the high initial prevalence with any vaccination program, before the application of testing and slaughtering was initiated. An accelerated bovine brucellosis eradication strategy based on the testing-slaughtering method with 0.6% initial prevalence was studied [16]. The results of this study indicated that the BCR value was higher than 1 after the fourth year, a total benefit of $236 million while a total cost of $43 million for the tenth year. A comparison with our study could not be made as the strategy planned in our study was not the same as Kouba [16]. The reason for Kouba's observation that the financial benefit of eradication is high is that he considered not only losses in the animal production system but also the benefits obtained by brucellosis eradication which is the status acquired by free from brucellosis. By the application of a mass vaccination program for cattle and small ruminants for ten years, 51.856 human beings could be protected from brucellosis. For this program, a figure of 3.2 BCR and $18.3 million NPV was determined. Despite the fact that we took not only human health but also animal production losses into consideration, the BCR for all scenarios and strategies in this study is lower than Zinstag et al.[17].

By considering cattle brucellosis in Turkey for a period of 20 years, Yurtalan performed the economical analysis of different control strategies [18]. The most rational strategy determined in this study financially was "vaccination of whole population for every year". The 6.77 BCR and $175.102.324 NPV achieved in his study are much higher values than any strategy in any scenario in our study. The main reasons for this inconsistency could be differences between initial prevalence and the periods of the application of these strategies. Yurtalan [18], determined 0.62 BCR and $136.423.313 NPV for another strategy in which test and slaughtering methods were applied for four years; also, he determined 0.77 BCR and $-70.171.774 NPV for a different strategy in which test and slaughtering methods were combined with vaccination and applied for a period of three years. Due to there being no similarities between any strategies in our study, no comparison could be made.

It is a well-known fact that testing, culling, vaccination and notification are most effective control methods for brucellosis. It is indicated that reducing the level of testing would have a major effect on the rate of spread of infection, should it be important [19]. Abortion notification is a very important additional means of surveillance. It may be feasible to eradicate *B. ovis* from flocks with moderate to high (10% to 38%) prevalence of infection by culling on the basis of 2 sequential tests. Vaccination was found to be more effective as a control strategy when the prevalence of flock infection was high (greater than 15%); however, it did not substantially reduce *B. ovis* transmission when the prevalence of flock infection was low, less than 10% [20].

It is suggested that to make a good economic assessment of a disease the problem should be approach as a system, if relevant epidemiological, medical and

economic variables are not taken into consideration to evaluate the impact the result will not be reliable and the benefits for producers and consumer of animal products will not be as good as expected [21].

The results of these studies indicated that fighting with infection is financially rational for expected and pessimistic scenarios, and BCR is bigger than one in all of the strategies for the mentioned scenarios. Also, the second strategy in which it was designed to continue for ten years with cattle and eight years with small ruminants is more advantageous than the presently applied strategy (3rd strategy). Nevertheless, it is very important to consider a strategy that is optimal financially, but may not be suitable technically or in respect to public health.

Different control strategies, periods, resources and methods could be planned for the financial and/or economical analysis of brucellosis and infection like it in further studies. It is also of great importance to know the incidence and prevalence of the disease, social-economical structure of livestock enterprises, legal regulations, and technical and financial resources of authorized ministries in the relevant countries. However, use of current and reliable data in the estimation of benefits and costs of infection control strategies should not be forgotten.

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RESEARCH ARTICLE

Investigation of the Effects of Alpha Lipoic Acid Application on Total Antioxidant and Oxidant Status, Paraoxonase, and Total Sialic Acid Levels in Laminectomized Rabbits

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Summary

The objective of this study is to investigate the effect of intramuscular administration of alpha-lipoic acid (ALA) on total antioxidant (TAS), total oxidant (TOS), paraoxonase (PON) and total sialic acid (TSA) levels in laminectomized rabbits. In the study, twenty four white New Zeeland rabbits weighing 3-4 kg were used. The investigation was carried out with three experiment and one control group consisting of six animals of each. No intervention has been done to the control group. Animals in the Group I received 50 mg/kg/day IM ALA (Thioctacid 600 T, M, EDA, Hamburg) without undergoing laminectomy. Group II underwent laminectomy; Group III underwent laminectomy and treated with IM ALA. The experiment lasted 45 days and blood samples were taken on 0, 15th, 30th, and 45th days from the animals. TAS, TOS, PON and TSA levels were measured in the serum. We conclude that ALA treatment would be beneficial in laminectomized rabbits in order to suppress inflammatory process and to expedite recovery.

Keywords: *ALA, Laminectomy, TAS, TOS, PON, TSA, Rabbit*

Laminektomi Yapılan Tavşanlarda Alfa-lipoik Asit Uygulamalarının Total Antioksidan Kapasite, Total Oksidan Kapasite, Paraoksonaz ve Total Sialik Asit Düzeyleri Üzerine Etkilerinin Araştırılması

Özet

Bu çalışma, laminektomi yapılan tavşanlarda intramüsküler Alfa Lipoik Asit (ALA) uygulamasının total antioksidan kapasite (TAS), total oksidan kapasite (TOS), paraoksonaz (PON), ve total sialik asit (TSA) düzeylerini üzerine olan etkilerini araştırmak amacı ile yapıldı. Çalışmada, 3.5-4 kg ağırlığında 24 adet beyaz Yeni Zelanda erkek tavşan kullanıldı. Araştırma, herbiri 6 adet hayvan içeren 1 kontrol ve 3 deneme grubu olmak üzere 4 grup halinde yürütüldü. Kontrol grubuna hiçbir müdahale yapılmadı. Grup I'deki hayvanlara laminektomi yapılmaksızın 50 mg/kg/gün İM ALA (Thioctacid 600 T, M EDa, Hamburg, Germany) enjekte edildi. Grup II'deki hayvanlara sadece laminektomi yapıldı. Grub III'e İM ALA+laminektomi uygulandı. Deneme toplamda 45 gün sürdürüldü. Denemenin 0., 15., 30.,ve 45. günlerinde vena auricularisten kan alındı. Elde edilen serum örneklerinde TAS, TOS, PON ve TSA düzeyleri belirlendi. Sonuç olarak, laminektomi yapılan tavşanlarda yangısal sürecin baskılanması ve iyileşmenin hızlandırılması için eksojen ALA kullanılmasının faydalı olabileceği kanısına varıldı.

Anahtar sözcükler: *ALA, Laminektomi, TAS, TOS, PON, TSA, Tavşan*

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INTRODUCTION

Alpha lipoic acid (ALA) has been described as a potent biologic antioxidant, a detoxifying agent, and a medicine for diabetes. It is used for the treatment of age-related cardiovascular and neuromuscular insufficiencies, and plays an important role as a modulator in various inflammatory signal pathways. Effect of ALA on inflammation originated from cytokines have been investigated and has been discovered that it was an inhibitor of NF-kappa β [1], matrix metalloproteinase-13 (MMP-13) and growth factor-p (TGF J-3) [1]. It has been reported that antioxidant property of ALA, which inhibits the liver fibrosis, arised from its natural tiol-antioxidant peculiarity [2]. Furthermore, ALA and its reduced form, dihidrolipoic acid (DHLA), play roles in the brain and other tissues as antioxidant defenders [3]. Anti-inflammatory properties of ALA have rarely been investigated in humans until today. It has been reported that ALA administration of 50 mg/day for 4 weeks results in 15% of reduction in Interleukin-6 (IL-6) levels which is an important indicator of inflammation, as well as an organizer of the expression of inflammatory cytokines as Interleukin 1 (IL-1) and Tumor Necrosis Factor α (TNF-α) [2].

Generally, oxidative stress is related to effect of increased free radical molecules resulting from metabolic disorders and other illnesses [4-6]. It has also been reported that free radicals were continuously produced in metabolic settings, and their levels significantly increase even in the physiologic circumstances [4]. It has been reported that some health problems occurred when the equilibrium was impaired between the oxidant and antioxidant substances [5-7]. In order to evaluate oxidative stress, although analyses of antioxidant parameters can be performed for this purpose, new methods like TAS have recently been developed [8,9]. To determine the oxidative index (OSI), ratio of the total peroxide value to total antioxidant capacity is calculated[1] It has been reported that only the TAS measurement can be used to determine the balance between the pro-oxidant and oxidant substances [6,10,11]. When poliansaturated fatty acids were oxidized with free radicals, lipid peroxide radicals emerge via LOOH producing chain reactions concomitant with the oxidation [12].

Reactive oxygen species and nitrogen-bearing free radicals (ROS and RNS) are being produced continuously during the aerobic metabolism and their levels increase significantly in the pathologic conditions [13,14]. Increase in the free radical levels also cause increases in the catabolic reactions in cells for the protection of homeostasis in the organisms [15]. Increased free radical levels cause cell and tissue destructions in the organism and elicits a status called "oxidative stress"[4,14,15].

Sialic acid (SA) is a derivative of neuroaminic acid [16,17] and abundantly present in all cell membranes [17-20]. It has been reported that SA concentration had increased rapidly in the pathologic conditions like inflammation, tissue destruction, and tissue proliferation [17]. Due to this fact, evaluation of SA concentration may be an important sign in the diagnosis of inflammatory diseases [19,21-24]. Although the mechanism in the increase of serum SA level during the inflammatory conditions is not clear, many investigators indicated the role of AFPs which share the same structure with SA [17,19,20,25]. Since acute phase reactants are glycoprotein in structure, it has been reported that increase in these proteins affects the TSA levels [20] but not reflects the increases in SA levels exactly, which also bears glycoprotein structure [19].

Paraoxonase (PON1) is a member of the protein family. PON1 was primarily synthesized in liver and some of it, together with high density lipoproteins, was secreted into the plasma. PON1, beside the posphorilated insecticides (e.g chlorpirifos, oxon, diaxozon) and active metabolites hydrolyses nervous agents like sarin, soman, and VX [26,27]. Due to its antioxidant capacity, plays a protective role in phosphate intoxications and exerts a modulating effect in cardiovascular diseases.

One of the phisiologic effects of PON1 is catching the low density lipoprotein particles (LDL) as well as the oxidized metabolized lipid byproducts. PON1 prevents the phospholipids included in HDL from oxidation as well [26-28].

The objective of this study is to investigate the effects of intramuscular ALA administration on TAS, TOS, PON and TSA levels in laminectomized rabbits.

MATERIAL and METHODS

Experimental Design

Twenty four adult New Zealand white male rabbits weighting 3.5-4 kg were used in this study. The study was accomplished at the Kafkas University, Local Ethical Committee of Animal experiments with approval (KAU-HADYEK 2012/85). All animals received humane care as outlined in the "Guide for the care and use of laboratory animals" [29]. The animals were deprived of food for 24 h before surgery, but were allowed free intake of water. The investigation was carried out with three experiment and one control group consisting of six animals of each. The animals in Group I received 50 mg/kg/day IM ALA (Thioctacid® 600 T, M, EDA, Hamburg, GERMANY) without undergoing laminectomy. Group II underwent laminectomy without treatment; Group III underwent laminectomy and was applied 50 mg/kg/day IM ALA. The duration of the experiment was 45 days and blood samples were taken on 0, 15th, 30th, and 45th days from the animals. TAS, TOS, PON and TSA levels were measured in the serum. Preoperative and postoperative analgesic and antibiotic prophylaxis was given for three groups of animals.

Surgery

Animals were anesthetized with intramuscular 9 mg/kg xylazine HCl (Rompun® Bayer, Istanbul, Turkey) and 60 mg/kg ketamine HCl (Ketalar®. Pfizer. Istanbul, Turkey) at 1/0.5 proportion, 0.1 mL/100 g body weight. Additional dose was applied for extending the anesthesia time if necessary; that was the 20% of the original dose of above mentioned medications. Following anesthesia, animals were stabilized on the operation table in prone position. The lumbar region was shaved and cleaned with the antiseptic providon iodine. The rectal temperature was recorded continuously and tried to be kept around 37°C during the whole surgical procedure. Following a 4-cm midline skin incision starting from the L5 level, the lumbar fascia was opened bilaterally from midline and bilateral subperiostal dissection of paravertebral muscles was carried out.

The L5 level was determined by palpation of the iliac wings. L3 and L4 total laminectomy was carried out with a 1-mm Kerrison rongeur under an operating microscope at 10x magnification (Möller-Wedel®, Wedel. Germany), following the removal of spinous processes. The ligamentum flavum and epidural fat tissue were removed and the dura mater exposed. Following hemostasis, the operation field was irrigated and cleaned with physiological saline solution. Then after, operation was done.

Collecting the Blood Samples

For the biochemical analyses, blood sample were taken from the vena auricilaris from all groups included in the study prior to application (0); and on 15th, 30th, and 45th days after the application. Blood samples were centrifuged at 3.000 rpm, serum samples were harvested, and stored in the -25°C freezer until they were analyzed.

Biochemical Analyses

Total sialic acid analyses (TSA): Serum TSA analyses were performed spectrophometrically [30].

Measurement of the Total Antioxidant Status: Serum TAS level was determined spectrofotometrically on an auto-analyzer (Aeroset®, Abbott®, Illinois, USA) using commercial kits (Rel assay diagnostic kits®, Gaziantep, Turkey) as described by Erel [31] The assay had excellent precision values lower than 3% and results were expressed as mmol Trolox Eq/L.

Measurement of Total Oxidant Status: Serum TOS level of was measured spectrofotometrically on an auto-analyzer (Aeroset®, Abbott®, Illinois, USA) using commercial kits (Rel assay diagnostic kits®, Gaziantep, Turkey) as described by Erel [32]. The assay was calibrated with hydrogen peroxide and the results were expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 Eq/L).

Oxidative Stress Index: Percentage ratio of TOS to TAC level was assessed as OSI. For calculation, the resulting unit of TAC was converted to mmol/L, and the OSI value was calculated according to the following formula; OSI (Arbitrary Unit) = TOS (μmol H_2O_2 Eq/L)/TAC mmol Trolox Eq/L) [32].

Measurement of Paraoxonase and Arylesterase Activity: Paraoxonase activity was measured in the absence (basal-activity) and presence of NaCl (salt-stimulated activity), using paraoxon substrate. The rate of paraoxon hydrolysis (diethyl p-nitrophenylphosphate) was measured by monitoring the increase of absorbance at 412 nm. Paraoxonase activity was expressed as U/L serum [33,34].

Statistics

It was subjected to normality. Normality results were analyzed by nonparametric method and then multiple groups were examined by kruskal wallis method. After these analysis, dual comparision were done by Mann-Whitney U method. Analyses were performed with SPSS for Windows (version 20.0; SPSS, Chicago, IL) in a PC.

RESULTS

TAS, TOS, PON, TSA OSI and Paraoxonase levels obtained from this study are shown in *Table 1*.

In the group II, significant increases were obtained on TSA, TOS and OSI levels in all treatment dates comparing with the values obtained before the operation ($P<0.001$), but significant decreases on TAS and PON levels were seen ($P<0.001$). It was determined statistically an important decrease in levels of TAS and PON activity as parallel to increase of the levels of OSI, TOS and TSA especially in the Group II compared to control Group from 15th day to finish of applications. It was determined statistically an important increase in levels of TAS and PON activity as parallel to decrease of the levels of OSI, TOS and TSA especially in the Group III compared to Group II from 15th day to finish of applications.

In the animals consisting the only Group I, values obtained from the samples taken from all treatment dates revealed nonsignificant decreases on TSA and TOS levels; but contrarily, significant increases on TAS and TOS values ($P<0.001$).

DISCUSSION

In this study, ALA, which its anti-inflammatory specifications has rarely been searched until today, has been administered to laminectomized rabbits via intramuscular course and effects of the substance on total antioxidant capacity (TAS), total oxidant capacity (TOS), paroxonase (PON), and total sialic acid levels

Table 1. *TAS. TOS. OSI. PON and TSA values obtained from the study (mean±std.deviation)*
Tablo 1. *Çalışmada elde edilen TAS. TOS. OSI. PON ve TSA değerleri (ortalama±std.deviasyon)*

Parameters	Groups	Days				P
		0	15	30	45	
TAS (mmol Trolox Eq/L)	Control (n=6)	0.652±0.028	0.645±0.016 B	0.648±0.034 B	0.646±0.048 B	-
	Group I (n=6)	0.645±0.016 B	0.715±0.034 A.a	0.726±0.067 A.a	0.737±0.042 A.a	P<0.001
	Group II (n=6)	0.648±0.034 A	0.515±0.075 B.c	0.504±0.074 B.c	0.540±0.038 B.c	P<0.001
	Group III (n=6)	0.653±0.029 A	0.601±0.040 B.b	0.612±0.021 B.b	0.627±0.021 AB.b	P<0.008
	P	-	P<0.001	P<0.001	P<0.001	
TOS (µmol H_2O_2 Eq/L)	control (n=6)	0.552±0.028	0.545±0.016 C	0.548±0.034 Bc	0.546±0.048 C	-
	Group I (n=6)	0.545±0.016	0.530±0.029 C	0.533±0.021 C	0.531±0.024 C	-
	Group II (n=6)	0.543±0.026 B	0.677±0.021 A.a	0.650±0.024 A.a	0.656±0.033 A.a	P<0.001
	Group III (n=6)	0.541±0.022 A	0.595±0.035 B.b	0.571±0.013 B.b	0.581±0.012 B.b	P<0.001
	P	-	P<0.001	P<0.001	P<0.001	-
OSI	Control (n=6)	0.846±0.006	0.844±0.003 C	0.845±0.007 Bc	0.844±0.010 B	-
	Group I (n=6)	0.844±0.003 A	0.741±0.037 AB.c	0.738±0.050 AB.c	0.632±0.259 B.c	P<0.05
	Group II (n=6)	0.838±0.015 B	1.341±0.278 A.a	1.316±0.263 A.a	1.219±0.092 A.a	P<0.001
	Group III (n=6)	0.829±0.045 C	0.990±0.025 A.b	0.933±0.043 B.b	0.927±0.0467 B.b	P<0.001
	P	-	P<0.001	P<0.001	P<0.001	-
PON (U/L)	Control (n=6)	210.1±21.9	208.9±13.7 B	210.2±14.6 B	210.4±19.8 B	-
	Group I (n=6)	210.0±10.3 B	236.8±7.7 A.a	236.7±2.5 A.a	235.5±4.4 A.a	P<0.001
	Group II (n=6)	210.8±15.9 A	160.5±9.6 C.d	163.6±6.2 BC.d	172.1±6.4 B.c	P<0.001
	Group III (n=6)	209.2±12.0 A	184.4±5.6 B.c	190.2±7.7 B.c	201.5±7.8 A.b	P<0.001
	P	-	P<0.001	P<0.001	P<0.001	-
TSA (mg/dl)	Control (n=6)	60.25±2.12	60.50±1.69 C	59.50±1.51 C	59.37±1.40 C	-
	Group I (n=6)	60.50±2.00	58.75±1.90 C	58.62±2.06 C	58.12±1.24 C	-
	Group II (n=6)	60.12±5.11 D	89.12±2.85 A.a	84.12±3.27 B.a	80.25±2.96 C.a	P<0.001
	Group III (n=6)	60.25±2.60 D	78.12±3.22 A.b	74.12±2.58 B.b	71.00±2.72 C.b	P<0.001
	P	-	P<0.001	P<0.001	P<0.001	-

ABCD: *Statistical significance of days in the groups;* ***a.b.c.d:*** *Statistical significance between the groups*

have been investigated. Intramuscular ALA application also exerted positive effect on TSA, TAS, TOS and PON values.

Analysis of sialic acid provides important clues for the diagnosis and estimation of the prognosis of the diseases, and is being used increasingly nova days [17,25]. In our study show that, plasma SA levels of laminectomized animals were higher than the levels of laminectomy + ALA administered, and only ALA administered animal groups. High levels of plasma SA values in laminectomized animals may be considered as the result of more pronounced tissue damage in this group comparing with the Group III. It has been reported that SA concentration rapidly increases during the pathological circumstances since tissue destruction and proliferation ocur [17]. We propose that, in accordance with the reports published by other investigators previously [18], these changes in SA levels originate from the over secretion of the lipid-bounded sialic acid compounds exerted by the sialoprotein synthesis in the liver [18] and increased sialidase enzyme activity [35] during the inflammation [36].

After the ALA treatment, low levels of TSA and TOS values were obtained due to prevention of inflammatory effect of laminectomy, especially after 15th day of administration. In this experiment, it has been detected that inflammation and stress caused by laminectomy operation had induced increases in TOS levels. In a study conducted by Gutteridge and Halliwell [37], authors proposed that lipid peroxidation levels might have been provoked to rise by a defect in the antioxidant defense mechanism. We consider that high TOS and low TSA values were the consequences of tissue damage and lipidperoxidation in laminectomized animals arising from the operation. This finding is parallel to the data from a previous study reporting the preventive effect of ALA against tissue damage. It has been detected that Groups III resulted in small increase on TOS (P<0.001), but significant increase on TSA values only in the laminectomized group comparing with other groups. We share

the same opinion with others [2,3] that this outcome resulted from an effect exerted by ALA hindering the production of the reactive oxygen species (ROS) and other free radicals. It has been reported that ALA could be used against the destructive effect of ROS, and this application evoked increased TAS levels [2,3].

While statistically significant decreases in PON values were obtained in laminectomized group comparing with the other three (control, Group I, Group III), significant increase was observed in PON values in the group treated only by ALA ($P<0.001$). In the Group III, PON values obtained on 15th, 30th, and 45th days of treatment showed significant decreases in comparison with the values obtained prior to operation ($P<0.001$). We share the opinion with the others that decreased values of PON in Group I might have been the consequence of the close relation between the free sulphydril groups and the antioxidant capacity, and the antioxidant effect during the conditions of inflammation and stress [38,39]. PON1which has paraoxonase, arylesterase and lactonase activities, is closely associated with high density lipoproteins $(HDL)_1$ and catalyzes the hydrolysis of a variety of aromatic carboxylic acid esters and several organophosphates and also a variety of lactones and cyclic carbonate esters, including naturally occurring lactones and pharmacological agents [40]. Paraoxonase activity which has decreased in oxidative stress conditions and belong to PON1 is used as an indicator in both toxicological studies and clinical cases [38-43]. In the present study, it was determined statistically an important decrease in PON1 activity as parallel to OSI levels especially in the Group II from 15th day to finish of applications.

The measurement of TAS and TOS using novel automated systems is likely to show the systemic production of all free radicals [31,32]. It has not run accrossed to the applied alpha lipoic acid on laminectomy. In this study were evaluated effect of alpha lipoic acid on TAS and TOS levels in laminectomy application. Additionally, the present study was not designed to analyze the possible discriminatory capacity of TOS/TAS (OSI) for laminectomy in the general population. The clinical value of the associations observed in our study will be analyzed as detail in another study.

As a result, in this study it has been proposed that laminectomy had caused alterations on TSA, TAS, TOS, and PON levels; on the other hand, administration of ALA had supported the antioxidant capacity of the plasma by increasing TAS and PON levels, but decreasing TSA and TOS levels in animals. We conclude that, in order to suppress the inflammatory process and to accelerate the healing, IM ALA treatment proves a promising method.

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RESEARCH ARTICLE

Comparison of Genotypic Diversity and Vancomycin Resistance of Enterococci Isolated from Foods and Clinical Sources in Adana Region of Turkey [1][2]

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Summary

In this research, genetic diversity and vancomycin resistance patterns were studied and also evaluated as a possible transfusion of vancomycin resistance that may be found from foods to clinical settings between 51 *Enterococcus* spp. isolated from food and 50 human clinical originated *Enterococcus faecium* strains in Adana Region. Identification and antimicrobial susceptibility tests were performed by Vitek-II system and disc diffusion method respectively. Minimum inhibitory concentrations of clinical isolates were confirmed by E-test and the presence of *vanA* and *vanB* genes were investigated by PCR method. Apart from one isolate, none of the food enterococci were resistant to vancomycin, and none of them carried *vanA* and *vanB* resistance genes. All clinical isolates were resistant to vancomycin, and 84% of these isolates carried *vanA*; 2%, *vanB*; and 14% neither *vanA* nor *vanB* genes. Genetic diversity within each group; 6 clusters of colonization isolates and 5 clusters of food isolates were found to be closely related by Pulsed Field Gel Electrophoresis method. Although no genetic relation was found among foods and human clinical infection isolates; 2 clusters of foods and human intestinal isolates were found to be closely related. Finally, vancomycin sensitive *E. faecium* strains from food colonized in humans acquired *vanA* and *vanB* resistance genes and are thought to be a reservoir for vancomycin resistance. These results revealed that food enterococci should be carefully monitored in food industry in terms of their genetic relation to infection species.

Keywords: *Enterococci, Food, PFGE, Vancomycin resistance*

Türkiye'nin Adana Bölgesinde Gıda ve Klinik Kaynaklı Enterokokların Genotipik İlişkilerinin ve Vankomisin Direnç Özelliklerinin Karşılaştırılması

Özet

Bu çalışmada, Adana Bölgesinde gıdalardan izole edilen 51 *Enterococcus* spp. ile klinik orjinli 50 *Enterococcus faecium* türlerinin vankomisin direnç paternleri ve genetik ilişkileri çalışılmış, ayrıca vankomisin direncinin yayılmasında gıda kaynaklı olası bir geçişin olup olmadığı araştırılmıştır. Tanımlama ve antimikrobiyel direnç testleri sırasıyla Vitek-II ve disk diffüzyon metodları ile araştırılmıştır. Klinik izolatların minimum inhibitor konsantrasyonları E-test ile doğrulanmış; tüm izolatlarda *vanA* ve *vanB* direnç genleri PCR metodu ile araştırılmıştır. Bir izolat dışında, tüm gıda izolatlarının vankomisine dirençli olmadığı ve hiçbir suşun *vanA* ve *vanB* geni taşımadığı saptanmıştır. Klinik izolatların tümünün vankomisine dirençli olduğu, %84'ünün *vanA*, %2'sinin *vanB* genlerini taşıdığı; %14'ünün *vanA* ve *vanB* direnç genlerini taşımadığı belirlenmiştir. Pulsed Field Gel Electrophoresis metodu ile yapılan genetik ilişkilendirmede; 6 kolonizasyon kümesi ve 5 gıda kümesi kendi içlerinde yakın ilişkili bulunmuştur. Gıda ve klinik enfeksiyon izolatları arasında genetik ilişki saptanmamışken, 2 adet kolonizasyon ve gıda kümeleri arasında yakın ilişki bulunmuştur. Sonuç olarak, Adana bölgesinde gıda kaynaklı vankomisine duyarlı *E. faecium* türlerinin insanlarda kolonize olabildikleri, *vanA* ve *vanB* direnç genleri kazanarak vankomisin direncinin yayılmasında rezervuar olabilecekleri düşünülmektedir. Bu sonuçlar, gıda endüstrisinde gıda kaynaklı enterokokların enfeksiyon etkeni izolatlar ile genetik olarak ilişkileri açısından dikkatle izlenmesi gerektiği sonucunu ortaya çıkarmıştır.

Anahtar sözcükler: *Enterokok, Gıda, PFGE, Vankomisin direnci*

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INTRODUCTION

Enterococci live as commensals of the gastrointestinal tract of warm-blooded animals and are the most abundant Gram-positive cocci in humans [1,2] as well as in soil, waters, raw plant and animal products. *E. faecium* and *E. faecalis* are important in food microbiology because of the lypolitic, esterolytic activities, using citrate and making aromatic compounds in foods. They also produce good organoleptic features in some foods. Thanks to these properties, enterococci are used together with lactic acid bacteria in some fermented dairy and meat products as starter cultures as well as probiotics to improve the microbial balance of the intestinal tract in humans and animals and can be used in the treatment of gastroenteritis in humans and animals [2]. However, the *Enterococcus* genus has more beneficial effects in food industry and is not considered "generally recognized as safe" (GRAS) due to its use as an indicator of fecal contamination and the frequent association with food-borne illnesses by biogenic amines production [2,3] but is recognized as important nosocomial pathogens causing endocarditis, bacteremia, and central nervous system infections as well as neonatal, respiratory tract, urinary tract, and other infections [2,4] which may be linked to the presence of antibiotic resistance and virulence properties.

Enterococci are able to acquire resistance determinants through gene transference by mobile genetic elements. Resistance of enterococci to therapeutically important antibiotics gain resistance to the glycopeptides vancomycin and teicoplanin, often associated with high-level resistance to amino glycosides [2]. The emergence of vancomycin-resistant enterococci, belonging predominantly to *E. faecium*, has resulted in cases of untreatable infections [1,2]. Vancomycin resistance is encoded by the *vanA* gene cluster carried on the mobile genetic element Tn1546. Transfer of resistance can be fulfilled by conjugative plasmids [1,2]. Dissemination of antimicrobial resistance genes through clonal expansion and horizontal transmission is so important for infectious disease specialists. Compared to animal products, fruits and vegetable foods are usually consumed raw, so they are more effective in transmission of antibiotic resistance traits since animal products are consumed by cooking, and in this way enterococci are inactivated. However, only few studies on the incidence of antibiotic resistance among enterococci from fruits and vegetable foods have been reported [5,6].

Accurate species identification and strain typing are important to evaluate the genetic diversity among enterococci populations and to select nonpathogenic bacteria for further use in food technology and probiotics [2,7]. Genetic typing techniques such as Pulsed-Field Gel Electro-phoresis [PFGE] analysis of *SmaI* macro restriction profiles are considered to be the "gold standard" for genotyping of enterococci with more discriminative power than other techniques [8,9].

Risk factors for VRE (vancomycin resistant enterococci) colonization may be patient-, hospital-, environment-, and antibiotic-related. Although many studies have been performed in Europe and the USA on the prevalence, incidence, epidemiology, and risk factors of VRE, data obtained in the Middle East and Asia are very rare [1,10].

The objectives of the present study were to determine the genetic diversity and vancomycin resistance of enterococci from food and clinical origins in Adana region of Turkey and to have a better understanding of the different reservoirs in the emergence and the spread of vancomycin resistance.

MATERIAL and METHODS

This study has an ethics report from "Turkish Republic, University of Cukurova, Faculty of Medicine Ethical Board of Scientific Research" with 15 decision code and number 6 on 10.03.2011.

Materials

In this study, a total of 80 food samples [*n*=28 cheese, *n*=10 fruit (raw and pickled olives and tomatoes), *n*=16 vegetables (lectures, packed salads, cabbages and purple cabbages), *n*=21 sucuk (a traditional Turkish meat product) and *n*=5 chicken meat] were purchased from various markets to determine whether enterococci were present in the food samples. The analyses were carried out during the day with an evaluation of a total of 50 enterococci isolates obtained from various clinical specimens provided by the Central Laboratory of Balcalı Hospital, Adana-Turkey during 2010-2011. Of these isolates, 25 were isolated from clinical specimens in patients with nosocomial infection, 25 from stool or rectal specimens in patients with intestinal colonization. All clinical isolates selected at the hospital were VRE.

Methods

Identification of Enterococci

For identification purposes; food samples were homogenized before used. After homogenization, samples (10-25 g) were weighed, and their appropriate dilutions were prepared. For *Enterococcus* spp., isolation diluted samples were cultivated on kanamycin aesculin azide agar (KEA agar) (Merck KGaA, Germany) then Slanetz agar (Merck KGaA, Germany) and incubated at 37°C, for 24-48 h [6,11]. For isolation of *Enterococcus* spp., Gram positive cocci isolated from kanamycin aesculin azide agar (KEA) medium were morphologically evaluated, and strains isolated from colonies showing typical enterococci morphology were subject to the following tests to fulfill identification

procedures: Catalase production, Gram staining, gas production from glucose and growing in 6.5% NaCl. Clinical isolates were routinely grown on Columbia agar (Becton-Dickinson, Sparks, MD) supplemented with 5% defibrinated sheep blood (supplied from Experimental Surgery Center of Medical Faculty) at 37ºC for 24 h. All suspected colonies were identified by VITEC automated identification system in Balcali Hospital Central Laboratory, The Faculty of Medicine, Çukurova University (Biomerieux, Durham, North Carolina, USA).

All identified isolates (food and clinical) were stored in Brain Hearth Broth (BHI) including 10% sheep blood and 10% glycerol at -20ºC until the completion of genotypic analysis.

Characterization of Vancomycin Susceptibility Test

Vancomycin susceptibility testing of isolates from food samples was performed using disk diffusion method by CLSI [12] guidelines, using antibiogram discs of vancomycin (Oxoid ltd.)(concentration of vancomycin disc is expressed in 30 µg mL^{-1}). The strains were cultivated on Mueller-Hinton Agar (Merck KGaA, Germany), and then antibiotic discs were located through a dispenser. After incubation (24 h, 37ºC), bacteria strains were classified as resistant, intermediate and sensitive by CLSI document criteria by measuring inhibition zone diameters around the antibiotic discs [12].

Vancomycin resistance patterns of clinical strains and suspected food isolates were evaluated by using Gram-positive antibiotic susceptibility cards (Biomerieux Vitek-2-AST-P534-SA-France) in VITEK-2 automated (Biomerieux, Durham, North Carolina, USA) identification system. The results were recorded following 18-24 h of incubation at 37ºC, and were evaluated by producer instructions using the breakpoints for enterococci proposed by the CLSI [12]. *Enterococcus faecalis* 1047387 (vancomycin sensitive) and *Enterococcus faecium* 1045803 (vancomycin resistant) collected from Balcali Hospital Central Laboratory as reference strains were used in antibiotic resistance tests.

Detection of vanA and vanB Genes

Genomic DNA was extracted mechanically by the "Mickle Sytem" (The Mickle Lab.Engeneering Co. Ltd. Gomshall, Surrey, UK) by producer instructions after overnight cultures in 5% defibrinated sheep-blood agar of enterococci. A spectrophotometer (UV-VIS Spectrophotometer CHEBIOS) was used for quantitation of DNA samples. Extracted DNAs of enterococci were stored at -20ºC until within use as a template for PCR amplifications.

PCR was performed to screen vancomycin resistance genes (*vanA*, *vanB*) as described previously [13,14]. The specific primers and PCR conditions were presented in *Table 1*. Amplicons were analyzed by electrophoresis using 2% agarose gels [PegGOLD Universal Agarose, 91052 Erlangen Deutschland, 2%(w/v)] containing 0.5% ethidium bromide in TBE buffer (40 mM tris, 20 mM boric acid and 1 mM EDTA, pH 8.3) for 30 min at 120 V in the presence of a 50-bp DNA ladder (Fermentas SMo.323-Lithuania). The gel was photographed on a UV transilluminator (Kodak Gellogic-1500 imaging system).

Minimal Inhibitory Concentrations (MICs) Testing

MICs of the antibiotic vancomycin (Va) was determined by VITEK-2 AST compact panel (Biomerieux Vitek-2-AST-P534-SA-France) and E-test (Biodisk, Solana, Sweden) in Mueller-Hinton agar, and the results were interpreted based on CLSI criteria [12].

Hemolytic Activity Testing

For hemolytic activity, all species were cultivated in sheep blood agar. After incubation (24 h, 37ºC), bacteria strains were categorized as α-hemolytic, β-hemolytic and non hemolytic [15].

Pulsed Field Gel Electrophoresis (PFGE) Analysis

The clonal relationship among isolates was established by PFGE method. Genomic DNA was prepared in agarose plugs according to methods previously described [16] and run on a CHEF-DR II (Bio-Rad Laboratories, Nazared, Belgium) machine, and the electrophoresis conditions used in this study were described previously [17]. In the first block, the initial switch-time was 3.5 s, the final switch-time was 20 s, and the run-time was 12 h, and in the second block, the initial switch time was 1 s, and the final switch time 5 s for 8 h, at 6 V/cm^2. *SmaI* was the enzyme used for cleaving the DNA (Lambda Ladder PFG Marker, New England BioLabs Inc.). Our index strains isolated from blood samples of two bacteriaemic patients in nosocomial infection outbreak were formed in 2011 and placed in our library and used as a molecular size marker and an internal

Table 1. *List of primers and amplification conditions used in the present study*
Tablo 1. *Çalışmada kullanılan primerler ve amplifikasyon şartları*

Gene	Primers sequence (5'-3')	Product Size (bp)	Amplification Conditions	Reference
vanA	TCT GCA ATA GAG ATA GCC GC	375	Initial cycle of 94ºC for 5 min; 30 cycles of 94ºC for 30 s, 48ºC for 30 s, 72ºC for 30 s; 1 cycle of 72ºC for 7 min	[14]
	GGA GTA GCT ATC CCA GCA TT			
vanB	GTG ACA AAC CGG AGG CGA GGA	527	Initial cycle of 94ºC for 5 min; 30 cycles of 94ºC for 40 s, 58ºC for 60 s, 72ºC for 30 s; 1 cycle of 72ºC for 30 min	[13]
	CCG CCA TCC TCC TGC AAA AAA			

control. GelCompar II software system (version 5.0; Applied Maths, Sint-Martens Latem, Belgium) was used to calculate the percentage of similarity (Dice coefficient) of *SmaI* pulsed-field gel electrophoresis (PFGE) banding patterns. Primarily, normalization was fulfilled using three standards bands in every picture of agarose gel. Unweighted pair group method with mathematical averaging (UPGMA) was used for creating dendograms and cluster analysis of PFGE profiles. Band and profile tolerance were used 1.5% for calculating similarity coefficent. Isolates were considered closely related if their PFGE banding patterns were ≥80% similar, and were indicated with capital letters, and if subtypes were in the same cluster, they were shown with numbers.

RESULTS

Among the food samples tested, a total of 51 isolates from 80 food samples were identified as presumptive enterococci. Of these, 51 isolates were identified as the following species; *E. faecium* (20 isolates, 39.2%), *E. faecalis* (12 isolates, 23.5%), *E. casseliflavus* (7 isolates, 13.8%), *E. gallinarum* (4 isolates, 7.8%), *E. durans* (7 isolates, 13.8%) and *E. raffinosus* (1 isolates, 1.9%). From a starting collection of 50 clinical isolates (corresponding to 50 patients), were identified as *E. faecium*. Most of isolates (9, 36%), (2, 8%), (2, 8%), (2, 8%) and (10, 40%) in nosocomial environment were from intensive care internal medicine, pediatric hematology, brain surgery, urology and other clinics respectively, while most of the intestinal isolates (15, 56%), (4, 16%), (2, 8%) and (4, 20%) were from pediatric hematology and oncology, brain surgery intensive care, burn unit and other clinics respectively.

In the present study, 39 (76.4%) of food enterococci were found to be sensitive to vancomycin, and 11 (21.5%) of these were found intermediate sensitive to vancomycin. Only one strain identified as *E. casseliflavus* and collected from a lettuce sample was found to be resistant to vancomycin. It's MIC value was found 64 µg/mL. All clinical *E. faecium* strains were found to be resistant to vancomycin and teicoplanin, and their MIC values were found over 128 µg/mL (*vanA* type).

The results of prevalence of genes encoding vancomycin resistance in enterococci isolated from food and clinical samples showed that, none of the food isolates carried *vanA* and *vanB* resistance genes, and 84% of clinical enterococci carried *vanA*, 2% of them *vanB,* and 14% of them carried neither *van A* nor *van B* genes.

Hemolytic activities of enterococci showed that non-hemolytic, α-hemolytic and β-hemolytic in food isolates were 32 (62.7%), 13 (25.4%) and 6 (11.7%) respectively. All human intestinal isolates were found non-hemolytic; but 50% of clinical *E. faecium* isolates were found to be α-hemolytic and 14% were β-hemolytic.

PFGE Analysis

The genome of 25 *E. faecium* strains leading to endogenous colonization strains from stool or perirectal swabs and the genome of 20 *E. faecium* strains from food samples were investigated by PFGE. It was found that band sizes of them ranged from 10 to 300 kb *(Fig. 1)*. According to band patterns, "A1-Gamma" was gathered in the so-called 13 different clonal clusters and 16 unique isolates *(Fig. 2)*. The largest two clusters were composed of four and three subtypes and named "I" and "G". In addition, closely related 11 clusters were included into two subtypes and named "A, B, C, H, J, L, S, T, U, W, Beta," forming clusters of food and human intestinal colonization isolates.

Isolates belonging to PFGE types "A, B, C, W, Beta" were also obtained from samples of different food origins. Human intestinal *E. faecium* isolates were shown to be closely related in PFGE types of "G, H, I, J, S, T," by using PFGE method. Food and intestinal enterococci isolates were found to be closely related in PFGE groups of "L and U" *(Fig. 2)*. Furthermore, this study found no clonal relation between *E. faecalis, E. durans, E. casseliflavus* and *E. gallinarum* isolated from food.

As a result of the PFGE investigation, based on band patterns of 25 *E. faecium* strains of clinical infection and 20 *E. faecium* strains of food origin, the clonal relationship could not be determined between food and clinical infection isolates. Both groups formed a separate group.

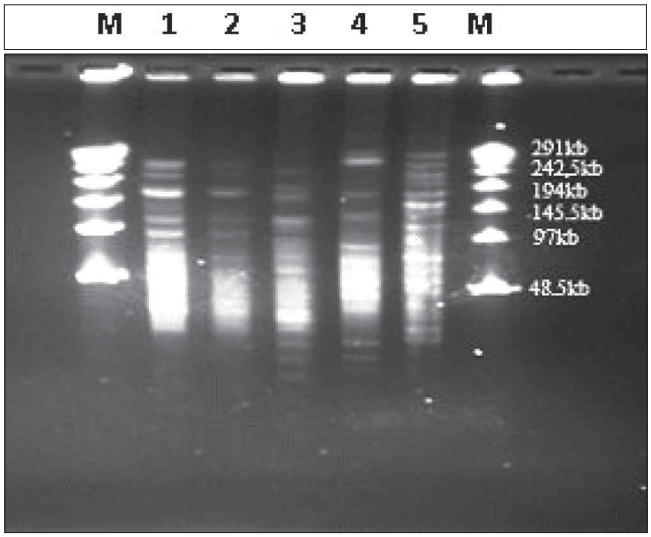

Fig 1. PFGE of *SmaI*-digested genomic DNA from five *Enterococcus* spp. isolates from different food sources. From left to right, M: γ ladder (New England BioLabs Inc.), 1- *E. faecium (cheese)*, 2- *E. faecium*, *(cheese)*, 3- *E. casseliflavus (sucuk)*, 4- *E. faecalis (sucuk)*, 5- *E. faecium (white cheese)*

Şekil 1. Farklı gıda kaynaklarından izole edilmiş beş adet *Enterococcus* spp.'nin *SmaI* ile kesilmiş genomik DNA'larının PFGE ile elde edilen jel görüntüleri. Soldan sağa, M: γ ladder (New England BioLabs Inc.), 1- *E. faecium (peynir)*, 2- *E. faecium (peynir)*, 3- *E. casseliflavus (sucuk)*, 4- *E. faecalis (sucuk)*, 5- *E. faecium (beyaz peynir)*

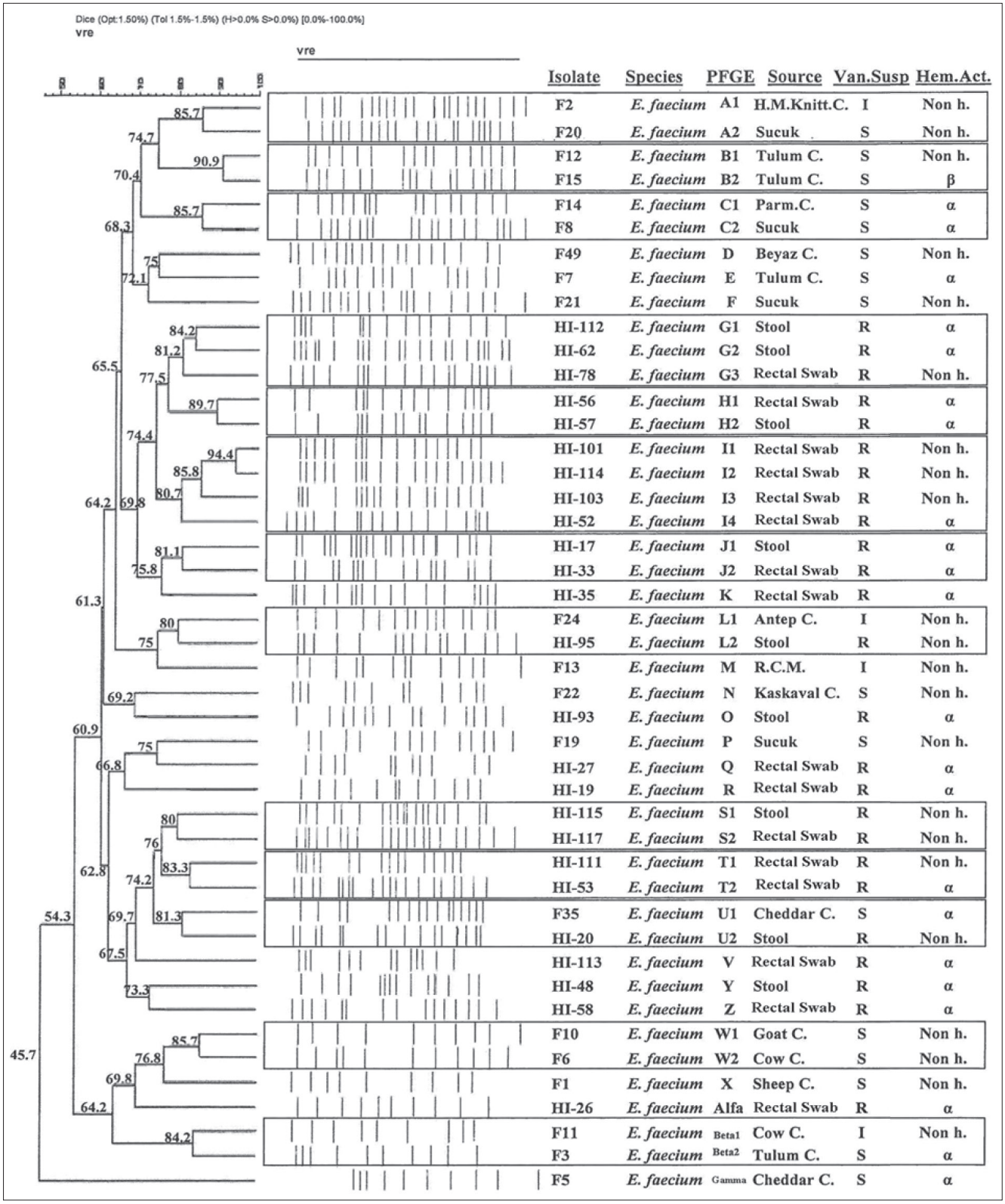

Fig 2. Dendrograms showing the similarity index among the 45 strains of *Enterococcus faecium* from human intestinal (25 strains) and foods (20 strains) are included. PFGE types are indicated for isolates tested by pulsed field gel electrophoresis (PFGE). Clusters sharing 80% or bigger similarity are shown boxed; F; Food isolate, HI; Human Intestinal Isolate, C; Cheese, R.C.M.; Raw Chicken Meat, Hm Knitt.; Home made Knitting Cheese, Van. Susp.; Vancomycin Susceptibility, Hem. Act; Hemolytic Activity, S; Sensitive, I; Intermediate, R; Resistant

Şekil 2. Gıda kaynaklı (20 suş) ve klinik kolonizasyon etkeni (25 suş) olan 45 *E. faecium* suşları arasındaki benzerlik indeksini gösteren dendogramlar. PFGE tipleri, Pulsed Field Gel Elektophoresis yöntemi ile elde edilmiştir. %80 ve üzeri benzerlik gösteren gen kümeleri kutu içine alınmıştır. F; gıda izolatı, HI; klinik kolonizasyon izolatı, C; peynir, R.C.M.; çiğ tavuk eti, Hm Knitt.; ev yapımı örgü Peyniri, Van. Susp.; vankomisin direnç özelliği, Hem. Act; Hemolitik aktivite, S; hassas, I; orta derecede hassas, R; dirençli

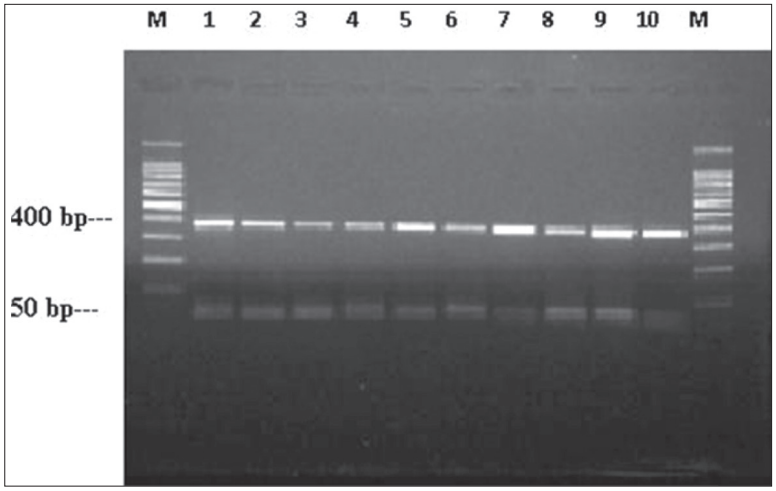

Fig 3. Agarose gel electrophoresis showing positive amplification of 375 base fragments specific for *van*A gene of VRE strains from clinical specimens. Lane M: Size marker (50-bp DNA ladder); lanes 1-10: clinical VR*E. faecium* strains

Şekil 3. Klinik kaynaklı VRE suşlarının 375 bp'de *van*A genine özgü pozitif amplifikasyon fragmentlerini gösteren agaroz jel elektroforezi. M: Moleküler belirteç (50-bp DNA ladder); 1-10: klinik kaynaklı VR*E. faecium* suşları

DISCUSSION

In this study, *E. faecium* was the most abundant species in human and food samples. Our results are different compared to the studies performed previously [6,18]. The species found most abundant in their study were *E. faecalis,* whereas the species found most abundant in our study were *E. faecium*. The studies show that enterococci can also be found in a variety of food products such as dairy products, meat and vegetables. The dominant flora can vary according to products or environment [18].

In sucuk, raw chicken meat, fruit and vegetables, the strains of *E. faecalis* are the second prevalent species, which could be explained by the fact that these foods are manipulated by hands suggesting a possible contamination during manufacturing process. Our results showed different species prevailing in the different reservoirs similar to some other studies [10,19]. As expected, *E. faecium* was the most prevalent species irrespective of the source, whereas *E. faecalis, E. durans, E. gallinarum, E. casseliflavus* and *E. raffinosus* were only isolated from food samples. Some studies show that, *E. faecium* dominant in hospitals by undergoing clonal selection either alone or together with Staphylococci rapidly increases the incidence of nosocomial hospital infections and is accepted as epidemic or hospital-acquired [20,21]. Similarly, all our clinical strains were classified as *E. faecium.*

Only one *E. casseliflavus* isolated from lettuce was resistant to vancomycin and did not carry *vanA* and *vanB* resistance genes. This might be an intrinsic resistance of *E. casseliflavus,* and its MIC value of vancomycin was 64 µg/mL. This study also investigated the prevalence of genes encoding vancomycin resistance in enterococci isolated from food and clinical samples. None of food isolates carried *vanA* and *vanB* resistance genes. Data on the incidence of vancomycin resistance within different types of food (especially dairy and meat products) enterococci occur at different extents depending on the geographical area [22]. In a recent study conducted in Turkey, *vanA* gene was found in only one *E. faecalis* strain isolated from a cheese sample and was intermediately resistant to vancomycin [23].

When all the clinical *E. faecium* strains are analyzed by using PCR for the detection of *vanA* and *vanB* genes, 84% of them carried *vanA*, and 2% of them *vanB,* and 14% of them neither *vanA* nor *vanB* genes *(Fig. 3)*. The results of our clinical isolate genotypes have been found to be similar to the previous studies (generally the most clinical isolates carried *vanA* genes) performed in Turkey [24,25]. However, different studies showed variable results concerning the detection rates of *vanA* and *vanB* genes in Middle East Countries such as; in Kuwait hospitals, all VRE strains carried the *vanA* genotype and *vanB* gene was not detected in any of the isolates [26]. Sharifi et al.[27] reported that high prevalence of *E. faecium* harbored vancomycin resistance with *vanA* genotype (89.5%) in North West Iran, which also showed that all VRE strains carried the *vanA* genotype, and *vanB* gene (6.3%) was detected as phenotypically sensitive in their isolates [27].

The emergence of antibiotic resistant enterococci in food samples would be the massive use of antibiotic in agriculture (e.g., avoparcin as animal growth promoters). Avoparcin was legally banned in 1998 in European Countries, and VRE isolation rate has increased and showed some differences depending on the geographical areas [28]. Biavasco et al.[19] reported that, the role of different reservoirs in the spread of glycopeptide resistance in European Countries was not obvious. While VRE infections acquired in several research hospitals in Turkey have been reported since 1999, the role and mechanism of the reservoirs in the spread of glycopeptide resistance was not explicit in our country, either. Few studies have been performed on vancomycin resistance and genetic diversity of enterococci clinical isolates in Turkey as well as Middle East Countries [10,24,29].

In this study, we compared GRE (Glycopeptide Resistant Enterococci) isolates of food and clinical origins (a broad

collection of *E. faecium* isolates from different human origins; wound fluid, sterile sites, urine and intestinal origin). PFGE results showed a polyclonal distribution of vancomycin resistance of isolates in the different reservoirs; however, the presence of some clones in different reservoirs was observed. For example, closely related clones were isolated from both foods (PFGE groups of L, U) and intestinal samples, suggesting food isolates may have the ability of human colonization. These results implied that, some *E. faecium* isolates from stools and perirectal swaps were highly related but not indistinguishable from food isolates. This may suggest that some human isolates collected in this study might be the reisolation of commercial starter culture isolates. Similar results were found by Vankerchoven et al.[5] for clinical and probiotic enterococci isolates, and Vancanneyt et al.[9] for (potentially) probiotic *L. rhamnosus* isolates.

Our results are different from those of Vancanneyt et al.[9] in terms of vancomycin susceptibility and hemolytic activities of closely related genomic groups. In their study, they differentiated two main genomic groups (I and II) among *E. faecium* by a combination of RAPD-PCR and AFLP (amplified fragment length polymorphism) methods. They reported that human clinical strains, antibiotic resistant strains, and beta hemolytic strains were found only in genomic group I. By contrast, in our study, vancomycin resistance and hemolytic activities of closely related food and human intestinal colonization isolates were found to be different *(Fig. 2)*. Interestingly, closely related two intestinal *E. faecium* strains (HI-115 and HI-117) carried neither *vanA* and nor *vanB* resistance genes. On the other hand, intestinal *E. faecium* strain (HI-95) carried *vanB* gene and was closely related to a food strain (F24). In addition, other related intestinal *E. faecium* strain (HI-20) carried *vanA* gene and was closely related to another food strain (F35). These results suggested that, food enterococci can acquire *vanA* or *vanB* gene in colonization section or these food isolates might be human originated.

We could not find a relationship between foodborne enterococci isolates and clinical VRE infection isolates. Foodborne and clinical isolates formed a separate group by PFGE. Our results are similar to the results of Abriouel et al.[6]. They found isolates of clinical samples clustered in separate groups with LH-PCR (length heterogenity-polimerase change reaction) typing methods.

As a result, the genetic relation between food and intestinal *E. faecium* isolates show that commercial strains used in the cheese-making production or/and contamination occur via staff during the cheese-making process. It may affect the genetic profile of the strains. The clonal relationship between each food isolates suggested that the same commercial starter cultures may be used in cheese and sucuk- making process. The genetic relation of vanA type (carrying *vanA* gene) in human intestinal isolates suggested that occasional clonal dissemination can occur in hospital environments. Finding 7 *E. faecium* [3 urine, 1 CSF (cerebrospinalfluid) and 3 intestinal isolates] strains with non *vanA* and non *vanB* genotype, but resistant to vancomycin show that other resistant genes (*vanC, vanD,* etc.) might also be important in the vanA phenotype of VRE isolates or some polymorphism might occur in the detection of resistance genes. More genotyping studies are needed to understand the mechanism of VRE dissemination between food (including starter cultures) and clinical origin in our country as well as in the geographical area of Middle East in the future.

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RESEARCH ARTICLE

The Effect of Vermiculite as Litter Material on Some Health and Stress Parameters in Broilers [1]

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Summary

This study was conducted to determinine the effect of vermiculite as the litter material on some health and stress parameters such as alanin aminotransferaz (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), glucose (GLU), cholesterol (CHOL), superoxide dismutases (SOD), malondialdehyde (MDA), catalase (CAT), footpad burn (FPB), gait score (GS), tonic immobility duration (TI) and feathering score (FS) in broilers. In present study, 195 day-old chickens were divided into three treatment groups placed on litter materials such as woodshavings (WS), mixture of woodshavings-vermiculite (WSV) and vermiculite (V), respectively. The experiment lasted for 42 days. At the end of the experiment, it was ascertained that the litter material did not have significant effect on ALT, AST, TP, ALB, GLU, CHOL, CAT and feathering score, but had significant effect on FPB, GS, TI, SOD and MDA values ($P<0.01$). FPB and GS were lower in the pens littered with V and WSV than in the pen littered with WS. The foot health was positively affectef by the use of V and WSV as litter material. TI of WS group was longer than those of V and WSV groups. SOD, MDA values from oxidative stress parameters were found lower ($P<0.01$) in the V and WSV groups than that of WS group. Consequently, vermiculite may be used as litter material in the rearing of broiler without adverse health problem.

Keywords: *Broiler, Litter material, Vermiculite, Health, Stress*

Vermikülitin Broilerlerde Altlık Olarak Kullanımının Bazı Sağlık ve Stres Parametreleri Üzerine Etkisi

Özet

Bu çalışmada, farklı altlık materyallerinin ALT, AST; TP, ALB, GLU, CHOL, SOD, MDA, CAT, ayak taban yanığı, yürüme skoru, tonik immobilite ve tüylenme skoru gibi bazı sağlık ve stres parametreleri üzerine etkisini belirlemek amaçlanmıştır. Araştırmada 195 adet günlük civciv kullanıldı. Civcivler üç farklı altlık materyali (talaş, talaş-vermikülit karışımı ve vermikülit) kullanılan bölmelere yerleştirildi. Deneme 42 günlük yaşa kadar sürdürüldü. Denemenin sonunda ALT, AST, TP, ALB, GLU, CHOL, CAT ve tüylenme skoru bakımından altlık materyalleri arasında fark bulunamadı ($P>0.05$), ayak taban yanığı, yürüme skoru, tonik immobilite, SOD ve MDA değerleri bakımından ise altlık grupları arası fark önemlidir ($P<0.01$). V ve WSV gruplarında WS grubuna göre FPB ve GS daha düşüktür. V ve WSV altık olarak kullanımı ayak sağlığının olumlu etkilemiştir. TI süresi WS grubunda V ve WSV gruplarından daha yüksek bulunmuştur. Oksidatif stres parametrelerinden SOD, MDA değerleri V ve WSV gruplarında WS grubundan daha yüksektir. Sonuç olarak, broylerlerde sağlık üzerine zararlı bir etkisi olamadığından, vermikülit atlık olarak kullanılabilir.

Anahtar sözcükler: *Broyler, Altlık, Vermikülit, Sağlık, Stres*

INTRODUCTION

All of the broiler raising is carried out as flocks on floor with litter. In view of emerging demands related to the new animal welfare rules implemented in European Union Member States, some modifications have been made in floor-based growth systems (i.e. free range systems) [1]. Especially broiler production is performed intensive and

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widely in the world, considering the importance of the litter is better understood. Litter material should also have low thermal conductivity, to retain warmth and act as insulation. It should therefore be capable of drying quickly and be soft and compressible, absorb moisture and buoyant [2].

The researchs for alternative poultry litter sources continue because availability of softwood shavings is limited. In many areas, decreased supplies and increased demand for wood shavings has increased the cost of these products and has resulted in a search for supplemental or alternative litter sources [3]. Several alternative litter materials have been tested with favourable results [4].

Wood shavings, commercial litter, rice hulls, sand and vermiculite were used as litter material by Miles et al.[5], they reported that vermiculite was a new litter material with high moisture absorption capacity but, it caused more ammonia production than wood shavings, commercial litter, rice hulls and sand.

Arunlertaree et al.[6] reported that when vermiculite and hydrated sodium calcium aluminosilicate were added to feed, they bind aflatoxin B1 on account of toxin-binding characteristics of the vermiculite.

Vermiculite is a siliat mineral that is obtained from volcanic magma resources. High heat treatment creates an expantion in volume, an increase in permeability and a descrease in weight. The obtained product is very light and sterile. With thermal insulated and fire-resistant features, vermiculite is used as the land regulator in agriculture. The chemical composition of vermiculite is: SiO_2 38-46%, AL_2O_3 10-17%, MgO 16-35%, CaO 1-5%, K_2O 1-6%, Fe_2O_3 6-13% TiO_2 1-3% and H_2O 8-16%. Material has a relatively high water-holding capacity (200-325% of weight and 20-50% by volume) and thermal conductivity (0.065-0.062 watt) and it has a gold colored, accordion shaped physical appearance. Although it has the same function as perlite, vermiculite has better thermal properties and lesser dust ratio than perlite [7].

Either consumer or broiler health can be negatively affected by unsuitable use of litter material [8]. Also litter materials should be free of other substances as chemicals, disease organisms and moulds that may damage the birds' health [2]. Litter materials compose about 4% of total consumption of broilers during growing period [9]. Litter material must be free of any contaminants, that can be absorbed by edible parts of the chicken.

According to Knowles et al.[10], modern management techniques associated to the genetic characteristics of rapidly-growing broilers have compromised their welfare, as well as their walking ability. A major welfare concern for the poultry industry is leg deformaties and lameness. It is critical to understand that lame birds experience pain. To assess lameness in a flock, use the gait scoring [11]. Foot sores and hock burns are related to leg disorders. Birds with leg problems spend more time sitting and, if the litter is wet and dirty with faeces, this results in burns and sores. Foot and hock burns in turn reduce walking activity because they make walking painful. Litter substrate significantly affected some of the indices of leg weakness measured [12]. Contact dermatitis is a 'relatively widespread' problem which can affect many of the birds in some flocks, and that it is associated with crowding, restricted movement, leg weakness and poor litter quality [13]. The material used as litters should protect birds from the impact and the friction on the poultry house floor, and this is particularly important when footpad lesions are considered, as their incidence is closely related to the quality and quantity of litter material. High litter moisture may lead to cycles of wetting and drying that compact the material and causes burns and footpad dermatitis in broilers. However, the incidence of dermatitis was significantly different ($P<0.05$) among litter materials, possibly due to differences in moisture content [14]. Footpad dermatitis, lesions on the back of the legs and feet, respectively, which may be superficial or progress into deep ulcers may also develop indirectly by deteriorating litter quality [15]. When birds lie in wet litter, ammonia produced by the decomposing organic material may irritate the skin [16].

Duration of TI has been used as a measurement for evaluating fearful behaviour and may be used as a criteria for measuring well-being and levels of stress of chickens [17].

Several factors may influence the feathering of broilers, particularly feed nutritional levels and environmental temperatures. Edens [18] found that chickens reared in cold environments presented higher feathering index, and the authors consider it an important characteristic for the maintenance of thermal homeostasis.

Formation and transformation of metabolic events takes place in liver. Measurement of ALT and AST activities is important to determine hepatic disorders. Serum total protein, albumin, total protein, glucose, cholesterol level is important for liver functions. Oxidative stress may occur as a result of increase of free radical production and decrease of antioxidant defense. Oxidative stress can be determined with SOD, MDA and catalase (CAT) measurements [19].

The objective of this study was to evaluate the effects of vermiculite as a litter material on some health and stress parameters of broiler chickens.

MATERIAL and METHODS

The experiment was carried out at Ataturk University in Research and Application Unit of School of Veterinary Faculty in accordance with approval by Ataturk Universitesi Local Etics Committee for Animal Experiments (Number: 2011/84). One hundred and ninety five (n=195) 1-d-old

(Ross 308) male commercial broiler chicks were used in the experiment. The chicks were weighed and assigned at random to 15 floor pens (13 chicks per pen) at a density of 12 broiler/m^2, in a naturally-ventilated. Chicks were brooded with one suspended electric brooder in each pen during the first 2 weeks. Birds were fed (mash form) with a starter diet from 1 to 21 d of age (24% of crude protein and 3075 kcal/ME/kg) and grower diet from 22 to 42 d of age (20% of crude protein and 3200 kcal/ME/kg). During the experiment feed and water were provided *ad libitum*. Feeder and drinker spaces were identical in each pen, and lighting was continuous (24L:0D).

The experiment consisted of 3 treatments with 5 replicates, and animals were allocated in a complete randomised design into the treatments. The 3 treatments were woodshavings (WS), a mixture of 50% woodshavings - 50% vermiculite (WSV) and vermiculite (V).

All the birds were assessed for tonic immobility (TI) at 40 d of age, and assessed for walking ability or gait score (GS) and feathering scores (FS) at 41 d of age. After slaughter at 42 d of age, carcasses were also assessed for prevalence of footpad burn (FPB). Bird feet were also assessed for prevalence of footpad burn. Feet were gently washed with a wet cloth before scoring. A scale from 1 (no lesions) to 4 (very severe lesions) was used to evaluate degree of burn, inflammation, wounds and scratches. With an aim to determine the gait score**,** each chicken was taken out of its cage and allowed to walk alone along the passageway for observation. Those reluctant to move were gently prodded. Scoring was made from "0" to "5" (GS from 0, for a perfectly normal bird, to 5, for a bird that could not walk at all) as described by Kestin et al.[20]. Chickens were caught avoiding any harm, and were transferred to a silent room, where they were restrained on their back in a cradle like U-shaped apparatus to determine tonic immobility periods as described by Jones and Faure [21]. All the birds were given feathering scores average of back, breast, wing and tail using scores of 1 to 4 for feather coverage with 1 representing maximal uncovered and 4 covered.

The blood that taken from V. subcutenea ulnaris of 10 chicks from each group, transferred to anticoagulant vacuum tubes, centrifuged at 3.000 rpm, +4°C for 10 min and stored at -20°C until the biochemical analysis. Serum SOD and MDA levels were measured according to methods reported by Sun at al.[22], Yoshioka at al.[23], CAT levels was measured according to Goth [24] with Biotek ELISA Reader. ALT, AST, TP, ALB, GLU and CHOL levels in serums were determined with Mindray perfect Plus 400 auto analyzer by ready kits.

The difference among the in terms of parameters related to footpad burn, gait score, tonic immobility and feathering score were determined with Kruskal Wallis variance analysis, the significance of difference was tested with Mann-Whitney U test. Blood biochemistry data were subjected to analysis of variance for a complete randomised design using the General Linear Models procedure of SPSS software [25]. A probability of $P<0.05$ was used for statements of significance using a Duncan multiple range comparison test.

RESULTS

The effect of use of vermiculite, wood shavings and vermiculite-wood shavings mixture as litter material in broilers on footpad burn score, gait score, tonic immobility and feathering score are presented in *Table 1* and *Fig. 1*, and the effect of litter materials on some biochemical parameters are given in *Table 2*.

DISCUSSION

Litter material significantly affected some of the indices of leg weakness measured. Footpad burn and gait scores were lower in the pens littered with V (1.06±0.05, 0.2±0.08) and WSV mix (1.02±0.01, 0.06±0.03) than in the pens littered with WS (1.98±0.12, 1.34±0.15). The use of V and WSV mixtures as litter material effected foot health positively. Although difference between V and VSW groups was not significant, observation of FPB frequency and GS in WS group was increased. So, the broilers in WS group were negatively affected by the kind of litter material.

Table 1. *Footpad burn score, gait score, tonic immobility and feathering scores of broilers reared on different litter materials*
Tablo 1. *Farklı altlık materyallarinde yetiştirilen broylerlerde ayak taban yanığı, yürüme skoru, tonik immobilite ve tüylenme skorları*

Parameters[1]	Litter Treatments[2]						P
	V		WS		WSV		
	X±Sx	Median	X±Sx	Median	X±Sx	Median	
FPB	1.06±0.05^B	1.00	1.98±0.12^A	2.00	1.02±0.01^B	1.00	0.000
GS	0.20±0.08^B	0.00	1.34±0.15^A	1.00	0.06±0.03^B	0.00	0.000
TI	172.25±31.86^B	128.00	310.60±35.29^A	360.00	153.75±29.33^B	118.50	0.005
FS	2.92±0.03	3.00	2.93±0.03	3.00	2.89±0.03	3.00	0.503

*[1] **FPB** = footpad burn score; **GS** = gait score; **TI** = tonic immobility; **FS** = feathering score; [2] **V** = vermiculite; **WS** = woodshavings (control); **WSV** = a mixture of 50% woodshavings - 50% vermiculite, [AB]: Means within rows with different superscripts are significantly different*

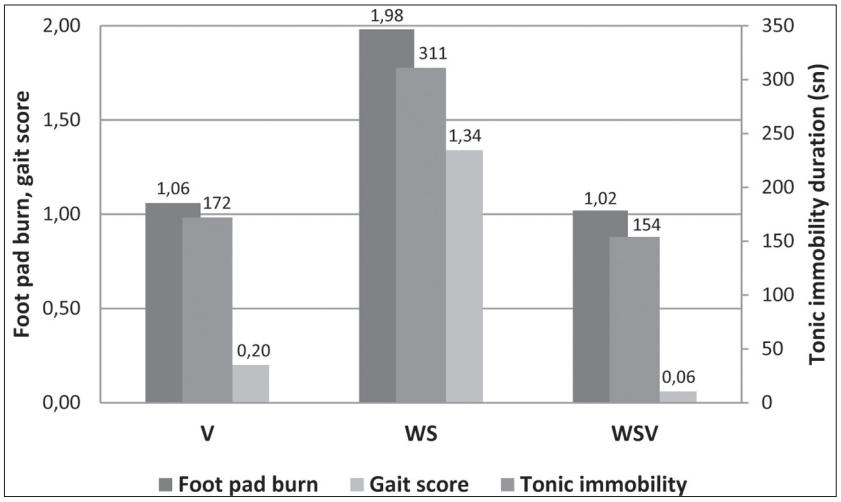

Fig 1. Footpad burn, gait score and tonic immobility duration of broilers reared on different litter materials

Şekil 1. Farklı altlık materyallerinde yetiştirilen broylerlerde ayak taban yanığı, yürüme skoru ve tonik immobilite skorları

Table 2. *Some blood biochemical parameters of broilers reared on different litter materials.*
Tablo 2. *Farklı altlık materyallerinde yetiştirilen broylerlerde bazı biyokimyasal kan parametreleri*

Biochemical Parameters	Litter Treatments			SEM	P
	V	WS	WSV		
ALT (U/l)	6.28	6.10	6.02	1.96	0.636
AST (U/l)	364.20	363.64	361.62	5.90	0.949
TP (g/dl)	3.06	3.05	3.02	0.03	0.747
ALB (g/dl)	1.39	1.42	1.40	0.02	0.797
GLU (mg/dl)	252.20	253.16	252.72	2.94	0.974
CHOL (mg/dl)	131.56	130.62	131.68	1.89	0.911
SOD (U/ml)	4.34^{B}	6.52^{A}	4.21^{B}	0.21	0.000
MDA (mol/l)	4.45^{B}	5.04^{A}	3.97^{B}	0.17	0.001
KATALAZ	54.43	59.89	53.35	2.01	0.065

^{AB} Means within rows with different superscripts are significantly different

Miles et al.[5] reported that V has not only higher water holding capacity than WS, but also it has higher NH_3 production capacity than WS. While the number of broiler with FPB was expected to increase related to ammonia production with use of V as litter, present study pointed out a decrease in the number of broiler with FPB and a positive affect on GS *(Fig. 1)*.

Sorbara et al.[26], comparing citrus pulp with wood shavings as litter material for broilers, did not find any significant difference in the incidence of footpad lesions between treatments. Santos et al.[27], in a study on the incidence of footpad lesions in broilers, concluded that the most probable cause of these lesions was excessive litter moisture. Su et al.[12], noticed that wood shavings is better litter material than chopped straw on walking ability and the incidence of FPB, comparing chopped straw with wood shavings as litter material for broilers.

As to findings obtained from present study, the effect of litter type on the tonic immobility duration was found significant (P<0.05). Tonic immobility of WS group (310.6±35.29) was longer than V (172.25±31.86) and WSV groups (153.75±29.33). WS group chickens were more fearful than the V and WSV group chickens. Campo et al.[28] confirmed that the development of footpad dermatitis had an increasing effect on the fearfulness of cocks kept in cages. Parallel to findings of present study, TI duration was found lower in groups, that has lower FPB levels.

The effect of litter type was not significant (P>0.05) on the feathering score of broilers in this study. Several factors may influence the feathering of broilers, particularly feed nutritional levels and environmental temperatures. Edens [18] found that chickens reared in cold environments presented higher feathering index, and researcher considered it an important characteristic for the maintenance of thermal homeostasis. Differences among the V, WSV and WS groups in terms of feathering index were not significant. That may be caused insignificant difference among the kind of litter materials in terms of termal insulation.

ALT, AST, TP, ALB, GLU, CHOL and CAT were not significant in V, WSV and WS groups (P>0.05). The most common used litter material in the world is wood shavings. Indifference between wood shavings and vermiculite pointed out

that vermiculite may be used as litter material in broilers without adverse health effects. Davis [29] reported that vermiculite did not have not negative effect on health as a result of study in rotends.

While TP and ALB levels in groups raised on wood shavings were compatible with the study findings performed by Yalçınkaya et al.[30], serum AST and CHOL levels were found higher than those of Yalçınkaya et al.[30]. Seven et al.[31] found lower GLU, TP, CHOL levels and higher ALB levels than those of present study. Serum TP and ALB values obtained from our study were parallel with the results of some other researchers [32,33].

SOD and MDA parameters varied significantly due to the kind of litter material in the present experiment *(Table 2)*. SOD, MDA values of oxidative stress parameters were found lower (P<0.01) in the V (4.34±0.21, 4.45±0.17) and WSV (4.21±0.21, 3.97±0.17) groups than that of WS group (6.52±0.21, 5.04±0.17). The study result showed that a lower value of MDA in V group in comparison to WS group because of decreasing effect of V on oxidative stress. Arivuchelvan et al.[34] found lower serum MDA level in the group reared on WS as litter material than that of other materials used as litter.

Consequently, the use of vermiculite and vermiculite-wood shavings mixture as litter material in broiler did not have any adverse effect on health and stress parameters, and V and WSV provided better conditions than WS in terms of some health and stress parameters. But, furter studies on the effects of different ratios of wood shavings and vermiculite mixture on performance of broilers should be researched.

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RESEARCH ARTICLE

Gebe Farelerde Perifer Kan ve Endometriyum Dokusunda T-lenfosit, Null Lenfosit ve Asit Fosfataz Pozitif Lenfositlerin Oran ve Dağılımları

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Özet

Bu çalışma, farelerde gebeliğin T-lenfosit, null lenfosit ve asit fosfataz (ACP-az) pozitif lenfositlerin perifer kandaki oranı ve endometriyumun desidua bazalis bölgesindeki dağılımı üzerindeki etkilerinin belirlenmesi amacıyla yapıldı. Bu amaçla, 12-14 haftalık fareler, gebe olmayan-kontrol grubu ile gebeliğin birinci, ikinci ve üçüncü haftalarının ortasına karşılık gelecek şekilde erken, orta ve geç gebelik dönemi (sırasıyla gebeliğin 3., 10. ve 17. günleri) olmak üzere 4 gruba ayrıldılar (n = 6). En düşük T-lenfosit oranı perifer kanda (%43.83) ve desidua bazalis dokusunda (10.83 adet/0.1 mm^2) sırasıyla gebeliğin erken ve orta dönemlerinde tespit edildi. Perifer kan ACP-az pozitif lenfosit oranlarında gebeliğin ikinci haftasında istatistiksel olarak önemli bir yükselme gözlenirken (%44.33); desidua bazalis dokusunda en düşük ACP-az pozitif lenfosit sayısı (5.50 adet/0.1 mm^2) gebeliğin erken döneminde gözlendi. Hem perifer kanda (%11.50) ve hem de desidua bazalis dokusunda (7.83 adet/0.1 mm^2) en yüksek null hücre oranı erken gebelik döneminde tespit edildi. En düşük perifer kan lenfosit oranı (%56.00) erken dönemde gözlendi. Dönemler arasında bazı farklar olsa da gebeliğin lenfosit, T-lenfosit, null lenfosit ve ACP-az pozitif lenfositlerinin sayı ve dağılımlarını etkilediği sonucuna varıldı.

Anahtar sözcükler: *ACP-az, ANAE, Fare, Gebelik*

The Proportion and The Distribution of T-lymphocytes, Null Lymphocytes and Acid Phosphatase Positive Lymphocytes of The Peripheral Blood and Endometrium in Pregnant Mice

Summary

This study was performed to determine the effects of pregnancy on the proportion of T-lymphocytes, null lymphocytes and acid phosphatase (ACP-ase) positive peripheral blood lymphocytes and the distribution of the mentioned cells in the decidua basalis region of endometrium in the pregnant mice. For this purpose, mice at 12-14 weeks of age were divided into four groups as non-pregnant control, and at the middle of the first, second, and the third week of the pregnancy, corresponding to early, middle, and late (3rd, 10th, and 17th days of pregnancy) gestational stages respectively (n = 6 for each group). The lowest T-lymphocytes percentage was determined at early and middle pregnancy in the peripheral blood (43.83%) and decidua basalis (10.83 number/0.1 mm^2), respectively. There was a statistically significant increase in the proportions of the peripheral blood ACP-ase (+) lymphocytes (44.33%) at the mid-gestational period while the lowest ACP-ase positive lymphocyte numbers (5.50 number/0.1 mm^2) in the decidua basalis was observed at early pregnancy. The highest null cell rates were found at early gestation either in the peripheral blood (11.50%) or in the decidua basalis (7.83 umber/0.1 mm^2). The lowest percentage of peripheral blood lymphocyte (56.00%) was recorded at the early pregnancy. It was concluded that the number and the distribution of the lymphocyte, T-lymphocyte, null lymphocyte, and ACP-ase positive lymphocyte were affected by pregnancy although there were some differences among the gestational periods.

Keywords: *ACP-ase, ANAE, Mice, Pregnancy*

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GİRİŞ

Gebelik, zigot ve devam eden süreçte fötüs tarafından eksprese edilen ve anne için yabancı olan antijenlere karşı maternal bağışıklık sistemin toleransı ile karakterize fizyolojik bir durum olarak tanımlanmaktadır. Temelini lenfosit alt tipleri ve doğal katil hücrelerinin (NK) kan ve endometriyum dokusundaki sayı ve dağılımları ile ürettikleri sitokin düzeylerindeki değişimlerim oluşturduğu bu süreç maternal tolerans olarak da adlandırılır [1,2].

Mahmoud ve ark.'nın [3] sağlıklı gebe ve gebe olmayan kadınlarda yaptıkları bir çalışmada, gebelik süresince perifer kan toplam lenfosit sayılarının yanı sıra B-lenfosit sayısı ile doğal katil hücrelerin sayısında belirgin düşüşler gözlenirken; Medina ve ark.'nın [4] farelerde yaptığı bir çalışmada da gebelikle birlikte B-lenfosit öncüllerinin ve B-lenfosit yapımının düştüğü bildirilmiştir. Nakamura ve ark.[5] da insanlarda gebelik boyunca sitotoksik T-lenfositlerinin aktivitelerinin azaldığını bildirmektedirler.

Gebeliğin bağışıklık sistemi üzerindeki etkilerinin araştırıldığı pek çok çalışma, histolojik açıdan en belirgin değişimlerin uterus mukozasında olduğunu göstermiştir. Martinez ve ark.'nın [6] keçilerde yaptıkları bir çalışmada gebe olmayan uterusların içerdiği lenfositlerin pek çoğunun T-lenfosit olduğu; buna karşın gebe uterusların karunkular bölgesinde tüm lenfosit alt tiplerinin büyük çoğunluğunun gözden kaybolduğu bildirilmiştir.

Lizozomal bir enzim olan alfa-naftil asetat esteraz (ANAE) enziminden [7], başta insan [8] olmak üzere, sığır [9], tavuk [10], köpek [11] ve farelerde [12] T-lenfositlerin ayrımında yararlanılır. Asit fosfataz (ACP-az) ise miyelositler, polimorf nükleer lökositler, lenfositler, plazma hücreleri, megakaryositler, kan pulçukları ve mononükleer fagositik sistem hücrelerinde bulunan lizozomal bir enzimdir [13].

Bu çalışma, insanlarda ve çiftlik hayvanlarında elde edilmesi son derece sınırlı olan sağlıklı gebe uterus endometriyum dokusunda T-lenfosit, null lenfosit ve asit fosfataz (ACP-az) pozitif lenfositlerin dağılımında gebelikle birlikte meydana gelen değişimlerin farelerde belirlenmesi ve bundan sonra maternal tolerans konusunda yapılacak olan çalışmalara katkı sağlamak amacıyla yapıldı.

MATERYAL ve METOT

Materyal

Hayvan Materyali

Çalışma, Selçuk Üniversitesi Meram Tıp Fakültesi Etik Kurulu'nun 31.08.2009 tarih ve 2009/50 sayılı kararı ile alınan Etik Kurul Onayı ile gerçekleştirildi. Çalışmanın materyalini erişkin (12-14 haftalık) dişi fareler oluşturdu. Dişi ve erkek fareler, 1 adet erkek 4 adet dişi birlikte olacak şekilde gruplara ayrılarak aynı kafese alındılar. Bir gece boyunca çiftleşmeye bırakılan fareler günlük olarak vajinal tıkaç oluşumu yönünden kontrol edildiler. Vaginal tıkaç oluşan dişi fareler gebeliklerinin sıfırıncı gününde kabul edilip ayrı kafeslere aktarılarak takibe alındılar. Fareler, her grupta 6'şar adet olacak şekilde gebe olmayan-kontrol grubu ile gebeliğin birinci, ikinci ve üçüncü haftalarının ortalarına karşılık gelecek şekilde erken, orta ve geç gebelik dönemi (sırasıyla gebeliğin 3, 10. ve 17. günleri) olmak üzere 4 gruba ayrıldılar. Sakrifiye edilen farelerden perifer kan ve implantasyon bölgesinden uterus dokusu örnekleri alındı.

Metot

Alınan kan örneklerinden 6'şar adet froti hazırlanarak havada kurutulduktan sonra ANAE ve ACP-az enzimi demonstrasyonu ve May Grünwald-Giemza boyaması için -10°C'deki glutaraldehid-aseton tespit solüsyonunda (pH = 4.8) 3 dk. süreyle tespit edildiler. Frotilerden ikişer adedi, inkübasyon solüsyonları içerisinde 37°C'de 2 saat kontrollü bir şekilde boyandılar. Lenfositlerde kırmızı ya da kahverengi granüllerin ortaya çıkmasını takiben inkübasyon işlemi sona erdirildi ve ardından %1'lik methyl-green ile çekirdek boyası uygulandı [14]. Kalan 2 froti ise perifer kan lenfosit oranlarının belirlenmesi amacıyla klasik May Grünwald-Giemza boyama metodu ile boyandılar.

Uterus dokusundan alınan örnekler enzim demonstrasyonları için 24 saat formal-sükroz (+4°C, pH 6.8) solüsyonunda tespit edildikten sonra 22 saat de Holt solüsyonunda (+4°C) bekletildiler. Doku örneklerinden kriyostatta (Leica, CM 1510 S) 12 µm kalınlığında seri kesitler alındı. İnkübasyon solüsyonları içerisinde oda sıcaklığında 15 dak. süreyle kontrollü bir şekilde bekletilen kesitlerdeki lenfositlerde kırmızı-kahverengi granüllerin ortaya çıkmasının ardından %1'lik methylgreen ile çekirdek boyası uygulandı [15].

Hücre Sayımları

Enzim demonstrasyonu yapılan kan preparatlarının her birinde toplam 200 adet lenfosit sayılarak enzim pozitif lenfosit oranları belirlendi. Tüm preparatlar DFC-320 model kamera ataçmanı olan Leica DM-2500 model ışık mikroskobu ile incelendikten sonra gerekli bölgelerin dijital görüntüleri kaydedildi. Kriyostatta 36 µm aralıklarla alınan 3 seri uterus kesitinin desidua bazalis'e karşılık gelen 10 farklı bölgesinden toplam 0.1 mm^2'lik alanda, ANAE demonstrasyonu yapılan preparatlarda T-lenfosit ve null lenfositler sayılırken; ACP-az demonstrasyonu yapılanlarda ACP-az pozitif lenfositler sayıldı.

İstatistik Analizler

Doku kesitlerinden elde edilen sayısal veriler SPSS 10 [16] programı yardımıyla One-Way Anova testi ve ardından çoklu karşılaştırma testlerinden DUNCAN testiyle analiz edilerek grupların ortalama değerleri arasındaki farkların önem dereceleri belirlendi.

Perifer kan enzim pozitivite oranları ile lenfosit oranları ise Açı (Arc Sinus) dönüşüm metodu kullanılarak analiz edildi. Bu metoda göre transforme edilen parametrelerinin birbirleriyle karşılaştırmalarında SPSS 10 istatistik programı yardımıyla DUNCAN testi kullanıldı. Verilerin tablolaştırılmasında dönüşüm öncesi gerçek değerler kullanıldı.

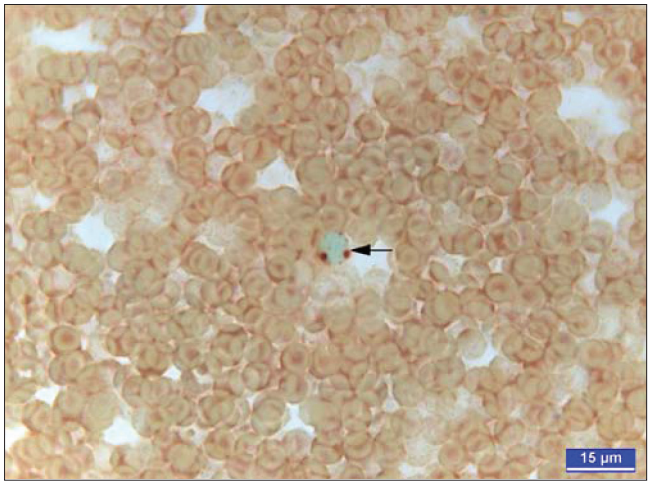
BULGULAR

Enzim Histokimyasal Değerlendirmeler

Gerek frotiler ve gerekse kriyostat kesitleri üzerinde yapılan ışık mikroskobik incelemeler sonucunda, lenfositlerde 2 farklı ANAE enzimi aktivitesi gözlendi. Sayıları 1-4 arasında değişen kahverengi granüller içeren lenfositler T-lenfosit olarak değerlendirilirken (*Şekil 1* ve *Şekil 4*); sayıları 5-8 arasında değişen kahverengi granüller içeren lenfositler "null lenfosit" olarak değerlendirildi (*Şekil 2* ve *Şekil 5*). ACP-az enzimi aktivitesinin ise 1-3 adet pembe-kırmızı granül şeklinde olduğu dikkati çekti (*Şekil 3* ve *Şekil 6*).

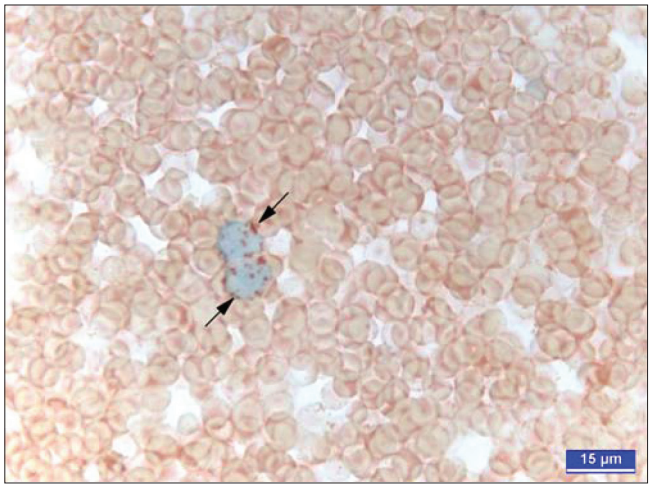
Enzim Histokimyasal Bulgular

Perifer Kan: Perifer kanda yapılan enzim histokimyasal boyamalar sonucunda kontrol grubunda %64.67 olan T-lenfosit oranının gebeliğin 1. haftası içerisinde belirgin bir biçimde düşerek %43.83'e gerilediği; kontrol grubunda %5.66 olan null lenfosit oranının ise yine erken dönemde en yüksek seviyesine ulaşarak %11.5'e çıktığı tespit edildi. Kontrol grubunda %67.5 olan lenfosit oranının ise gebeliğin ilk haftasında %56'ya düştüğü dikkati çekti. Buna karşın ACP-az pozitif lenfosit oranının ise gebeliğin ilk haftası içinde istatistiksel olarak anlamlı olmasa da %36.83'ten %34.66'ya düştüğü, gebeliğin ikinci haftası içinde ise belirgin bir biçimde yükseldiği (%44.33), ancak doğuma yakın dönemde tekrar başlangıç değerlerine (%35.5) gerilediği belirlendi *(Tablo 1)*.



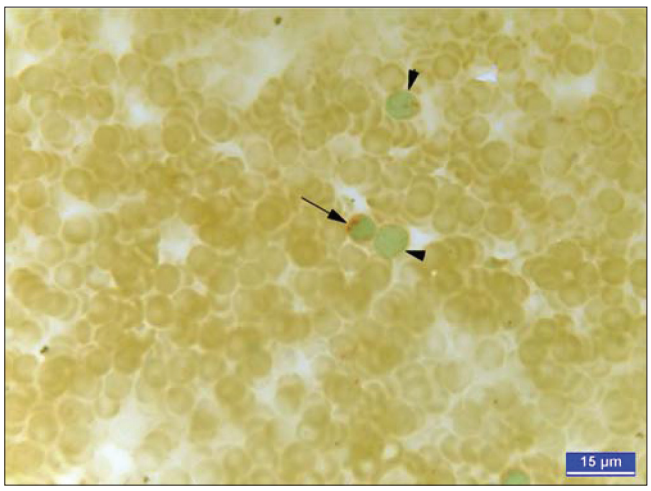
Şekil 1. Kontrol grubundan bir fareye ait perifer kan frotisinde ANAE demonstrasyonu. *Ok:* T-lenfosit, Büyütme çizgisi: 15 µm

Fig 1. A peripheral blood T lymphocyte in animal from control group. ANAE demonstration. *Arrow:* T lymphocyte, Bar: 15 µm



Şekil 2. Gebeliğin erken dönemindeki bir fareye ait perifer kan frotisinde ANAE demonstrasyonu. *Oklar:* Null lenfositler, Büyütme çizgisi: 15 µm

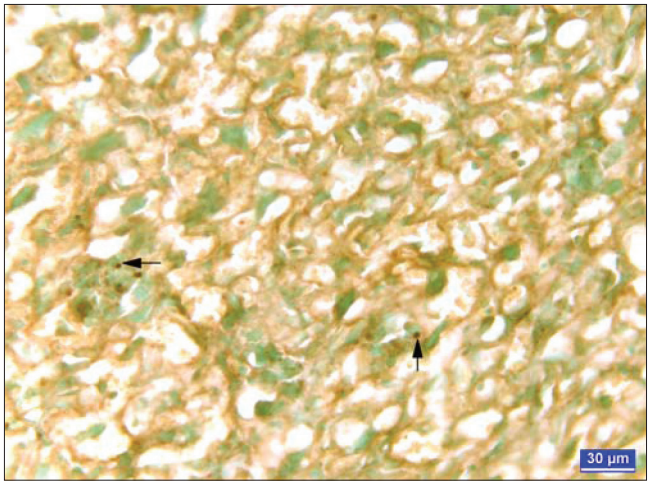
Fig 2. A peripheral blood null lymphocyte in animal at the early pregnancy period. ANAE demonstration. *Arrows:* Null lymphocytes, Bar: 15 µm



Şekil 3. Kontrol grubundan bir farenin perifer kan frotisinde ACP-az enzimi demonstrasyonu. *Ok:* ACP-az-pozitif lenfosit, *Ok başları:* ACP-az negatif lenfositler, Büyütme çizgisi: 15 µm

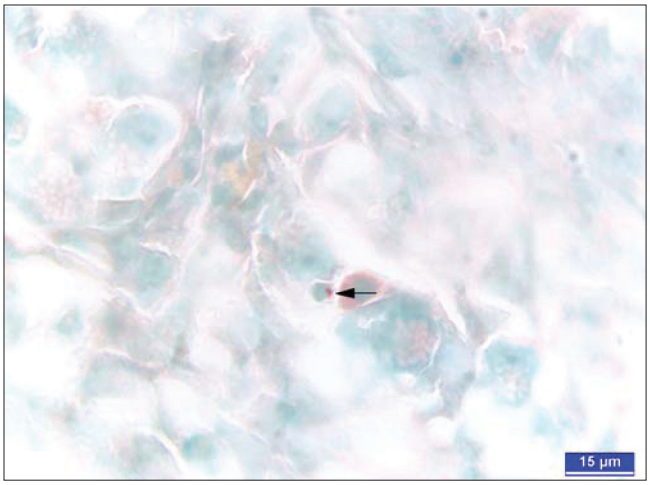
Fig 3. An ACP-ase-positive peripheral blood lymphocyte in animal from control group. ACP-ase demonstration. *Arrow:* ACP-ase-positive lymphocyte, *Arrow heads:* ACP-ase-negative lymphocytes, Bar: 15 µm

Endometriyum: Endometriyum'un desidua bazalis'e karşılık gelen bölgesinden yapılan hücre sayımları sonucunda kontrol grubunda yer alan hayvanların birim alanındaki T-lenfosit sayıları 17.67 adet/0.1 mm^2 olarak tespit edildi. Gebelikle birlikte düşüşe geçen ve tüm gebelik süresince düşük seyreden bu sayının gebeliğin orta dönemindeki hayvanlarda 10.83 adet/0.1 mm^2 ile en düşük değerine gerilediği dikkati çekti. ACP-az pozitif lenfositlerin sayıları da benzer biçimde tüm gebelik boyunca kontrol grubundan düşük değerlerde seyrederken en düşük değerine 5.5 adet/0.1 mm^2 ile gebeliğin erken döneminde ulaştığı tespit edildi. Buna karşın kontrol grubunda 3.83 adet/0.1 mm^2 olan null lenfosit sayısının gebeliğin erken döneminde belirgin bir biçimde artarak 7.83 adet/0.1



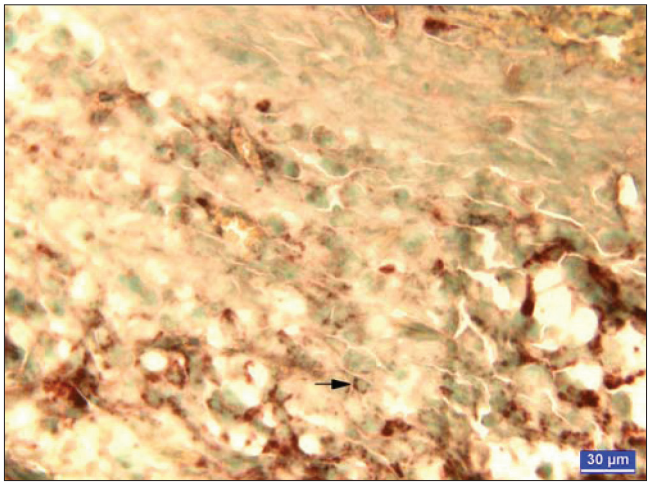
Şekil 4. Gebeliğin orta dönemindeki bir farenin endometriyumundan alınan kriyostat kesiti. ANAE demonstrasyonu. *Oklar:* T-lenfositler, Büyütme çizgisi: 30 µm

Fig 4. A cryostat section from endometrium of animal at middle gestational period. ANAE demonstration. *Arrows:* T lymphocytes, Bar: 30 µm



Şekil 6. Gebeliğin geç dönemindeki bir farenin endometriyumundan alınan kriyostat kesiti. ACP-az demonstrasyonu. *Ok:* ACP-az pozitif lenfosit, Büyütme çizgisi: 15 µm

Fig 6. A cryostat section from endometrium of animal at late gestational period. ACP-ase demonstration. *Arrow:* ACP-ase positive lymphocyte, Bar: 15 µm



Şekil 5. Gebeliğin erken dönemindeki bir farenin endometriyumundan alınan kriyostat kesiti. ANAE demonstrasyonu. *Ok:* Null lenfosit, Büyütme çizgisi: 30 µm

Fig 5. A cryostat section from endometrium of animal at early gestational period. ANAE demonstration. *Arrow:* Null lymphocyte, Bar: 30 µm

mm²'ye ulaştığı, ancak devam eden süreçte kontrol grubuna yakın değerlere gerilediği dikkati çekti *(Tablo 2)*.

TARTIŞMA ve SONUÇ

Gebeliğin perifer kan hücreleri üzerindeki etkilerinin araştırıldığı çalışmalar çoğunlukla lenfositler üzerinde yoğunlaşmıştır. Agricola ve ark.'nın [17] kısraklarda yaptıkları bir çalışmada gebelik süresince perifer kan toplam lökosit sayılarının yanı sıra, toplam T-lenfosit, yardımcı T-lenfosit ve sitotoksik T-lenfosit sayılarında düşüşlerin meydana geldiği gözlenmiştir. Pisek ve ark.'nın [18] koyunlarda yapmış oldukları bir çalışmada ise gebelik süresince perifer kan toplam akyuvar sayısında önemli düşüşlerin meydana geldiği ve düşüşün asıl kaynağının nötrofil ve lenfosit sayılarındaki düşüşler olduğu kanıtlanmıştır. Sur ve ark.'nın [14] gebe sığırlarda yaptıkları çalışmada da perifer kan lenfosit oranının I. trimesterde belirgin bir biçimde düştüğü ortaya konulmuştur. Bu çalışmada da perifer kan lenfosit oranlarının sadece gebeliğin erken dönemlerinde belirgin bir biçimde düştüğü, ancak ilerleyen günlerde yükselişe geçerek kontrol grubu farelere yakın değerlere ulaştığı görülmüştür *(Tablo1)*.

Alfa naftil asetat esteraz (ANAE) enzimi, içerisinde insan ve farelerin de yer aldığı pek çok türde T-lenfositleri için

Tablo 1. *Gebeliğin farklı dönemlerinde perifer kan lenfosit, T lenfosit, null lenfosit ve ACP-az pozitif lenfosit oranları*
Table 1. *Proportion of peripheral blood lymphocyte, T lymphocyte, null lymphocyte, and ACP-ase positive lymphocyte in different gestational stages*

Gruplar (n=6)	Lenfosit (%) ± SE	T-lenfosit (%) ± SE	Null Lenfosit (%) ± SE	ACP-az + Lenfosit (%) ± SE
Kontrol (Gebe olmayan fareler)	67.50±1.258^{a}	64.67±1.308^{a}	5.66±0.421^{a}	36.83±2.821^{a}
Grup 2 (Erken dönem)	56.00±1.064^{b}	43.83±2.167^{b}	11.50±0.670^{c}	34.66±1.584^{a}
Grup 3 (Orta dönem)	65.00±3.00^{a}	44.17±1.167^{b}	8.83±1.424^{b}	44.33±1.406^{b}
Grup 4 (Geç dönem)	67.16±1.922^{a}	61.33±1.406^{a}	10.66±0.666^{bc}	35.50±1.910^{a}
P	*	**	**	*

a-c: *Aynı sütunda farklı harfler taşıyan gruplar arasındaki farklılıklar istatistiksel açıdan önemlidir. * P<0.05, ** P<0.001*

Tablo 2. *Gebeliğin farklı dönemlerinde desidua bazalis dokusunda T lenfosit, null lenfosit ve ACP-az pozitif lenfositlerin dağılımı (adet/0.1 mm²)*
Table 2. *Distribution of T lymphocyte, null lymphocyte, and ACP-ase positive lymphocyte in decidua basalis of different gestational stages (number/0.1 mm²)*

Gruplar (n=6)	**T-lenfosit ± SE**	**Null ± SE**	**ACP-az ± SE**
Kontrol (Gebe olmayan fareler)	17.67±0.715[a]	3.83±0.5426[a]	14.83±0.477[a]
Grup 2 (Erken dönem)	13.67±1.145[b]	7.83±1.166[b]	5.50±0.763[c]
Grup 3 (Orta dönem)	10.83±1.515[b]	3.33±0.714[a]	7.66±0.760[b]
Grup 4 (Geç dönem)	12.67±1.085[b]	3.83±0.307[a]	7.66±0.666[b]
P	*	**	**

a-c: *Aynı sütunda farklı harfler taşıyan gruplar arasındaki farklılıklar istatistiksel açıdan önemlidir. * P<0.05, ** P<0.001*

spesifiktir [8-12]. Gerek perifer kan ve gerekse dokulardaki lenfositlerde iki farklı ANAE enzimi pozitivitesi söz konusudur. Sayıları 1-4 arasında değişen granüllerin oluşturduğu boyanma şeklinin T-lenfositleri için spesifik olduğu bildirilmektedir [8]. Akbulut'un [19] insanlarda yapmış olduğu çalışmada ANAE histokimyası ile T-lenfosit olarak değerlendirilen hücrelerin perifer kandaki oranı hamile olmayan sağlıklı bayanlarda %70 olarak bulunurken; hamilelikle birlikte bu oranın %58-60'lara kadar düştüğü görülmüştür. Sur ve ark.'nın [14] sığırlarda yaptıkları bir çalışmada da ANAE histokimyası ile demonstre edilen perifer kan T-lenfositlerinde gruplararası istatistiksel farklar olmasa da en düşük oranın ilk trimesterdeki hayvanlarda gözlendiği bildirilmektedir. Sur ve ark.'nın [20] koyunlarda yaptıkları bir başka çalışmada ise ANAE pozitif lenfosit oranı diğer gruplara nazaran gebeliğin ilk ayında %63.5'lik oranla en düşük seviyede gözlenmiştir. Bu çalışmada da kontrol grubunu oluşturan farelerin ANAE histokimyası ile demonstre edilen perifer kan T-lenfosit oranları %64 olarak bulunurken, gebeliğin ilk günlerinde bu rakamın %43'e kadar gerilediği dikkati çekmiştir *(Tablo 1)*.

ANAE histokimyası ile lenfositlerde tespit edilen bir diğer boyanma şekli ise çok sayıda dağınık yerleşimli granüllerin oluşturduğu ve "null" lenfositleri için özel olduğu ileri sürülen boyanma şeklidir [8]. Null lenfositlerinin, matur T- ve B-lenfositlerine ait reseptörler taşımayan ancak doğal katil (natural killer cell-NK) hücrelerinin öncüllerinin yanı sıra farklı gelişim aşamalarındaki T- ve B-lenfosit serilerine ait hücreleri de içeren bir lenfosit alt tipi olduğu bildirilmektedir [21,22]. Akbulut'un [19] insanlarda yapmış olduğu çalışmada null lenfosit olarak değerlendirilen hücrelerin oranı kontrol grubunda %2 olarak bulunurken, gebeliğin I. trimesterindeki bayanlarda bu hücrelerin oranının önemli derecede artarak %11'e yükseldiği bildirilmiştir. Sur ve ark.'nın [14] sığırlarda yaptıkları bir çalışmada da en yüksek perifer kan null lenfosit oranının %8.1'lik ortalama ile yine gebeliğinin ilk trimesterindeki hayvanlarda tespit edildiği bildirilmiştir. Sur ve ark.'nın [20] koyunlarda yaptıkları bir başka çalışmada ise en yüksek null lenfosit oranı %12.75'lik ortalama ile yine gebeliğinin ilk ayındaki koyunlarda tespit edilmiştir. Bu çalışmada da null lenfosit olarak değerlendirilen perifer kan lenfositlerinin oranı kontrol grubunda %5.66 iken, gebeliğin ilk günlerinde bu oranın %11.5'e kadar yükseldiği ve gebelik süresince de kontrol grubundan yüksek seyrettiği tespit edilmiştir *(Tablo 1)*. Gebelikte NK hücrelerinin perifer kandaki oranlarında gözlenen değişimlerin klinik açıdan önemli olduğu bildirilmektedir. Andalip ve ark.'nın [23] sağlıklı bir gebelik süreci geçiren kadınlar ile tekrarlayan spontan düşük (RSA) geçmişi olan kadınlarda yaptıkları bir çalışmada sağlıklı gebelerin perifer kan NK oranı %9.21 olarak bulunurken RSA geçmişi olanlarda bu oranın %13.48 olduğu tespit edilmiştir. Bu bilgiler dikkate alındığında, çalışmada elde edilen bulguların ileride yapılacak olan klinik çalışmalar için de önemli olduğu düşünülmektedir.

Lenfosit histokimyasında kullanılan bir diğer enzim de asit fosfataz (ACP-az) enzimidir. Sur ve ark.'nın [14] sığırlarda yaptıkları çalışmada gebeliğin ilk ve son dönemlerinde ACP-az pozitif lenfosit oranlarında belirgin düşüşlerin varlığı dikkati çekmiştir. Yine Sur ve ark.'nın [20] koyunlarda yapmış oldukları bir başka çalışmada en düşük ACP-az pozitif lenfosit oranının gebeliğin son dönemindeki hayvanlarda tespit edildiği bildirilmiştir. Bu çalışmada ise ACP-az histokimyası ile elde edilen sonuçlar çiftlik hayvanlarından elde edilen sonuçlardan oldukça farklı bulunmuştur. Zira gebelik süresince ACP-az pozitif lenfosit oranlarında anlamlı bir düşüş tespit edilmemiş; aksine gebeliğin orta dönemindeki farelerde bu oranın belirgin bir biçimde yükseldiği görülmüştür *(Tablo 1)*. Bu durumun türler arası farktan ileri gelebileceği düşünülmektedir.

Gebeliğin bağışıklık sistemi üzerindeki etkilerinin araştırıldığı pek çok çalışma, histolojik açıdan en belirgin değişimlerin uterus mukozasında olduğunu göstermiştir. Diğer sistemlere ait mukozalarda olduğu gibi uterus mukozası da normalde T- ve B-lenfositlerinin yanı sıra makrofajlar, dendritik hücreler ve NK hücrelerini içerir [24,25]. Bununla birlikte hormonal değişimlerin etkisi altında olan uterus dokusu, gerek seksüel siklus, gerekse gebelikle birlikte hücresel anlamda yeniden düzenlenir [26]. Karaca ve ark.'nın [27] keçilerde yapmış oldukları bir çalışmada ANAE-pozitif lenfositlerin preimplantasyon dönemindeki hayvanların uterus dokusundaki sayısal dağılımlarının gebe olmayan hayvanlara göre daha az olduğu ileri sürülmektedir. Bu çalışmada da desidua bazalis'e karşılık gelen endometriyum bölümünden alınan kriyostat kesitlerinde birim alandaki T-lenfosit ve ACP-az pozitif lenfosit sayıları dikkate alındığında belirgin değişimler göze çarpmaktadır.

Özellikle T-lenfosit sayılarında gebelikle birlikte başlayan ve tüm gebelik boyunca devam eden düşüşler oldukça belirginken *(Tablo 2);* benzer şekilde ACP-az pozitif lenfosit sayılarında da özellikle gebeliğin ilk haftasında gözlenen düşüşler, uterus endometriyumunda gebelikle birlikte meydana gelen hücresel değişimler açısından değerlendirildiğinde oldukça dikkat çekicidir *(Tablo 2)*.

Bu çalışmada farelerden elde edilen bulgular, insan [3,5,19,23], kısrak [17], keçi [6,27], sığır [14], koyun [18,20] ve farelerde [4] daha önce yapılan benzer çalışmalardan elde edilen bulgularla uyumlu bulunmuştur. Özellikle gebeliğin ilk dönemlerinde perifer kan lenfosit oranlarında tespit edilen ve hem perifer kan T-lenfosit oranında hem de desidua bazalis bölgesinin birim alanındaki T-lenfosit sayılarında meydana gelen paralel düşüşler en dikkat çekici bulgular olarak karşımıza çıkmaktadır. Seksüel siklusun diöstrus evresinde korpus luteumdan salgılanan ve eğer gebelik şekillenmişse bu süre içerisinde de salgılanmaya devam eden progesteron hormonun lenfositlerin çoğalmasını baskıladığı bilinmektedir. Gerek luteal fazda ve gerekse gebelik süresince lenfositlerdeki progesteron reseptörlerinin arttığı yönündeki bilgiler dikkate alındığında söz konusu düşüşlerin altında yatan mekanizma bir ölçüde açıklanabilir [28]. Buna karşın yine erken dönemde gözlenen null lenfosit oran ve sayılarındaki artışlar da (*Tablo 1* ve *Tablo 2*), annenin bağışıklık sisteminin bir tepkisi olarak değerlendirilebilir. İmplantasyon, bağışıklık sistemine ait hücrelerce belli bazı sitokinlerin salınmasıyla karakterize yangısal bir süreçtir [29]. Başlangıçta anneye ait bağışıklık sisteminin babaya ait antijenler de içeren plasenta dokusuna karşı tepkisel bir reaksiyonu gibi algılanabilecek olan bu durum, gerek progesteron hormonunun ve gerekse plasentaya ait hücrelerin salgılamış oldukları bir takım mediyatörler aracılığıyla önce bölgesel sonra da sistemik bir immün tolerans olarak karşımıza çıkmaktadır. Maternal tolerans olarak da bilinen bu durumun bir sonucu olarak, normal bir gebelikte annenin bağışıklık sisteminin embriyoya saldırmaması için hem perifer kan ve hem de endometriyumda bağışıklık sistemi hücrelerinin sayı, dağılım ve fonksiyonel aktivitelerinde bir takım değişimler söz konusudur [1,30]. Koç ve Kanter'in [31] gebe sıçanlarda yaptıkları bir çalışmada ANAE pozitivitesi ile demonstre edilen uterus NK hücrelerinin endometriyum dokusunda implantasyonun ikinci gününden itibaren arttığı ve 6. günde en yüksek seviyeye ulaştığı bildirilmektedir. Bazı araştırıcıların, null hücreler ile NK hücrelerinin birlikte değerlendirilebileceğini ileri süren çalışmaları da dikkate alındığında [32] bu çalışmada gebeliğin erken dönemlerinde tespit edilen perifer kan ve desidua bazalis dokusundaki null hücre sayısındaki artışlar, embriyonun maternal kabulü sürecinde meydana gelen en dikkat çekici hücresel değişim olarak ileri sürülebilir. Özellikle desidua bazalis dokusundaki null hücre sayısında gebeliğin ilerleyen günlerinde gözlenen düşüşler de embriyonun maternal kabulünün hücresel bir göstergesi olarak değerlendirilebilir *(Tablo 2)*.

İmplantasyon ile başlayan ve embriyonun kabulü ile devam eden süreçte annenin immün sisteminin, bir yandan hastalık yapıcı patojen etkenlere karşı işlevsel kalabilmesi bir yandan da gelişmekte olan embriyoya karşı tolerans geliştirmesi ileri derecede hassas bir dengenin sonucudur. Bu dengenin kurulması ve söz konusu mekanizmaların kusursuz işlemesi sağlıklı bir gebelik süreci için mutlaka gereklidir. Ayrıca bu mekanizmaların iyi anlaşılmasının infertilite ve tekrarlayıcı düşük vakalarının tedavisine ışık tutacağı gibi ilerleyen dönemlerde doku nakillerinde yaşanan sorunların çözümüne de katkı sağlayacağı düşünülebilir.

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RESEARCH ARTICLE

The Protective Role of Silymarine on Selenite-Induced Cataract in Rabbit Model

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Summary

The study was designed to investigate silymarine as preventive agent in selenite-induced cataract in rabbits. Eighteen rabbits were concluded and divided equally into negative control (I), positive control (II) and test (III) groups, each 6. After clinical and ophthalmoscope, bio-microscope slit lamp equipped with a digital camera and ultrasonographical examinations of rabbits' eyes, selenite sodium was administered subcutaneously on the neck in all rabbits at day 0 of the study. After induction of anesthesia, lateral recumbency and making a pore in globe 0.1ml of saline solution 0.9% in group II and 0.1ml of silymarine were injected into vitreous in group III. Any agent did not administrate in group I. Selenite sodium was injected in days third and sixth in all rabbits subcutaneously. The bio-microscopic slit lamp study showed grade one and two cataract in day 4 and 8, and grade three in day 11 in all rabbits in group I and II. In group III, three rabbits on the eighth day and four until the end of the eleventh day presented grade one cataract. Anterior lens capsule thickness was less in group III in comparison to group I and II, and posterior capsule thickness in group III was significant statistically by ultrasonography on day 20 and the anterior-posterior lens diagonal length in the experimental group comparing to the control groups was longer significantly. Selenite sodium showed cataractogenic character and silymarine as probable protective role on selenite –induced cataract in rabbit model.

Keywords: *Cataract, Selenite sodium, Silymarine, Rabbit*

Tavşan Modelinde Silymarinin Selenit ile Oluşturulmuş Katarakt Üzerine Koruyucu Rolü

Özet

Bu çalışma tavşanlarda selenit ile oluşturulmuş katarakt üzerine önleyici ajan olarak silymarinin araştırılması için tasarlanmıştır. Her biri altılı gruplardan oluşan 18 tavşan negatif kontrol (I), pozitif kontrol (II) ve test (III) grupları olmak üzere eşit şekilde ayrıldı. Tavşan gözlerinin klinik ve oftalmoskop, dijital kameralı biyo-mikroskop yarık (slit) lamba ve ultrasonografik muayene sonrası sodyum selenit çalışmanın 0. gününde tüm tavşanlara boyuna subkutan olarak uygulandı. Anestezinin başlatılmasından sonra, yan yatırılarak ve küre üzerine gözenek oluşturularak 0.1ml %0.9'luk tuz solusyonu grup II'deki ve 0.1ml silymarin grup III'deki hayvanların vitreouslarına enjekte edildi. Grup I'deki hayvanlara ise herhangi bir madde enjeksiyonu yapılmadı. Selenit sodyum subkutan olarak tüm tavşanlara üçüncü ve altıncı günde enjekte edildi. Biyo-mikroskop yarık (slit) lamba çalışması birinci ve ikinci derece kataraktın 4. ve 8. günlerde ve üçüncü derece kataraktın ise 11.günde grup I ve II'deki tüm tavşanlarda gözlemlendiğini ortaya koydu. Grup III'de, üç tavşanda sekizinci günde ve dördünde ise onbirinci günün sonunda birinci derece katarakt gözlemlendi. Yirminci gündeki ultrasonografiye gore anterior lens kapsül kalınlığı, Grup I ve II 'ye göre grup III daha az ve posterior lens kapsül kalınlığı grup III'de istatistiksel olarak anlamlı bulundu. Anterior-posterior lens diagonal uzunluğu deney grubunda kontrol grubuna gore önemli düzeyde daha uzun olarak bulundu. Çalışma sonuçları selenit sodiumun, tavşan modelinde selenit ile oluşturulmuş katarakt üzerinde kataraktojenik karakter ve muhtemel koruyucu rol oynadığını gösterdi.

Anahtar sözcükler: *Katarakt, Selenit sodyum, Silymarin, Tavşan*

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INTRODUCTION

The lens is a transparent biconvex structure that is stabilized in place by zonular fibers [1,2]. A cataract disease is the gradual clouding that develops in the eye's lens that over time leads to impair vision and complete cloudiness (advanced mature cataract) which may progress to blindness [2]. Cataract is a congenital or an acquired condition; the recent type is associated with aging and is influenced by some structural and biochemical changes in the lens [3].

Today, mature cataract is treated by various surgical methods [2]. However, the less severe form and the non-developed form of cataract in diabetic patients and elderly make with some complications [4,5]. Therefore, some agents such as vitamins and antioxidants are used to reduce the progression of the disease [6,7]. Vitamin E and ascorbic acid are examples of such antioxidants utilized [6]. Considering not having the reliable response, the effort of the authors for introducing the appropriate agent in this regard is continued. Since the category of silymarine possesses antioxidant compounds, the present study has investigated the use of silymarine compounds in reduction of selenite-induced cataract in rabbit model.

MATERIAL and METHODS

Eighteen rabbits of both genders (9 male and 9 female) weighing 1100±430 g were conducted in the experimental study. After clinical examinations and administration of anti-parasitic drugs at the research center, the rabbits' eyes were carefully examined, using ophthalmoscope, bio-microscope slit lamp equipped with a digital camera and ultrasonography upon arrival of the rabbits (day one) *(Fig. 1)*. The rabbits were kept under constant environmental conditions and nutritional care, another careful eye examination was performed in the day 14.

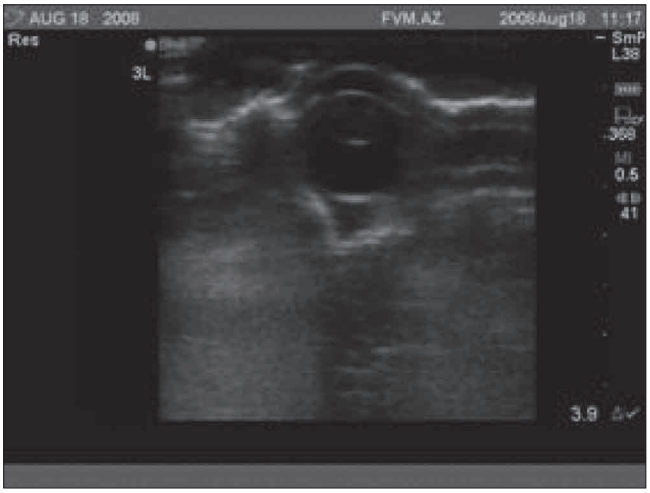

Fig 1. Ultrasonography of normal rabbit eye
Şekil 1. Normal tavşan göz ultrasonografisi

The structure of the eyes and normal lens diagonal size were performed to determine transcorneal ultrasonography (Sonosite Titan, USA) under physical restraint with minimum pressure applied on the head and the neck, while transducer horizontally placed on the surface of the cornea, in a position that the cornea, anterior and posterior capsules and the optic discs were at the same direction.

After ultrasonography examination, the eyes were rinsed using 0.9% normal saline solution. The rabbits were then divided randomly into three groups of six rabbits in each negative (I) and positive (II) control, and test (III) groups. The study was approved by the Animal Ethics Committee of the Iranian laboratory animal ethic frameworks under the referencecode IAEC 1-12/2.

Stage 1

A mixture of 99.3% pure selenite sodium (Merk, Germany) and 0.9% sterile normal saline solution having a density of 0.1% was prepared and 1 mg/kg of body weight (1 ml) was administered subcutaneously on the neck in all three groups of rabbit at day zero of the study.

Stage 2

Anesthesia was induced using combination of ketamine hydrochloride (35 mg/kg) and xylazine hydrochloride (5 mg/kg) intramuscularly. Then anesthesia rabbits in groups II and III were placed on their sides, and the eyes and the eyelids were irrigated with dilute solution of povidone-iodine. Prior to the administration of normal saline and silymarine (Madaus, Germany), a pore in the cornea using the gauge 27 needle from a distance of 2 mm of limbus was created to allow the release of pressure and neutralization after injection of silymarine into vitreous chamber.

In group I, no normal saline solution or silymarine was applied. In group II, 0.1ml of 0.9% normal saline solution and 0.1 ml of silymarine in group (test) III was injected into vitreous chamber under general anesthesia, respectively *(Fig. 2)*. Then topical antibiotic therapy using

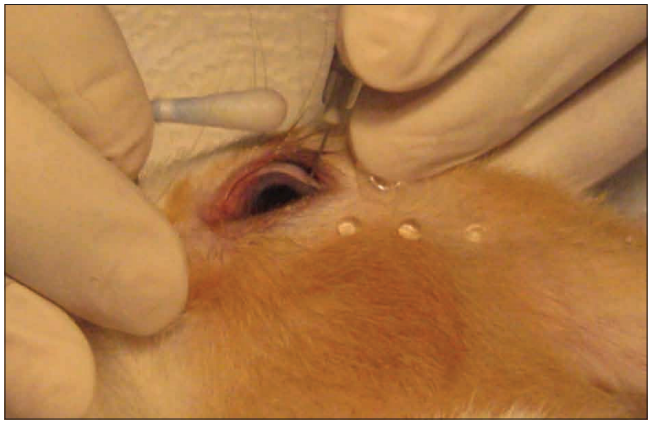

Fig 2. Injection in the vitreous chamber
Şekil 2. Vitreus boşluğa enjeksiyon

chloramphenicol eye drop was adminestrated post-injection every 6 h for 3 days.

Stage 3

Days third and sixth of the study, the same dosage of selenite sodium was administered subcutaneously to all 3 groups of rabbits.

Stage 4

For prevention infection, no ultrasonography of eyes was performed during three days after the injection. Ultrasonographic study was performed on day 20 after injection of silymarine to measure the diagonal of anterior and posterior of the lens capsules and diagonal of the anterio-posterior of the lens. Bio-microscopic slit lamp study of the eyes was performed with 3 day intervals for 20 days. The degree of opacification was assessed according to a study that described as follows: grade 0, absence of opacification (gridlines clearly visible); grade 1, slight degree of opacification (minimal clouding of gridlines and gridlines still visible); grade 2, diffuse opacification involving almost the entire lens (moderate clouding of gridlines and gridlines faintly visible); grade 3, extensive thick opacification involving the entire lens (total clouding of gridlines and gridlines not seen at all [7].

Stage 5

The blind method was applied to study the rabbits' eyes, and the results analyzed by the ANOVA.

RESULTS

The results suggested that the injection of selenite sodium with 3 days intervals and three times, did not cause any death in the rabbits of 3 groups. All rabbit tolerated the experimental studies. The study of the eyes with bio-microscopic slit lamp showed that cataract of grade one on day 4, grade two on day 8 and grade 3 until on day 11 in the eyes of all rabbits in group I and II post-injection of selenite sodium. It is noteworthy that the degree and severity of cataract from day 11 until the end of the study remained without changes. Cataract grade one was seen in three rabbits on the eighth day in the group (test) III, and four rabbits on day 11. The cataract was not observed in two rabbits of group III *(Fig. 3)*. Ultrasonographically, anterior lens capsule thickness was less in group III in comparison to group I and II, and posterior capsule thickness in group III was significant statistically ($P<0.05$). The anterior - posterior lens diagonal length in the group III comparing to the control groups was longer and showed a significant difference ($P<0.05$).

Results of ultrasonographic study of the eyes showed an increase in thickness and anterior and posterior capsule echogenicity in the 11 days which remained constant until the end of day 20 *(Fig. 4, 5, 6, 7, 8)*.

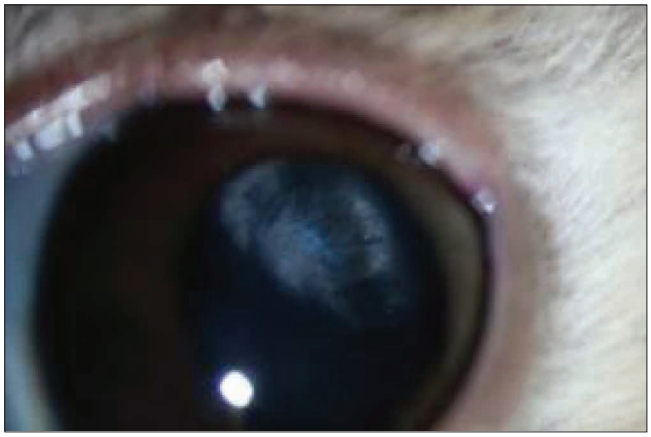

Fig 3. Grade one of cataract in rabbit eye
Şekil 3. Tavşan gözünde birinci derece katarakt

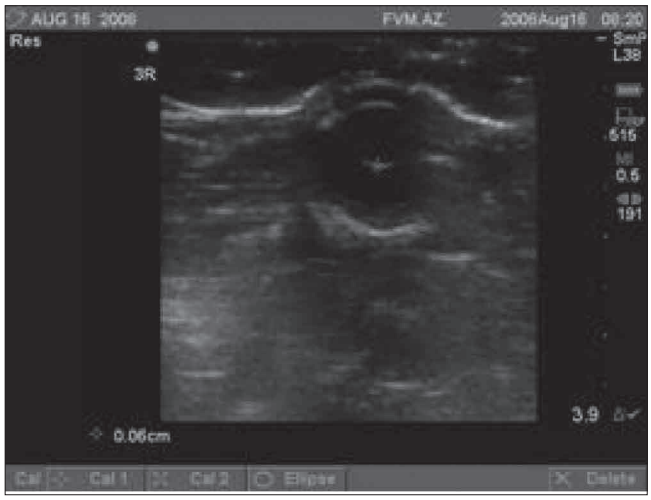

Fig 4. Increase of posterior lens capsule thickness following intraocular selenite sodium injection
Şekil 4. Göz içi selenit sodyum enjeksiyonu takiben posterior lens kapsül kalınlığı artışı

DISCUSSION

From past till now, different studies for induction the experimental cataract in various animal groups have been conducted [3,8,9]. There is a troublesome question that how could make the experimental cataract [8,10]. Many research implicated in finding out which medication and diet could induce cataract in animal model [10]. Medication or method for reduction or prevention of cataract in patients has not been known clearly [2].

There have been a lot of reports related to induction of acquired cataract due to the hyperglycemia, but laboratory trails for induction of cataract using hyperglycemia has been failed [1,2,8,11,12]. Investigations have induce temporary cataract in younger dogs and cats (under 6 months of age) using administering goat milk without argenine, but the cataracts was observed to be resolved after diet modifications [13]. In another study, it was shown that a diet

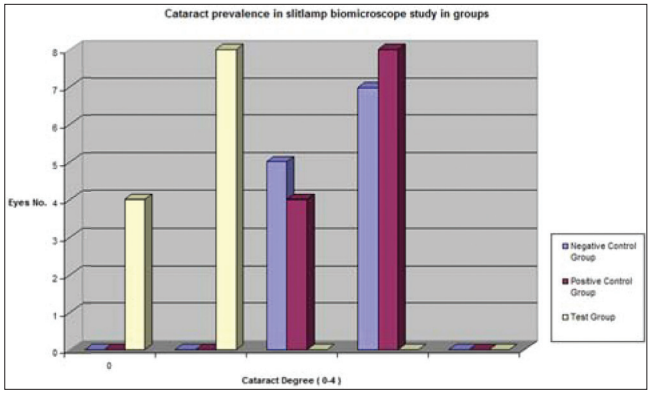

Fig 5. Cataract prevalence in slitlamp microscope study in groups
Şekil 5. Gruplardaki yarık (slit) lamba mikroskop çalışmasındaki katarakt yaygınlığı

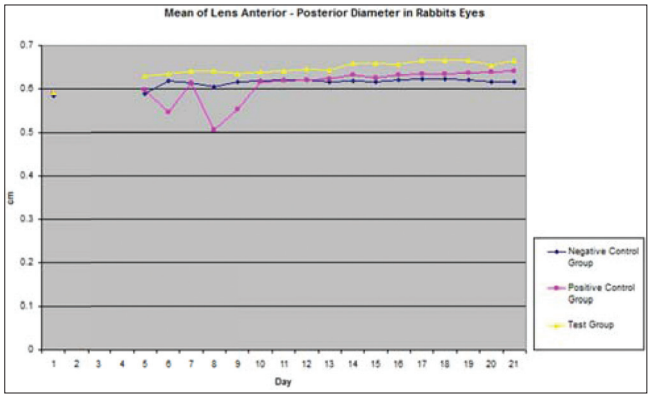

Fig 6. Mean of lens anterior-posterior diameter in rabbits' eyes
Şekil 6. Tavşanların gözlerinde lens anterior-posterior çap ortalaması

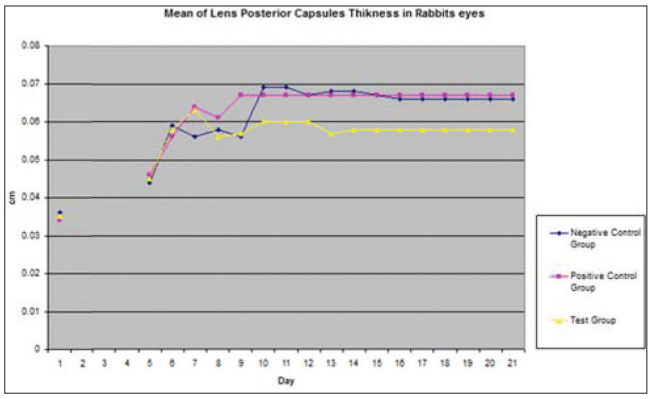

Fig 7. Mean of lens posterior thickness in rabbits' eyes
Şekil 7. Tavşanların gözlerinde lens posterior kalınlığı ortalaması

without methionine, resulted in irreversible cataract [12].

Reports revealed that the toxic agents such as paromomycin (aminoglycosides drug) result in acute renal failure and cataract in cats [2]. They believed that cats suffering from enteritis has impaired intestinal epithelial layer and absorb toxic substance. Absorption of the toxin of healthy colon epithelium is not possible [3]. Other studies have showed that corticosteroid drugs (dexamethasone),

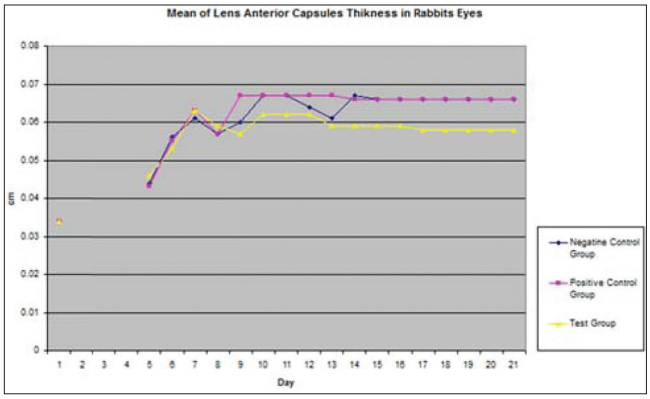

Fig 8. Mean of lens anterior thickness in rabbits' eyes
Şekil 8. Tavşanların gözlerinde lens anterior kalınlığı ortalaması

hydroxymetyglotaryl co-enzyme drugs, D-methylsulfoxide and diazoxid could also result in cataract in experimental dog models [14-16].

Investigators believe that cataract effects of the diazoxid drug and dexamethasone could be following hyperglycemia [4]. In other study, investigators showed that nephthalene in rabbits could cause induced-cataract in female rat model [4]. Many researches showed selenite sodium administration to infant rats induced cataract experimentally [4].

We have showed that injection of selenite sodium three times for three days with a dose of 1 mg/kg in rabbits during 11 days induced in different degrees of one to three. Therefore, the cataract effect of selenite sodium was statistically confirmed in our study. Pathological changes in the retina under the bio-microscopic slit lamp in any of three groups I, II, and III was not seen. Any signs of pathological changes in the liver, kidney, brain and spleen were not observed too. However, in our study, no gender predilection was found. Several studies have suggested that antioxidants retard the process of cataractogenesis by scavenging free oxygen radicals. The researchers have dedicated a lot of efforts using different agent to reduction cataract or the development of age-related cataract and or some of the acquired cataract conditions [17]. One research showed intraperitoneal injection of garlic aqueous extract in rat model appeared to effectively prevent selenite-induced cataract [18]. Doganay and his colleagues demonstrated that apricots reduced selenite induced cataract in Spraque-Dawley rat on the experimental cataract model [19]. The antioxidant effects and vitamin content play preventive role [20]. Various reports have shown that the use of enzyme inhibitors an anhydrous release carbon dioxide, ardostine antioxidants, N-steelcartozin and vitamin E, have reduced progression of cataract [4,20]. Geraldine and et al.[21] suggested that acetyl-L-carnitine (ALCAR) is able to significantly retard experimental selenite-induced cataractogenesis in Wister rat model.

We showed that increase of the anterior - posterior

diameter of the lens which is due to the antioxidant properties of silymarine increases elasticity of the lens and explains the increase of the anterior-posterior diameter in group III. The results of bio-microscopic slit lamp study confirmed that levels of cataract which were seen in groups I and II was more severe and significant than the group III, these findings also were confirmed by the ultrasonographical (increased capsule thickness).

In conclusion, our results demonstrated that selenite-sodium has cataractogenic effect on the experimental cataract model in rabbits and moreover silymarine has protective role on selenite-induced cataract in rabbit without gender predilection. We recommended that for achieving to reasonable results, histopathological study of globe and particularly lens and retina need to further studies.

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RESEARCH ARTICLE

Can Sequential Human Embryo Culture Media be Used in Bovine *in vitro* Embryo Culture? [1]

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Summary

The aim of the study was to investigate potential use of sequential human embryo culture media in culture of bovine embryos. Bovine oocytes were matured in Tissue Culture Medium-199 (TCM-199) for 22 h at 38.5°C and fertilized in modified Tyrode-Albumine-Lactate-Pyruvate medium (mTALP). The putative zygotes were randomly allocated to two embryo culture media groups; (1) Synthetic Oviduct Fluid (SOF) supplemented with fatty-acid free bovine serum albumin (FAF-BSA) (8 mg/ml) and (2) sequential human embryo culture media [Quinn's Advantage Medium-(QAM)] supplemented with FAF-BSA (8 mg/ml). Zygotes were cultured in SOF and sequential QAM for 9 days (5% CO_2, 5% O_2, and 90% N_2) at 38.5°C. Cleavage (73.3% and 72.2%), morula (37.6% and 33.2%) and blastocysts rates (23.9% and 22.9%) were similar among groups (P>0.05), but the total blastocyst cell number were significantly higher in blastocysts developed in SOF (101.6±4.0) than those in sequential QAM (87.4±3.2) (P<0.05). QAM may be suggested to use in culture as an alternative media in terms of supporting embryo development, but low cell number in blastocysts produced in QAM may suggest a possible low pregnancy rate.

Keywords: *Bovine, Embryo culture, SOF, QAM, Blastocyst quality*

Ardışık İnsan Embriyo Kültür Medyumu Sığır Embriyolarının *in vitro* Kültüründe Kullanılabilir mi?

Özet

Bu çalışmanın amacı, ticari insan embriyo kültür medyumlarının sığır embriyolarının kültüründe kullanılabilirliğinin araştırılmasıdır. Sığır oositleri, TCM-199'da 22 saat süreyle 38,5°C'de maturasyona tabi tutulduktan sonra, mTALP medyumunda fertilize edildiler. Muhtemel zigotlar rastgele 2 gruba ayrıldı; (1) sığır serum albümini (FAF-BSA) (8 mg/ml) ilaveli Sentetik Ovidukt Sıvısı (SOFaa) ve (2) sığır serum albümini (FAF-BSA) (8 mg/ml) ilaveli ardışık Quinn's Advantage Medium (QAM). Muhtemel zigotlar SOFaa ve ardışık QAM medyumlarında 38.5°C'de 9 gün süre ile kültüre edildiler (%5 CO_2, %5 O_2 ve %90 N_2). Yapılan istatistiksel değerlendirme sonucunda; bölünme oranı (%73.3 ve %72.2), morula (%37.6 ve %33.2) ve blastosiste ulaşma oranları yönünden (%23.9 ve %22.9) deneme grupları arasında önemli bir farklılık gözlenmemiştir (P>0.05). Buna karşın total blastosist hücre sayıları yönünden yapılan değerlendirmede, SOFaa medyumu (101.6±4.0) ve QAM medyumunda (87.4±3.2) kültüre edilen blastosistlerin hücre sayıları önemli oranda farklılık göstermiştir (P<0.05). Sonuç olarak, ardışık QAM medyumunun sığır embriyolarının *in vitro* kültüründe alternatif bir medyum olarak kullanılabileceği, ancak bu medyumdan elde edilen blastosistlerin total hücre sayılarının düşük oluşması sebebiyle beklenilen düzeylerde gebelik oranlarının elde edilmesinde yetersizlik şekillenebileceği düşünülmektedir.

Anahtar sözcükler: *Sığır, Embriyo kültürü, SOF, QAM, Blastosist kalitesi*

INTRODUCTION

In vitro embryo technologies have enabled the production in large numbers of embryos of superior breeds in various livestock animals, including cattle, and allows for embryo transfer at low costs. They have also enabled the production of embryos for scientific research purposes from slaughtered and/or live animals. Although

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several decades of research have gone into *in vitro* culture conditions that promote the maximal embryo yield are yet to be standardized [1,2]. *In vitro* embryo culture conditions depend on multiple parameters such as the composition of culture media and gases. An efficient culture media for *in vitro* embryo development should be formulated to protect from intracellular stress and include all demands of the embryo thereby embryo can maintain viability [1,3,4].

In vitro embryo production is widely used in the treatment of subfertility in human. Studies on the metabolism of the preimplantation cleaving human embryo enabled the formulation of culture media capable of sustaining normal development [3]. To satisfy all requirements of embryo, sequential culture media have been developed. Therefore, there are now commercially available, ready-to-use, and quality-controlled sequential commercial human embryo culture media [5-7]. These media were formulated specifically to prevent intracellular stress to the embryo. Additionally, these media take into account the changing carbohydrate and amino acid requirements of the embryo [1]. Due to these characteristics, these media are thought considered to support blastocyst development in culture of bovine embryos. Because, human and bovine are single ovulators and there are close similarities between human and bovine ovarian physiology, oocyte characteristics and reproductive functions. Moreover, bovine and human embryos are remarkably similar with respect to the micro-tubule timing of genome activation, metabolic requirements, interactions with the culture medium and duration of preimplantation development [2].

Synthetic oviductal fluid (SOF) is one of the medium commonly used for bovine embryo culture *in vitro*. Synthetic oviductal fluid has subsequently been modified by the addition amino acids [4,8,9]. Numerous studies have examined the ability of bovine embryos to develop *in vitro* using a wide variety of culture media. However, to our knowledge, no studies comparing the effects of commercially available human culture media on the development of bovine zygotes produced *in vitro* to the blastocyst stage have been reported. Therefore, the aim of the present study was to investigate potential use of sequential human embryo culture media (QAM) in bovine embryo culture and to compare the development of bovine zygotes to the blastocyst stage in SOF and sequential QAM culture media.

MATERIAL and METHODS

All chemicals and media used in this study were from Sigma-Aldrich Chemical Co. (Turkey) unless otherwise stated.

Collection and in vitro Maturation of Oocytes

Bovine ovaries at various stages of their oestrous cycle were collected from a local slaughterhouse and transported to the laboratory at approximately 35°C in physiological saline solution (0.9% w/v NaCl) supplemented with 0.1 µl/ml gentamycin sulphate. Cumulus-oocyte complexes (COCs) were recovered from follicles 2-8 mm in diameter by aspiration, using an 18 gauge needle and 10 ml disposable syringe. The COCs were collected in 3-4 ml Hepes-buffered Medium 199 containing Earle's salts and supplemented with 1% v/v antibiotic-antimycotic solution (10.000 IU penicillin, 10 mg streptomycin and 25 µg amphotericin B per ml). The COCs were assessed morphologically before *in vitro* maturation and only oocytes with compact, non-atretic cumulus investment and evenly granulated cytoplasm were selected for maturation. All COCs were washed three times in Hepes-buffered Medium 199, and then twice in maturation medium. Maturation medium were prepared as reported by Cevik et al.[10]. Maturation medium was sodium bicarbonate-buffered Medium 199 containing Earle's salts and L-glutamine supplemented with 5.5 µg/ml sodium pyruvate, 1% v/v antibiotic-antimycotic solution, 10% v/v heat-inactivated fetal calf serum (FCS). The COCs were placed in 500 µl of maturation medium (approximately 25-35 COCs per well) covered with 300 µl mineral oil in four-well dishes (Nunc, Roskilde, Denmark) and matured for 22 h in a humidified atmosphere of 5% CO_2 in air at 38.5°C.

In vitro Fertilization

After *in vitro* maturation, COCs were washed twice in Hepes-buffered Medium 199 and then twice in fertilization medium. Fertilization medium was modified TALP supplemented with 6 mg/ml FAF-BSA, 10 µl/ml pyruvate (0.2 mM) and 0.5 µl/ml antibiotic-antimycotic solution (pH 7.4 and 280-300 mOsm/kg) [11]. After washing, COCs were then transferred into 44 µl drops (approximately 15 COCs per drop) of fertilization medium added 2 µL heparin (10 µg/mL), and 2 µL PHE mix (penicillamine, 20 mM; hypotaurine, 10 mM; epinephrine 1 mM). Subsequently, 2-3 straws of frozen semen from tested bulls for IVF were thawed at 36°C for 1.0 min. The thawed semen was layered over 2 ml of both 45% and 90% Percoll discontinuous density gradient into a 15 ml conical tube and was centrifuged for 15 min at 1.200 g. After centrifugation, the supernatant above the sperm pellet was carefully removed. The pellet was resuspended with Sperm-TL medium (4 ml) and centrifuged for 5 min at 300 g. The pellet containing the motile sperm fraction was carefully collected from the bottom of the conical tube.

The sperm concentration was counted by hemocytometer using a phase-contrast microscope at a magnification of 400×. Sperm was then diluted to 50×10^6/ml spermatozoa with fertilization medium [11]. The sperm motility was visually checked for acceptable motility (i.e. at least 80% progressively motile). The oocytes were fertilized with 2 µl diluted semen per fertilization drops for 22 h in a humidified atmosphere of 5% CO_2 in air at 38.5°C.

In vitro Culture

After fertilization, the putative zygotes were washed three times in Hepes-buffered Medium 199. They were then vortexed to remove cumulus cells. The putative zygotes were randomly allocated to two embryo culture media groups; (1) synthetic oviduct fluid (SOF) culture media supplemented with 8 mg/ml fatty-acid free BSA (FAF-BSA), 10µl/ml BME (50×) essential amino acid solution, 20 µl/ml MEM (100×) non-essential amino acids solution (SOF); and (2) sequential commercial human embryo culture media (Quinn's Advantage Medium® (SAGE In-Vitro Fertilization, Inc Trumbull, C.T. USA) supplemented with 8 mg/ml FAF-BSA [6] (QAM).

The zygotes were washed twice and placed in 50 µl drops (approximately 15 zygotes per drop) of either SOF or sequential QAM media under mineral oil and cultured in a humidified atmosphere of 5% CO_2, 5% O_2 and 90% N_2 in air at 38.5°C. Following fertilization, embryos were cultured in Quinn's Advantage Cleavage Medium (QACM) supplemented with 8 mg/ml essentially fatty-acid free BSA for 72 h. Then developing embryos were cultured in Quinn's Advantage Blastocyst Medium (QABM) supplemented with 4 mg/ml essentially fatty-acid free BSA. The embryos in SOF media were cultured in the same drops until the end of the culture period (9 days). Cleavage, morula, and blastocyst development rates were evaluated from the zygotes (day 0 = *in vitro* fertilization) on days 4 and 9 using a stereomicroscope, and blastocyst rates on day 9 were present.

Determination of Total Cell Number of Blastocyst

The number of total cell of the blastocysts was determined as reported by Arat et al.[12]. Blastocysts in both groups were washed twice in Hepes-buffered Medium 199 and fixed in a solution of ethanol and acetic acid (volume ratio = 3:1) for 24 h. Subsequently, the blastocysts in both groups were stained with Hoechst 33342 for 15 min and the total number of cells in each blastocyst was determined by counting stained nuclei with the aid of a fluorescence microscope.

Statistical Analysis

Data were analyzed using by generalized linear models after appropriate transformation where necessary (proportion of cleaved zygotes, morula and blastocyst yields, arcsine-transformation; total cell numbers of blastocyst, $\log_{10}$ transformation) [13]. Significant differences between treatment means were tested using *Student's t-test*. The results are presented as untransformed mean ± SE values, and statistical significance was determined at the level of $P<0.05$.

RESULTS

In the present study, total number of 450 bovine cumulus-oocyte complexes (COCs) were used and maturated in standard maturation medium. Approximately 85% of COCs matured following *in vitro* maturation (IVM). *In vitro* matured oocytes (382) were subjected to the *in vitro* fertilization (IVF) procedure. A total number of 164 and 218 bovine embryos were cultured in QAM and SOF media, respectively.

Development rates of bovine embryos cultured in SOF and sequential QAM media are presented in the *Table 1*. There were no significant differences between two culture media in terms of cleavage rates (73.3% vs 72.2%, $P>0.05$). Similarly no significant differences in morula (37.6% vs 33.2%) and blastocyst formation rates (23.9%) vs 22.9%) were observed on day 9 of between two culture media ($P>0.05$).

The mean total cell numbers of bovine blastocysts developed in SOF and sequential QAM culture media are presented in the *Fig. 1*. The mean total cell numbers were 101.6±4.0 and 87.4±3.2 for blastocysts cultured in SOF and sequential QAM culture media, respectively. There were significant reduction in total cell number of blastocysts developed in sequential QAM culture media compared to those in SOF culture media ($P<0.05$).

DISCUSSION

The results of the present study indicate that bovine embryos produced by *in vitro* fertilization and cultured in SOF and Quinn's Advantage sequential culture media (QAM) supplemented with BSA showed similar developmental competence *in vitro*. To allow reliable comparison between SOF and the sequential QAM human culture media systems, all embryos were cultured with the same oil overlay and drop volume, and in the same incubator,

Table 1. *In vitro embryo development of bovine embryos cultured in SOF and sequential QAM culture media*
Tablo 1. *Ardışık QAM ve SOF medyumlarında kültüre edilmiş sığır embriyolarının in vitro gelişimi*

Culture media	No. of oocytes	Cleavage (%)	*In Vitro* Embryo Development Rates (%)		
			Morula	Blastocyst	Blastocyst / Cleavage
SOF	218	73.3±6.3	37.6±3.3	23.9±2.0	34.4±5.5
QAM	164	72.2±4.4	33.2±1.2	22.9±0.9	32.3±3.4

Values (mean ± SE) within columns with different letters are insignificantly different (P>0.05)

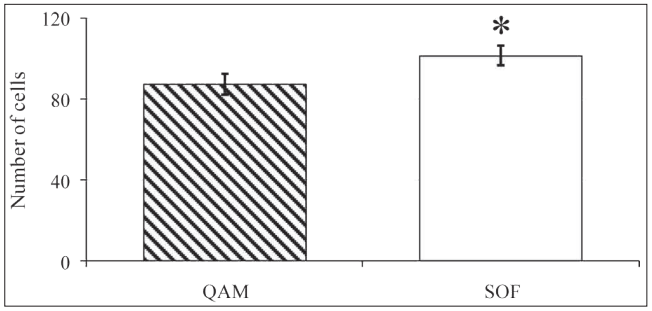

Fig 1. The mean total cell numbers of bovine blastocysts developed in SOF and sequential QAM culture media. Asterisk indicates significant differences between media groups (P<0.05)

Şekil 1. SOF ve ardışık QAM medyumlarında sığır blastosistlerinin total hücre sayıları

under the same oxygen conditions. However, sequential QAM culture media decreased embryo quality by reducing the total cell number of blastocysts, which is considered to be an indicator of viability and hence establishing a successful pregnancy, compared to blastocysts developed in SOF culture media.

Many studies have been performed to evaluate culture systems with respect to developmental competence, embryo quality and development rate in humans [6,7,14] and bovine [3,4,15,16]. Among these studies, mSOF culture medium containing BSA or FBS is widely used for producing bovine embryos [8,9,17,18]. However, comparisons between studies, even those evaluating the same culture system, remain problematic due to variations in culture parameters including type of overlay, oxygen tension, culture drop volume, serum supplement, combined procedures such as IVF/ICSI and many more [9,18-20].

In vivo, the developing embryo migrates from the oviduct to the uterine lumen where the fluid composition and gas atmosphere are likely to be different. Analysis of embryonic physiology and metabolism also shows that the requirements for exogenous substrates change with development. Therefore, sequential media may theoretically be more optimal for culture of developing embryos than single culture medium. Commercially available sequential media have been widely for embryo culture in various species and have shown better competence to support development of IVF embryos both *in vitro* and *in vivo* than single culture media in some previous studies [1,3,8]. In addition, some studies showed that a medium cultured for long periods of time can rapidly deteriorate, resulting in its inability to support embryo development [1,4,16]. Sequential culture media formulated for *in vitro* development of mammalian embryos overcome this problem [8,16]. Currently, there are different sequential media available for culturing mammalian embryos such as G1/G2 [8,21], Quinn's Advantage sequential medium and Sydney IVF medium [5,7].

Problems with pregnancies following *in vitro* culture have primarily been attributed to the presence of serum in the culture system [1,12,14,22]. These problems have included heavier birth weights (commonly referred to as the large lamb or calf syndrome, LOS), extended gestation periods, higher rates of abortion and increased rates of perinatal mortality [22]. Studies investigating culture of embryos with or without serum have determined that many of these problems with pregnancy and parturition are eliminated when serum is replaced in the medium with purified preparations of BSA [1,20]. Therefore, the need for a culture system that does not employ serum is essential for the extended application of *in vitro* culture procedures in a commercial setting.

As reported by Cooke et al.[6], one such serum-free culture system is commercially available the sequential QACM and QABM human culture media systems. These media were formulated specifically to prevent intracellular stress to the embryo thereby maintaining embryo viability. Additionally, these media take into account the changing carbohydrate and amino acid requirements of the embryo. As a result these media are able to support high rates of blastocyst development in culture of embryos from many species.

Perin et al.[23] reported that the percentage of blastocyst development by day 5 of culture of mouse zygotes was higher in the potassium-enriched simplex optimized culture medium (KSOM) compared with the G1/G2 sequential culture media. The notches of distributions of the total number of cells of the blastocysts produced in each culture condition overlapped, revealing no difference between the medians of these two distributions. Choi et al.[17] reported that the cleavage rates of equine zygotes cultured in either G1/G2 or Dulbecco modified Eagle medium (DMEM) both with and without BSA or 10% FBS were similar. In agreement with these observations, in our study, there was no significant difference in cleavage, morulae and blastocyst formation rates between culture media groups. However, cleavage and blastocysts formation rates of the bovine embryos in QAM media were reported to be lower than studies for human embryo culture [6,7].

Swain et al.[16] reported that the use of a single culture medium (NCSU23) and sequential G1.2/G2.2 media resulted in similar cleavage percentages for *in vitro* derived porcine embryos. In agreement with Swain et al.[16] in our results, the rates of cleavage and blastocyst formation in SOF and sequential QAM were similar, but total cell number of blastocysts developed in sequential QAM culture media was lower those developed in SOF culture media. It is known that the total cell number and morphology of blastocysts are currently the best predictors available for assessing embryo quality [8,12]. Higher cell number has been associated with increased embryo viability after transfer into a surrogate [16]. The blastocyst cell numbers of embryos in the present study were similar to those reported by Arat et al.[12] and Wang et al.[8].

Althought not reported here, we have observed that bovine embryos cultured in SOF media showed faster developmental speed and higher developmental rate to form early blastocysts on day 7 of culture in compared to sequential QAM. The difference in speed of blastocyst formation between culture media studied in the present study may explain the differences in total cell number in blastocyst produced in these culture media. The late formation of blastocyst may yield a lower number of cells in each blastocyst.

In conclusion, results of present study showed that bovine blastocysts can develop in sequential human embryo culture medium and serum-free sequential human culture system can be used for *in vitro* bovine embryo production. However, the QAM sequential human embryo culture system supports lower quality of *in vitro* derived bovine embryos, based on the total cell number, compared to those cultured in SOF media. Therefore, further research is needed to examine substrate requirements such as BSA and amino acids of commercially available sequential human embryo media system for support a higher percentage of bovine blastocyst development and to determine how metabolism of these blastocysts compare to embryos developed in different culture medium *in vitro* or *in vivo*.

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RESEARCH ARTICLE

Introducing A New Tool to Calculate Greenhouse Gas Emissions from Feedlot Cattle [1]

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Summary

Agriculture in Australia contributed 15.5% of total national greenhouse gas (GHG) emissions produced in 2009, mainly as methane (CH_4) and nitrous oxide (N_2O). In this study, a new tool (Feedlot greenhouse gas accounting framework also known as F-GAF) incorporating all components of the GHG emissions produced from feedlot systems was demonstrated. The objective of developing the F-GAF was to create awareness of the various sources of GHG emissions from feedlots in order to stimulate thinking and action aimed at reducing these emissions while further improving farming efficiency. It was found that the main source of total GHG emissions was CH_4 from enteric fermentation, contributing around 60% of the total emissions. The N_2O emissions were mainly produced from manure and contributed 30% of the total emissions. The F-GAF can be used as a practical tool to calculate GHG emissions from feedlot systems. Further studies can be conducted to incorporate mitigation options into the tool.

Keywords: *Australia, Calculator, Feedlot cattle, Greenhouse gas*

Açık Besi Sığırlarının Sera Gazı Üretiminin Hesaplanmasında Yeni Bir Araç

Özet

Avustralya'da 2009 yılında tarım ve hayvancılık kaynaklı sera gazı üretimi, başta metan (CH_4) ve nitroz oksit (N_2O) gazları olmak üzere, toplam ulusal sera gazı üretiminin %15.5'ini oluşturmuştur. Bu çalışmada, açık besi sistemlerinden üretilen sera gazı emisyonlarının bütün bileşenlerini içeren yeni bir aracın (F-GAF olarak da bilinen açık besi sığırlarının sera gazı hesaplanması sistemi) kullanılması tanıtılmaktadır. Bu aracın (F-GAF) geliştirilmesinin amacı, bir yandan çiftlik etkinliğinin iyileştirmesini sağlarken, diğer yandan sera gazlarının azaltılmasını amaç edinen düşünce ve çalışmaları stimüle etmek için açık besi sığırlarından üretilen sera gazı emisyonlarının kaynakları hakkında farkındalık yaratmaktır. Bu çalışmada toplam sera gazı üretiminin büyük bir çoğunluğunun (yaklaşık %60) enterik fermentasyon kaynaklı CH_4 gazı üretimine dayandığı tespit edilmiştir. Diğer yandan, gübre yönetimi kaynaklı N_2O gazı üretimi toplam sera gazı üretiminin yaklaşık olarak %30'unu oluşturmuştur. Bu çalışmada tanıtılan F-GAF açık besi sistemlerinden üretilen toplam sera gazı emisyonunun hesaplanmasında pratik bir araç olarak kullanılabilir. Ayrıca, sera gazı üretiminin azaltılması seçeneklerini F-GAF'a uyarlayacak çalışmalara ihtiyaç bulunmaktadır.

Anahtar sözcükler: *Açık besi, Avustralya, Hesaplayıcı, Sera gazı*

INTRODUCTION

In 2009, Australia produced a total of 545.8 million tonnes (Mt) of CO_2-eq greenhouse gas (GHG) emissions excluding land use, land use change and forestry sector (LULUCF) emissions. The agriculture sector produced 84.7 Mt CO_2-eq emissions (15.5% of total national emissions) of which livestock production emitted 58.1 Mt CO_2-eq (10.6% of total national and 68.5% of total agricultural emissions) [1]. The three main GHGs emitted at a farm

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scale contributing to global warming are methane (CH_4), nitrous oxide (N_2O) and carbon dioxide (CO_2) [2]. The global warming potentials (GWP) of CH_4 and N_2O are 21 and 310 times higher than CO_2, respectively [3].

Methane emissions are produced mainly from enteric fermentation and effluent ponds [4]. Enteric CH_4 comprises the highest proportion (64.6%) of the total agricultural emissions, producing 54.7 Mt CO_2-eq emissions in Australia [1]. On the other hand, agricultural soils emits 14.2 Mt CO_2-eq emissions or 16.8% of the total agricultural emissions [1]. It is estimated that 2.8 gigatonnes (Gt) CO_2-eq of N_2O is produced from the global agriculture sector every year. This accounted for 60% of the global anthropogenic N_2O emissions in 2005 [5]. The N_2O emissions contributing 18% of total t CO_2-eq output (or 2 t CO_2-eq/t milk solids (MS)) are derived from four major sources: effluent ponds; fertiliser; indirect emissions; and excreta. Indirect emissions are produced from ammonia (NH_3) and nitrate (NO_3) losses. Lastly, direct CO_2 emissions from livestock farms are mainly sourced from diesel and electricity consumption [6].

When assessing the GHG emissions from livestock systems, quantification of GHG emissions is necessary to provide a common platform of information. Mathematical models, such as statistical or dynamic empirical models estimating the CH_4 emissions, are advantageous in terms of not requiring extensive and costly experiments. However, the statistical and empirical models may not be able to predict CH_4 emissions in the systems other than those they were initially built on. This can be overcome by developing mechanistic models that use commonly measured input variables such as dietary variables. The dry matter intake (DMI) (kg/d) and the metabolisable energy intake (MEI) (MJ/d) are good predictors of enteric CH_4 emissions [7]. Australian emissions are assessed by the National GHG inventory (NGGI) method [8] based on IPCC guidelines on the basis of animal species and classes, seasonal and geographical impacts (on livestock and pasture production/or emissions). This method has been prepared by the National Greenhouse Gas Inventory Committee (NGGIC) [8] and adopted by the Australian Government Department of Climate Change Energy Efficiency (DCCEE) [9] as the Australian methodology to estimate GHG emissions from livestock production systems. It reflects country-specific information, revised IPCC guidelines for national GHG inventories [10] and emission factors, and they are believed to represent international practice [9].

The objective of developing the current Feedlot - Greenhouse Accounting Framework was to create awareness of the various sources of GHG emissions on feedlot industry in order to stimulate thinking and action aimed at reducing these emissions while further improving farming efficiency. By entering in some simple data, which most farmers are likely to know, the model presents the user with a GHG emission profile for their farm. The model also then breaks down these GHG emissions into the various sources, and where they originate from on the farm. The user can then conduct some "What if" scenarios to explore the GHG impact of changes to farm management. The framework is on a spreadsheet which utilises calculations, models and assumptions based on the Australian National Greenhouse Gas Inventory method, as published by the Australian Government DCCEE in April 2012 [6].

MATERIAL and METHODS

Feedlot Greenhouse Gas Calculator

The F-GAF is a part of a suite of tools calculating GHG emissions from Australian dairy, beef, feedlot, sheep and grains industries, which are named as Dairy GHG accounting framework (D-GAF), beef GHG accounting framework (B-GAF), northern beef GHG accounting framework (B-GAFN), feedlot cattle GHG accounting framework (F-GAF), sheep GHG accounting framework (S-GAF) and grains GHG accounting framework (G-GAF). The tool is based on a Microsoft excel workbook where the calculation of GHG associated with a particular production system is based on the categories identified in the national inventory [6], where the inventory method has been adjusted where appropriate to apply to a farm boundary. The four categories for which the emissions are calculated in the F-GAF are (i) CH_4 emissions from enteric fermentation, (ii) CH_4 emissions from manure management, (iii) N_2O emissions from different manure management systems (MMSs), and (iv) N_2O emissions from agricultural soils. A summary page is provided where a pie chart features proportions of different GHGs (t CO_2-e) emitted in each production system through the outputs of CO_2 emissions from energy use, CH_4 emissions from enteric fermentation, CH_4 emissions from effluent ponds, N_2O emissions from effluent ponds, N_2O emissions from N fertiliser, Indirect N_2O emissions, N_2O emissions from manure, faeces and urine. After deducting the CO_2-eq emissions from tree planting finally, the summary page provides a sum value for total GHG emissions produced in the production system. The inputs of the F-GAF consist annual data entered for different animal classes.

Data

Data were obtained from various sources. The number of cattle on feed in Victoria was reported by the Australian Lot Feeders' Association and Meat and Livestock Australia Statistics in March 2013 as 40373 [11]. A same was assumed to apply for all three animal classes, namely domestic, export and Japan ox. Average maximum daily feed intake of the feedlot cattle was 2.5% of live weight [12], equalling to 9, 12.3 and 14.1 kg, for domestic (360 kg), export (490 kg), and Japan ox (565 kg), respectively. The lengths of stay in the feedlot for the three animal classes were 75, 140 and 250 days for domestic, export and Japan ox, respectively.

Dry matter digestibility (DMD) of the forage was 80% for all animal classes. Live weight gain varied among the animal classes with being 1.7, 1.5 and 1.2 kg/day for domestic, export and Japan ox, respectively [6].

Assumptions

The tool was run for Victoria and was assumed to derive electricity from brown-coal. A high rainfall area was chosen in the tool where type of the trees planted was hardwood as an offset against the emissions. The proportions of the feed components were assumed to remain as they were reported in the DCCEE [6]. That is, total grains (included molasses) constituted 0.779 of feed whilst the proportions of other concentrates, grasses and legumes were 0.048, 0.138, and 0.035 of feed, respectively. Feed components comprised of cellulose, hemicellulose, soluble residue, and nitrogen at different proportions for different feed components [6].

Ash content required to calculate the CH_4 emissions from manure management was a fraction of 0.08 as reported in the DCCEE [6]. Density of CH_4 was a fixed value of 0.662 [6]. The conversion factor to convert the elemental mass of N_2O to molecular mass was 1.57. Standard reference weight for steers older than 1 year old for Victoria was used as 660 kg. The fraction of N volatilised in each manure management system (MMS) was 0.3 and the emission factor was assumed to be 0.1 [6].

The calculation of enteric CH_4 fermentation follows the national inventory equations on annual basis in the F-GAF. The methane conversion factor (MCF) is a dynamic calculation that is based on a summation of all systems allocations (%) by their specific MCF to come up with the final composite. In the F-GAF, IPCC drylot MCF value for 'warm' regions is used for Queensland and Northern Territory (0.05) and MCF value for 'temperate' regions is used for all other states (0.0015) [6]. Total N_2O emissions are calculated for each of the MMS practised in each production system. The manure management systems incorporated in the GHG accounting framework suite are solid storage and drylot. N_2O emissions from synthetic fertiliser application are calculated in all systems. N_2O emissions from organic fertiliser (manure) application were not calculated. The only N_2O emissions calculated from agricultural soils was indirect NH_3 emissions because feedlot managers do not deal with pasture and fertiliser (synthetic and organic = manure), and also the cattle in this system do not deposit their faeces and urine on pasture directly. The waste is scraped and stored straightaway.

In order to allow users to explore the carbon offset value of planting trees, an option is included in the model to choose the type of trees and the rainfall zone, with the total carbon removed by trees being subtracted off the farm greenhouse gas emission total. It is important to note that this is a guide only [13], as actual tree growth is affected by the local growing conditions. In addition, the age of the plantation has a great impact on the carbon sequestration, whereas a linear growth function has been assumed here where type of trees planted was assumed to be *Eucalyptus nitens* in Victoria region receiving 500-700 mm rainfall per annum. The CO_2 emissions from diesel consumption and electricity use have also been added as a guide only. Farm electricity source was assumed to originate from brown coal. Users are encouraged to check updates and seek advice for the interpretation of their results.

RESULTS

The total net farm emissions were 18718 t CO_2e/farm for domestic, 45459 t CO_2e/farm for export, and 91474 t CO_2e/farm for Japan ox per year. The amount of CH_4 from enteric fermentation and manure management, and the amount of N_2O emissions manure management systems and agricultural soils are provided in *Table 1*.

The proportions of the total CH_4, N_2O and CO_2 emissions were 62-64%, 35-38% and 0.004-1%, respectively for all animal classes *(Fig. 1)*.

In terms of the two major gasses the highest emissions were produced from enteric fermentation for CH_4 (63%: 60%: 59%) and manure for N_2O (30%: 33%: 33%) for domestic, export and Japan ox, respectively. The lowest amounts of emissions resulted from manure for CH_4 (2-3%) and indirect ammonia for N_2O (5%) for all animal classes *(Fig. 2)*.

The area of trees required to offset 10% of the feedlot emissions (1872, 4562 and 9133 t CO_2e/farm) was 89, 217 and 435 ha of fast growing *Eucalyptus nitens* grown in a medium rainfall zone for domestic, export and Japan ox, respectively. This reflected the 21 t CO_2e/farm reduction in total farm emissions for every 1 ha of land being planted tree after 1990.

Table 1. *Greenhouse gas emissions produced from feedlot production (t CO_2e/farm)*
Tablo 1. *Açık besi üretiminden kaynaklanan sera gazı üretimi (t CO_2e/işletme)*

Animal Class	Enteric Fermentation	Manure Management CH_4	Manure Management N_2O	Agricultural Soils	Total
Domestic	11.780	294	5.711	857	18.721
Export	27.368	1.026	14.914	2.237	45.624
Japan ox	54.109	2.408	30.207	4.531	91.335

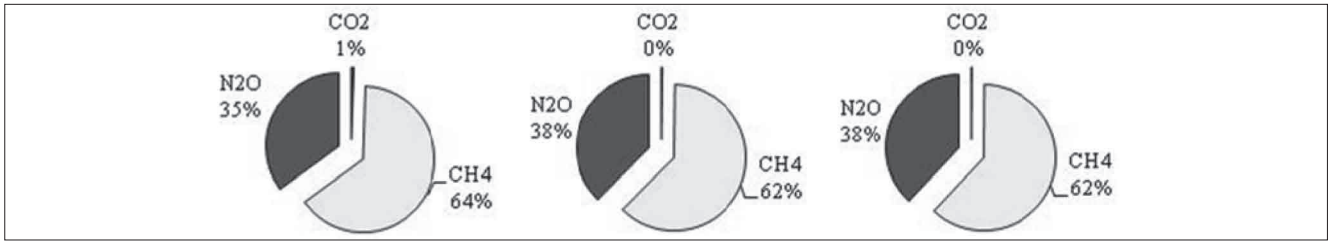

Fig 1. Proportion of CH_4, N_2O and CO_2 for the Domestic (left), Export (centre) and Japan ox (right) systems
Şekil 1. CH_4, N_2O and CO_2 gazlarının Domestic (sol), Export (orta) and Japan ox (sağ) sistemlerinde dağılımları

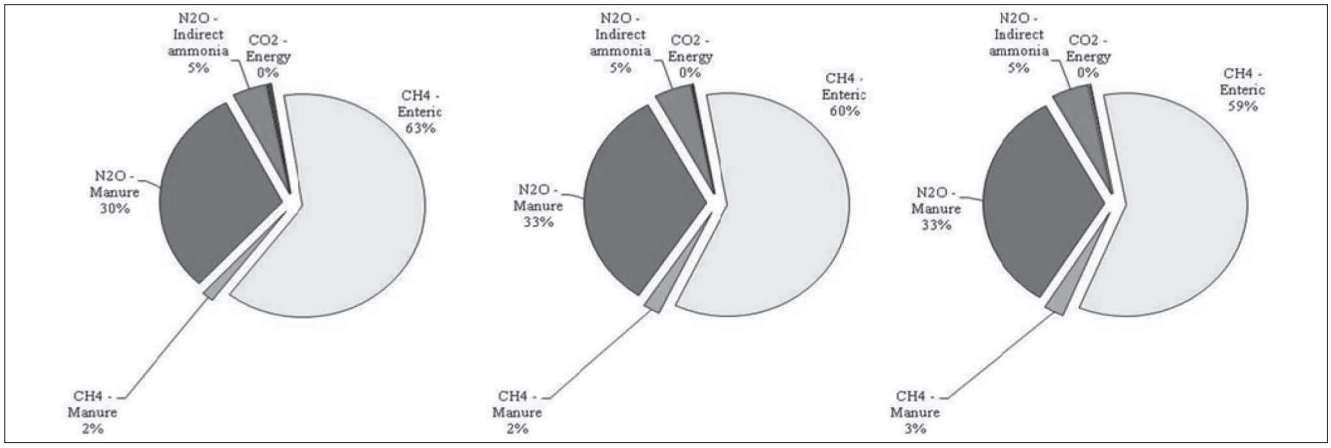

Fig 2. Greenhouse gas profile summary for all animal classes
Şekil 2. Bütün hayvan sınıfları için sera gazı profili

DISCUSSION

Greenhouse gas accounting frameworks for dairy, beef, sheep and cropping systems have been used widely to account for the GHG emissions produced in Australia. For instance, Bell et al.[14] used the S-GAF to assess the impact of future climate scenarios on productivity and GHG emissions from sheep grazing systems. Similar approach was used by Cullen and Eckard [15] using the D-GAF to evaluate the impact of future climate scenarios on productictivity and GHG emissions from pasture-based dairy production systems. A comparative study was conducted by Browne et al.[16] to estimate the GHG emissions from different agricultural production systems, utilising D-GAF, B-GAF, S-GAF and G-GAF. The F-GAF was developed in June 2012 and reported in this paper for the first time. There are also other tools available to calculate the GHG emissions from different agricultural systems such as FarmGAS allowing farmers, researchers and advisors to assess the impact of different farm management practices on farm GHG emissions [17] and enabling users to alter emission factors, feed factors for livestock, stubble management or manure management systems. Given that feedlot systems also require estimation of their GHG emission profiles, F-GAF presents an opportunity for feedlot farms to account for their GHG emissions.

The F-GAF is well suited to feedlot systems as it accounts for all CH_4 emissions produced from enteric fermentation, manure management, and N_2O emissions produced from manure management systems and agricultural soils. However, the calculation of total GHG emissions in F-GAF is only possible for a maximum of four groups of animals managed in a year. Systems managing more than four groups are advised to run the model for each group separately. It is important to note that if feedlot waste is applied to crop or pasture land, other calculators will be needed to account for manure applied to land. That is, in the F-GAF, no waste is assumed to apply to land directly.

In this study, enteric CH_4 was shown to be the major source of the GHG emissions produced from all feedlot systems by contributing to around 60% of the total GHG emissions. This is consistent with those reported by Christie et al.[18] analysing dairy farms and Bell et al.[14] estimating GHG emissions from sheep farms. Beauchemin et al.[19,20] also reported that around 63% of the total GHG emissions produced on beef production systems can be attributed to enteric CH_4 emissions. It is important to note that the manure management systems used in the current model were solid storage and drylot. Where there are different manure management systems, an assessment should be made more carefully to account for the emissions from different manure management systems. For instance, Öztürk and Ünal [21] reported three systems in dairy cattle farms in Turkey, namely collection, storage, and treatment. The differences among the three production systems

for total GHG emissions produced in this study can be due either to the length of stay or the live weights of the animals, which varied greatly between these classes. There were no differences among the animal classes in terms of the feed profile of the ration they were fed.

When assessing emissions produced in a livestock production system, it is important to choose the most relevant metric. For example, emissions produced per ha is a metric that can vary with climate, soil type and production system. It is a useful metric to describe the amount of emissions on a certain amount of farm area; however, it can provide no information about resource efficiency. To better measure the resource efficiency, emissions intensity can be used as a metric. Emissions intensity usually defined as emissions produced per unit of product is a technical metric which cannot reveal the economic value of the production. On the other hand, comparing the emissions produced per MEI may be a potentially better metric to use as it can also demonstrate the impact of different management practices, such as quality of the supplement fed to the animals on the farm [16]. However, the metric used will depend on the purpose of the study as well as the data availability.

In this study, an excel based spreadsheet was developed to calculate GHG emissions of typical Australian feedlot systems. The calculation of GHG emissions associated with feedlot farms was based on the categories identified in the Australian national GHG inventory published by the DCCEE in 2012 [6]. By utilising total feedlot cattle numbers published by the Australian Lot Feeders' Association and Meat and Livestock Australia Statistics in March 2013, it was found that the majority of the emissions resulted from enteric fermentation (around 60%). The main source of the N_2O emissions was manure contributing to around 30% of the total emissions. By using the calculator, a baseline strategy can be compared with a hypothetical farm management practice [18] such as changing the quality of the supplement and/or the quality and quantity of the pasture fed. The F-GAF spreadsheet model can be a practical tool for farmers and researchers to assess GHG emissions produced on feedlot systems. It can be further developed to include mitigation strategies to reduce the emissions of these systems.

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SHORT COMMUNICATION

Zoonotic *Trichuris trichiura* Infections in Non-Human Primates at Samsun Zoo, Turkey: First Molecular Characterization

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Makale Kodu (Article Code): KVFD-2013-9615

Summary

Trichuris trichiura were collected from rhesus macaque *(Macaca mulatta)* and Hamadryas baboon *(Papio hamadryas)* in Samsun Zoo, Turkey. DNA was isolated from individual worm in baboon for molecular characterization. The ITS region was amplified and sequencing in both directions using NC5-NC2 primers. Pairwise comparison between ITS region of the *T. trichiura* isolates from Turkey (KC877992) and other isolates China (AM992981, AM992985, AM992998), Czech Republic (JF690940, JF690941, JF690950), Netherlands (JF690948) and South Africa (GQ301551, GQ301553) showed differences ranging from 0.1% to 1.2%. With the present study, *T. trichiura* from Turkey were characterized for the first time by sequencing of the ITS.

Keywords: *Trichuris trichiura, Macaca mulatta, Papio hamadryas, Molecular Characterization, Samsun, Turkey*

Samsun Hayvanat Bahçesi'ndeki Primatlarda Zoonotik *Trichuris trichiura* Enfeksiyonu: İlk Moleküler Karakterizasyon

Özet

Samsun Hayvanat Bahçesi'ndeki rehesus maymunu *(Macaca mulatta)* ve babun'dan *(Papio hamadryas) Trichuris trichiura* toplanmıştır. Moleküler karakterizasyon için *Papio hamadryas*'dan toplanan ergin parazitin DNA'sı izole edildi. Ribosomal DNA'nın ITS gen bölgesi çoğaltıldı ve NC5-NC2 primer çifti ile iki yönlü dizi analizi yapıldı. *T. trichiura* Türkiye izolatı (KC877992) ile diğer bölgelere ait Çin (AM992981, AM992985, AM992998), Çek Cumhuriyeti (JF690940, JF690941, JF690950), Hollanda (JF690948) ve Güney Afrika Cumhuriyeti (GQ301551, GQ301553) izolatlarının tüm ITS gen bölgesi arasındaki uzaklık indeksi %0.1 ile %1.2 arasında değişiklik gösterdi. Bu çalışma ile Türkiye'de *T. trichiura*'nın ITS bölgesinin ilk kez moleküler karakterizasyonu yapılmıştır.

Anahtar sözcükler: *Trichuris trichiura, Macaca mulatta, Papio hamadryas, Moleküler Karakterizasyon, Samsun, Türkiye*

INTRODUCTION

Trichuriasis is caused by the nematode Trichuris trichiura that is a gastrointestinal nematode *in non-human primates and human. Trichuris* egg is frequently identified during routine faecal examination but is rarely clinical significance [1]. The life cycle is direct and infection is by ingestion of embryonated eggs. Humans and primates become infected directly by ingesting the embryonated eggs from contaminated hands, food, soil or water. PCR molecular techniques demonstrated that *T. trichiura* from primates and humans can be identified by their ITS sequences [2,3]. The ITS of the rDNA has been the target locus in many studies on helminths and is considered a good target locus for diagnosing indistinguishable stages of parasites, known to be different species. Generally, the entire ITS-sequence variation among individuals of the same species as well as the intra individual variation has shown to be ≤ 1% for a range of nematodes [4]. Little is known of the molecular characteristics of *T. trichiura* from Turkey. No

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rDNA ITS region study has previously been reported on the genus *Trichuris* in Turkey. In the present study, the entire first and second internal transcribed spacer (ITS-1 and ITS-2) regions of nuclear ribosomal DNA (rDNA) of *T. trichiura* from Turkey were amplified by polymerase chain reaction (PCR) and sequenced.

MATERIAL and METHODS

Parasitological Examination

The studied animals which naturally died were *Macaca mulatta* (rhesus macaque (one ♀) and *Papio hamadryas* (Hamadryas baboon) (one ♀) from Samsun Zoo, Turkey. Post mortem helminthological examinations of two primates were carried out between 2006 and 2010. Parasites were counted and identified morphologically. The collected parasites were preserved in 70% alcohol and were deposited in the collection of Department of Parasitology of the Veterinary Faculty of Ondokuz Mayis University, Samsun, Turkey (voucher OMUPAR.53.12.01).

DNA Extraction, PCR Amplification, and Sequencing

One male nematode from *Papio hamadryas* was randomly selected among the total samples for the molecular identification. Genomic DNA was extracted from individual male nematode using the DNA purification kit (Genomic DNA Purification Kit, Thermo Scientific) according to manufacturer's instructions. PCR targeting the ITS region (ITS-1, 5.8S, ITS-2) were performed. DNA content was determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific) at 260 nm. PCR was carried out in a final volume of 100 µl, 1.5 µl of DNA template (72.2 ng/µl), 10 µl of 10×Taq Buffer with KCl (Thermo Scientific), 6 µl of 25 mM of $MgCl_2$ (Thermo Scientific), 2 µl of 10 mM dNTPs (Thermo Scientific), 5 µl of forward and reverse primers (0.5 µM each), 0.5 µl of 2.5 U *Taq* DNA Polymerase (Thermo Scientific), and 70 µl of autoclaved distilled water. ITS-1, 5.8S and ITS-2 region were amplified using the forward primer NC5 (5′-GTAGGTGAACCTGCGGAAGGATCATT-3′) and reverse primer NC2 (5′-GGTTAGTTTCTTTTCCTCCGCT-3′) [3]. Negative control consisted of autoclaved distilled water. The PCR was performed in a Thermo PxE 0.2 thermal cycler (Thermo Scientific) and the conditions were as follows: 3 min at 94°C, then 35 cycles of 1 min at 94°C, 1 min at 55°C and 1 min at 72°C followed by a final elongation of 10 min at 72°C. PCR products were electrophoresed in 1.5% agarose gel (Prona) in a TBE buffer (Thermo Scientific), stained with ethidium bromide (Sigma) and visualized by UV illumination. The size of the amplified fragments was estimated by comparisons with the 200 bp DNA Ladder (Thermo Scientific). The ITS region products were sent to sequencing company (Genoks Ankara, Turkey) for purification and sequencing in both directions using NC5-NC2 primers.

Data Analysis and Phylogenetic Tree Construction

The obtained sequences were verified by forward and reverse comparisons, assembled and edited with using Contig Express in Vector NTI Advance 11.5 (Invitrogen). Resulting sequence data were identified via GenBank and aligned with previously characterized sequences of nematodes, using ClustalW in Mega 5.0 multiple sequence alignments [5]. Nucleotide composition was calculated using Mega 5.0 [6]. Genetic distances were calculated using the Kimura two-parameter model with pairwise deletion in Mega 5.0 [6]. Phylogenetic relationships of the parasite lineages were estimated using the neighbor joining (NJ) method in Mega 5.0 [6]. The NJ analysis was performed using a Kimura two-parameter correction model [7] and pairwise deletion option for gaps. Confidence in the NJ trees was determined by analyzing 1.000 bootstrap replicates [8] using the Mega program. The ITS region sequence of *T. trichiura* was deposited in GenBank under accession no. KC877992.

RESULTS

Parasitological Result

All specimens were morphologically determined to be *T. trichiura*. Number of infected animals and parasites are presented in *Table 1*.

Molecular Results

The amplification of the ITS region produced a fragment of approximately 1400 bp from nematode *(Fig. 1)*. The ITS PCR products were subjected to direct sequencing giving products 1248-bp long. Pairwise comparison between the entire ITS region of the *T. trichiura* isolates from Turkey (KC877992) and other *T. trichiura* and *Trichuris* sp. isolates China (AM992981, AM992985, AM992998), Czech Republic (JF690940, JF690941, JF690950), Netherlands (JF690948) and South Africa (GQ301551, GQ301553) showed differences ranging from 0.1 to 1.2 % *(Table 2)*. Phylogenetic relationships among *T. trichiura* isolates from *P. hamadryas* of the Turkey and the other *T. trichiura* isolates and *Trichuria* sp. as inferred by neighbor joining analysis of the ITS sequence, and based on the entire ITS fragment including ITS-1, 5.8S, and ITS-2 sequences are presented in *Fig. 2*.

Table 1. *Number of infected animals and parasites*
Tablo 1. *Enfekte hayvan ve parazit sayıları*

Hosts	*Trichuris trichiura*			
	♀	♂	Immature	Total
Macaca mulatta (♀ 4 years old)	7	12	-	19
Papio hamadryas (♀ 6 months old)	783	641	1458	2882

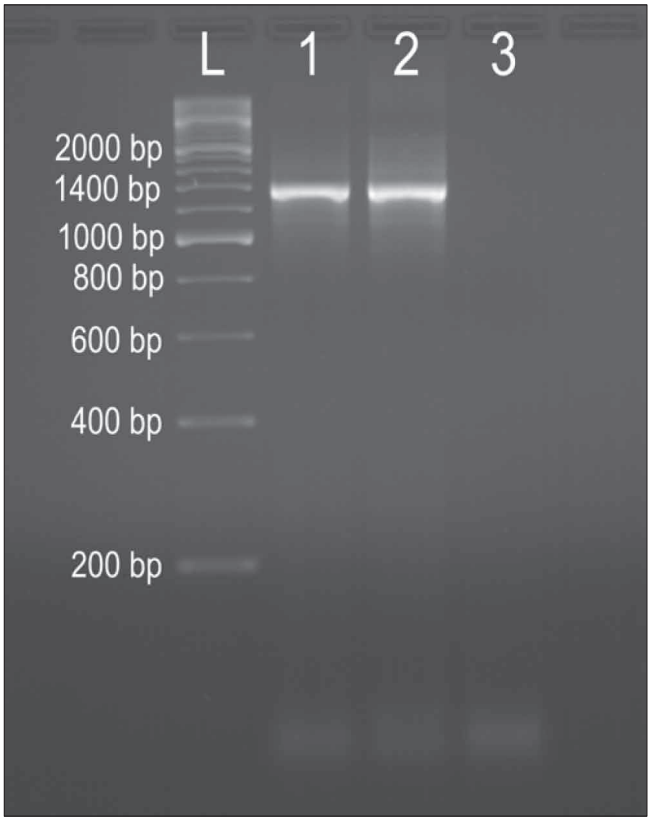

Fig1. PCR amplification of *Trichuris trichiura*. L: Ladder; 1-2: *T. trichiura*; 3: negative control

Şekil 1. *Trichuris trichiura*'nın PZR amplifikasyonu. L: Ladder; 1-2: *T. trichiura*; 3: negatif control

Table 2. *Pairwise comparison of nucleotide sequence differences (in percent) in the ITS among Trichuris isolate (Turkey) and various geographical isolates*
Tablo 2. *Trichuris Türkiye izolatı ile değişik coğrafik bölgelere ait izolatların ITS bölgesindeki nükleotit sekans farklılıklarının birbirlerine olan uzaklık indeksleri*

Accession Number	1	2	3	4	5	6	7	8	9	10
1. AM992981 (China)										
2. AM992985 (China)	0.005									
3. AM992998 (China)	0.003	0.005								
4. JF690940 (Czech Republic)	0.009	0.011	0.011							
5. JF690941 (Czech Republic)	0.003	0.003	0.000	0.011						
6. JF690948 (Netherlands)	0.005	0.005	0.002	0.012	0.002					
7. JF690950 (Czech Republic)	0.003	0.003	0.000	0.011	0.000	0.002				
8. GQ301551 (South Africa)	0.004	0.005	0.002	0.011	0.000	0.002	0.000			
9. GQ301553 (South Africa)	0.006	0.002	0.003	0.011	0.000	0.002	0.000	0.003		
10. KC877992 (Turkey)	0.006	0.002	0.004	0.012	0.002	0.003	0.002	0.004	0.001	

DISCUSSION

Zoological gardens exhibit wild animals for aesthetic, educational and conservation purposes. However, parasitic diseases constitute one of the major problems causing even mortality in these animals while in captivity, the effects of which range from sub-clinical to death [9]. In Turkey, there were a few studies about parasites of non-human primates [10-12]. In this study, we necropsied two non-human primates and identified parasites as *T. trichiura*. According to results of the study, parasites burden of monkeys were seen that too much. We think that the reason of this is the direct life cycle of *T. trichuira*. The other hand some human parasites originated in prehominid ancestors in Africa. Nematode species, such as *Enterobius vermicularis*, hookworms and *T. trichiura* are shared by humans and other close phylogenetic primates [13]. Furthermore, a phylogenetic analysis based on ITS sequence was performed for *T. trichiura* from *Papio hamadryas* in the present study. The phylogenetic trees showed all the sequences of *T. trichiura* and *Trichuris* sp. clustered together and separated from *T. ovis* and *T. discolor*. In the present study, *T. trichiura* from *Papio hamadryas* were characterized for the first time by sequencing of the ITS rDNA. The analyses revealed

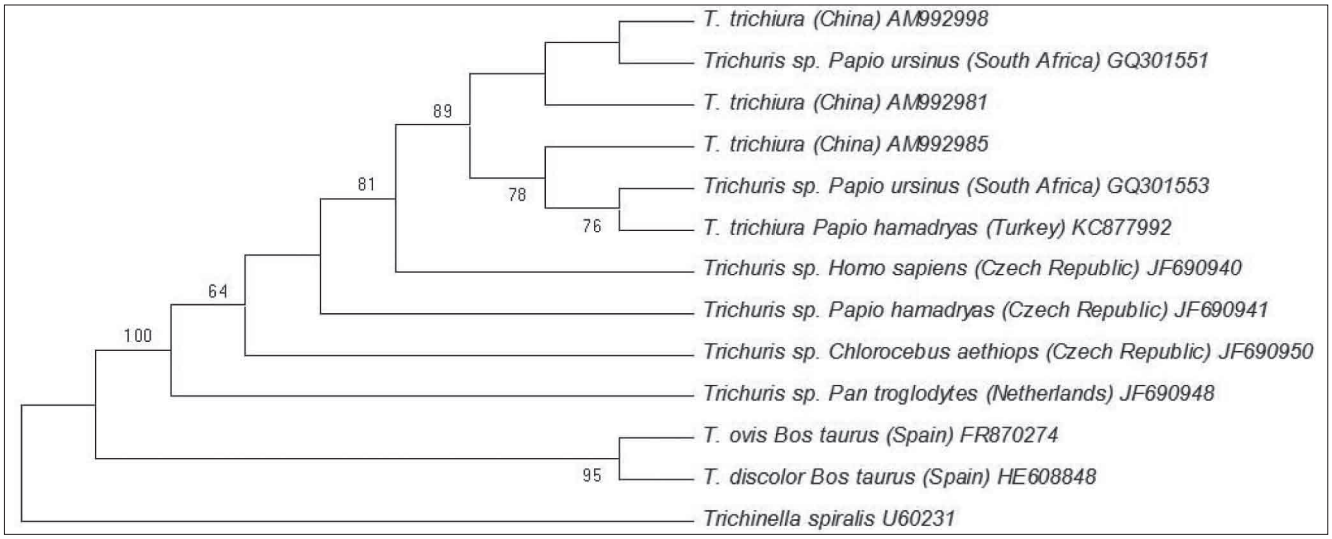

Fig 2. Neighbor joining analysis of the ITS sequence
Şekil 2. ITS dizisinin neighbor joining analizi

that the sequence obtained are highly similar to those of previously published for *T. trichiura* and *Trichuris* sp. from China (AM992981, AM992985, AM992998), Czech Republic (JF690940, JF690941, JF690950), Netherlands (JF690948) and South Africa (GQ301551, GQ301553). The high genetic similarity between the sequence of *T. trichiura* from the Turkey and those published data, from different geographical isolates, represents a wide range of distribution for this nematode and its humans and primates specificity. The presence of *T. trichiura* in monkeys at the zoo is a high risk to zoo keepers and also visitor's welfare because of its zoonotic character. Thus, we suggest that effective parasite control program should be established and stool control should be done regularly for primates.

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SHORT COMMUNICATION

Molecular Detection of Peste des Petits Ruminants Virus from Different Organs/Tissues of Naturally Infected Animals [1]

Murat ŞEVİK [1]

[1] *The part of this study has been presented in 1st International Biology Congress as a oral presentation in Bishkek, Kyrgyzstan, September 24-26, 2012*

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Makale Kodu (Article Code): KVFD-2013-9706

Summary

PPR virus (PPRV) detection rate in different organs/tissues of naturally infected sheep and goats was investigated. In order to achieve this, 24 animals, each from different flocks, with PPR suspect were examined for the presence of PPRV nucleic acid in different organ/tissues by reverse transcriptase PCR (RT-PCR) and real time RT-PCR. Virus neutralizing test (VNT) was used for the determination of PPRV specific antibody response. Real time RT-PCR and RT-PCR were found positive in all of lung, spleen, liver and lymph node samples tested in each of 16 VNT-positive animals and were negative in all samples from VNT-negative animals (n=8).

Keywords: *Peste des petits ruminants virus, Tissues, RT-PCR, Real time RT-PCR*

Doğal Enfekte Hayvanların Farklı Organ/Dokularında Koyun ve Keçi Vebası Virusunun Moleküler Tespiti

Özet

Doğal enfekte koyun ve keçilerin farklı organ/dokularından PPR virusunun (PPRV) tespit oranının araştırılmasının amaçlandığı bu çalışmada, her biri farklı sürüye ait PPR şüpheli 24 hayvan, farklı organ/dokularda PPRV varlığı yönünden konvansiyonel reverz transkriptaz polimeraz zincir reaksiyonu (RT-PCR) ve real time RT-PCR yöntemleri ile incelenmiştir. PPRV spesifik antikor yanıtını belirlemek için virus nötralizasyon testi (VNT) kullanılmıştır. Real time RT-PCR ve RT-PCR yöntemleri ile test edilen 16 VNT pozitif hayvanın bütün akciğer, dalak, karaciğer ve lenf nodülü örnekleri pozitif olarak tespit edilirken, VNT negatif hayvanlardan (n=8) elde edilen bütün akciğer, dalak, karaciğer ve lenf nodülü örnekleri negatif olarak belirlenmiştir.

Anahtar sözcükler: *Koyun ve keçi vebası virusu, Dokular, RT-PCR, Real time RT-PCR*

INTRODUCTION

Peste des petits ruminants (PPR) is an acute and highly contagious viral disease of small ruminants that is characterized by fever, an erosive stomatitis, broncho-interstitial pneumonia, diarrhoea and enteritis [1]. Clinical disease is seen in sheep and goats, and seroprevalence rate in sheep and goats rises with age. However, cattle, buffaloes and camels can become infected but there is little or no evidence of symptoms associated with their infection [2].

The causative agent, peste des petits ruminants virus (PPRV), is a member of the *Morbillivirus* genus in the *Paramyxoviridae* family. It is antigenically closely related to rinderpest virus (RPV), measles virus (MeV), canine distemper virus (CDV), and morbilliviruses of marine mammals [3]. Genetically, PPRV isolates can be grouped into four distinct lineages on the basis of partial sequence analysis of the fusion protein (F) and nucleoprotein (N) genes [4].

PPR occurs in West and Central Africa, Central and Southern Asia, the Middle East and Arabia [5]. It was first histochemically described in Turkey in 1993 [6]. Sequence analysis on the basis of the F gene of the isolates from

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outbreaks of PPR in Turkey has revealed that the virus was closely related to the members of lineage 4 [7].

Competitive ELISA and virus neutralization are routinely used for serological diagnosis of PPR, but gold-standard test remains the virus neutralizing test [8]. Virus isolation and differential neutralization in cell culture are slow, takes 2-3 weeks to complete, and of low efficiency. With advances in molecular biology, new diagnostic tests like PCR are available for detecting PPRV genomic material. Reverse transcription-PCR (RT-PCR) provides rapid, sensitive and reliable diagnosis of the disease [9]. However, these are being replaced by more sensitive and robust real time RT-PCR assays [10]. Organ (lymph node, spleen, lung, and liver) and swab specimens (ocular and nasal) can be used in PCR for the detection of PPRV genomic material [9]. In routine diagnostic laboratories all organ/tissues and swab specimens are not always available for testing. The aim of this study was to investigate PPR virus detection rate in different organs/tissues of naturally infected sheep and goats by reverse transcriptase PCR (RT-PCR) and real time RT-PCR. We used these two tests to compare the obtained results for each samples.

MATERIAL and METHODS

Samples and Positive Control

During January 2011 and May 2012, 24 animals, each from different flocks in 4 provinces (Konya, Antalya, Aksaray and Niğde), suspected to have PPR were submitted to the Veterinary Control Institute, Konya, Turkey. Blood serum samples were collected before necropsy. Except lymph nodes other organs/tissues were collected from all of cases. All tissue samples were kept at -85ºC prior to sample preparation and the RT-PCR assays. A total of 86 tissue samples of lung (n=24), liver (n=24), spleen (n=24) and mesenteric lymph nodes (n=14) collected from 24 animals (15 sheep and 9 goats), aged between 1 and 24 months, were tested. Lyophilized freeze-dried live PPR vaccine (Nigeria75/1 vaccine strain) obtained from the Division of Virology, Etlik Central Veterinary Control and Research Institute, Ankara, Turkey, was used as the positive control.

Virus Neutralizing Test

A virus-neutralization test for PPRV was performed in microtiter plates according the OIE recommendations [8]. Sera with VNT titres of >1:10 were considered positive.

RNA Extraction and Conventional RT-PCR

Viral RNA was extracted from tissue samples (25-30 mg) using a robotic extraction method (MagNA Pure LC 2.0 System, Roche Applied Science, Indianapolis, IN, USA) with the Magna Pure LC total nucleic acid isolation kit. One-step RT-PCR was used for the detection of PPRV RNA. Primers (PPRVF1b: 5´AGTACAAAAGATTGCTGATCACAGT and PPRVF2d: 5´GGGTCTCGAAGGCTAGGCCCGAATA) based on F protein coding gene of virus which amplify a 448 bp product [7] were used.

One-step Real Time RT-PCR

Real time RT-PCR amplification and detection was performed using LightCycler 2.0 real time PCR machine (Roche Applied Science, Indianapolis, IN, USA) with the one step RT-PCR kit (Qiagen, Germany). The primers in the N region PPRVF: AGAGTTCAATATGTTRTTAGCCTCCAT and PPRVR: TTCCCCARTCACTCTYCTTTGT and probe: Fam-CACCGGAYACKGCAGCTGACTCAGAA Tamra were used [10]. The samples that had a Ct value <35 were considered positive.

RESULTS

Of 24 sera tested, 16 (66.6%) were antibody-positive for PPRV by VNT. CPE of PPRV/N75/1 was observed at 4 days post-inoculation in wells. RT-PCR was found positive in all of lung, spleen, liver and lymph node samples tested in each of positive animals (n=16) and was negative in all samples from negative animals (n=8). The positivity rates were 100% (16/16), 100% (16/16), 100% (16/16) and 100% (11/11) for lungs, livers, spleens and lymph nodes, respectively *(Table 1)*.

The amplification of PPRV specific 448 bp fragment from nucleic acid of test samples and positive control (vaccine strain Nigeria75/1) were described as positive reaction *(Fig. 1)*.

Of the 86 tissue samples, 59 samples collected from positive animals (n=16) were positive for PPRV by real time RT-PCR and Ct values ranged from 18 to 33. All the 27 tissue samples collected from negative animals (n=8) were found negative for PPRV. Ct value of positive control (vaccine strain Nigeria75/1) was found 25. *Table 2* presents the mean Ct and SD values of tissue samples examined.

DISCUSSION

PPR was officially reported to OIE in Turkey for the first time in September 1999 [11], but there had been previous reports on its occurrence [6]. Serological studies carried out in Turkey show that prevalence of PPRV infection varies between 8.39% and 47.17% [7,12].

PPR can be confused clinically with contagious caprine pleuropneumonia (CCPP) or pasteurellosis, and hence the clinical observations for both diseases should always be confirmed by a laboratory test. Serological assays such as ELISA designed to detect the presence or absence of antiviral antibodies are used for confirmation of freedom from disease [8]. Neutralization and isolation of virus in cell culture is technically difficult and time-consuming and

Table 1. *RT-PCR results in various tissues of sheep and goats with PPR*
Tablo 1. *PPR ile enfekte koyun ve keçilerin farklı dokularının RT-PCR sonuçları*

Case	Species	Oral Cavity Lesions	RT-PCR Findings			
			Lung	Spleen	Liver	Lymph Node
1	Sheep (lamb)	p[a]	p	p	p	*[b]
2	Sheep (lamb)	p	p	p	p	*
3	Sheep (lamb)	p	p	p	p	*
4	Sheep (lamb)	p	p	p	p	p
5	Sheep (lamb)	p	p	p	p	p
6	Sheep (lamb)	p	p	p	p	p
7	Sheep	p	p	p	p	*
8	Sheep	p	p	p	p	p
9	Sheep	p	p	p	p	p
10	Sheep	p	p	p	p	p
11	Sheep	p	p	p	p	p
12	Sheep	p	p	p	p	p
13	Goat	p	p	p	p	p
14	Goat	p	p	p	p	p
15	Goat	p	p	p	p	p
16	Goat	p	p	p	p	*

[a] *Positive;* [b] *Not examined*

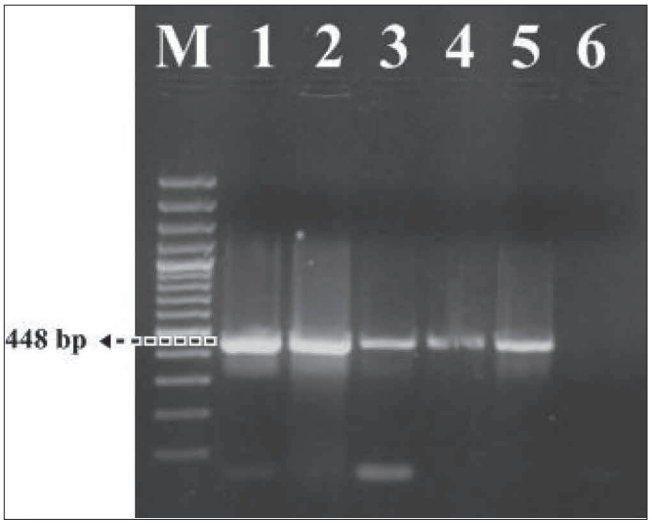

Fig 1. Agarose gel electrophoresis of RT-PCR product based on F gene of PPRV, **M:** Molecular marker of 100 bp, **Lane 1:** Positive control (Nigeria75 /1), **Lane 2-5:** Sample of lymph node, lung, liver, spleen, respectively, from the same animal, **Lane 6:** Negative control. Lymph nodule bands were comparably stronger than other tissue samples

Şekil 1. PPR virusunun F genine dayalı RT-PCR ürünlerinde agaroz jel elektroforezi, **M:** 100 bp moleküler marker, **1:** Pozitif kontrol (Nigeria75 /1), **2-5:** Sırasıyla aynı hayvana ait lenf nodülü, akciğer, karaciğer ve dalak örnekleri, **6:** Negatif kontrol. Lenf nodülü bandları diğer doku örneklerininkinden nispeten güçlü idi

Table 2. *Cycle threshold (Ct) values of tissue samples*
Tablo 2. *Farklı dokuların eşik döngüsü (Ct) değerleri*

Tissue Samples	n[a]	Mean Ct	± s.e.m[b]	SD[c]
Lung	16	25.73	1.45	5.03
Liver	16	26.04	1.19	3.77
Spleen	16	24.87	1.37	3.88
Lymph node	11	22.49	0.92	2.44

[a] *Number of samples;* [b] *Standard error of mean;* [c] *Standard deviation*

thus is not suitable as a routine diagnostic assay [4]. As an alternative to isolation, several RT-PCR and real time RT-PCR assays based on the fusion, the nucleoprotein, or the matrix protein genes were developed for the rapid and specific detection of PPRV [4,13]. In this study, one conventional RT-PCR targeting the F gene and one real time RT-PCR targeting the N gene were used to determine whether the organ/tissues had viral RNA.

For diagnosis of PPR, samples of conjunctival discharges, nasal secretions, buccal and rectal mucosae and anticoagulant-treated blood from live animals or lymph nodes, especially the mesenteric and bronchial nodes, lungs, spleen and intestinal mucosae from necropsied animals are recommended [8]. Success of detecting the virus in blood depends on the time of sampling (viraemic phase) and there are more chances of missing the presence of PPRV in the blood. Mahajan *et al.*[14] reported that the ocular and nasal swab samples are most valuable diagnostic material in case of live animals whereas tissue samples should be preferred in case of dead animals for diagnosis of PPRV infection. In a previous study, Albayrak and Alkan [15] have observed that maximum positive rate with tissue samples (50%, 22.58% and 17% in lymph nodes, spleen and lungs, respectively) followed by nasal (25%) and conjunctival (10%) swab samples whereas no blood and oral swab samples were positive for PPRV nucleic acid by RT-PCR.

Most of the time, tissue samples from necropsied animals have been submitted to the Division of Molecular Microbiology, Veterinary Control Institute, Konya, Turkey for molecular detection of PPRV. All required tissues/ organs are not available in every cases. Therefore, in this study, we aim to investigate detection rate of PPRV in different tissue samples (lungs, livers, spleens and lymph nodes) by RT-PCR and real time RT-PCR. RT-PCR and real time RT-PCR were found positive in all of lung, liver, spleen and lymph nodes samples tested in each of positive animals (n=16) and were negative in all samples from negative animals. Furthermore, the results of F gene-based conventional RT-PCR were consistent with the results of N gene-based real time RT-PCR. Therefore, these two methods are suitable for PPR diagnosis.

In the study, different organ/tissues samples from necropsied animals were used for diagnosis of PPRV

infection in small ruminants and the diagnostic value of lungs, livers, spleens and lymph nodes were assessed with RT-PCR and real time RT-PCR assays and it was concluded that in molecular diagnosis of PPR infection lung, liver, spleen and mesenteric lymph node samples can equally be used for PPR virus detection.

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LETTOR TO EDITOR

Agenesis of the Left Abdominal Wall in A Fetus Yeanling (Bir Oğlak Fetusunda Sol Abdominal Duvar Agenezisi)

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Makale Kodu (Article Code): KVFD-2013-10174

Dear Editor,

Several pathologies are encountered in the goat's embryo and fetus that they occur due to infectious and non-infectious reasons at pre-implantation, embryonic and fetal periods. Embryo and fetal diseases resulted in internal and external factors are described as "embryopathy". The well-known fetal diseases are schistosoma reflexum, perosomus elumbus, monster fetus and hybrid newborns [1,2]. The abdominal defects are commonly seen on the ventral and dorsal abdominal regions, which are suspected as a result of the amnion membrane deformity. Ventral abdominal defects result in partial or total side plate absence during the genesis of the vertebral column. These fissures can either extent from thorax to pelvis or form only on thorax, abdomen or pelvis [3]. We aimed to report an agenesis of the left abdominal wall in a fetus yeanling and thought to share its clinical appearance and radiological results with the veterinary practitioners.

A Siena breed, 4 year-old, female goat was presented to Uludag University, Faculty of Veterinary Medicine Emergency Clinics with a postpartum problem. The goat had a spontaneous yeanling birth one day ago; however, the birth pangs had not terminated since then. The owner had seen an intestinal segment and a rounded tissue protruding the vulva, which was suspected by the owner as mummy's abdominal organs. Clinically, the intestinal segments and urinary bladder was protruding from the ventral commissura of the vulva. Although vital parameters of the goat were normal, abdominal palpation was painful and she had a sensibility. Intravaginal examination diagnosed a dead fetus and its abdominal organ's protrusion from the mummy's vulva that the fetus was removed from the uterus as a single piece. Examination of the fetus demonstrates only a congenital absence on the anatomical structures of the left abdominal wall *(Fig. 1)*. The absence extents from last rib to tuber coxae craniocaudally, and from spinal process of the lumbal spine to ventral midline dorsoventrally. The intraabdominal organs were protruding from this defect and there was bilateral sacroiliac joint separation resulted from removing of the fetus from the birth canal. There was no other congenital malformation about the other systems (thoracic fissure, any organ hypoplasia and atresia, rudimental uterine, cryptorchidism, hydrocephalus, diaphragmatic rupture, cardiac anomalie etc.) on the fetus, macroscopically.

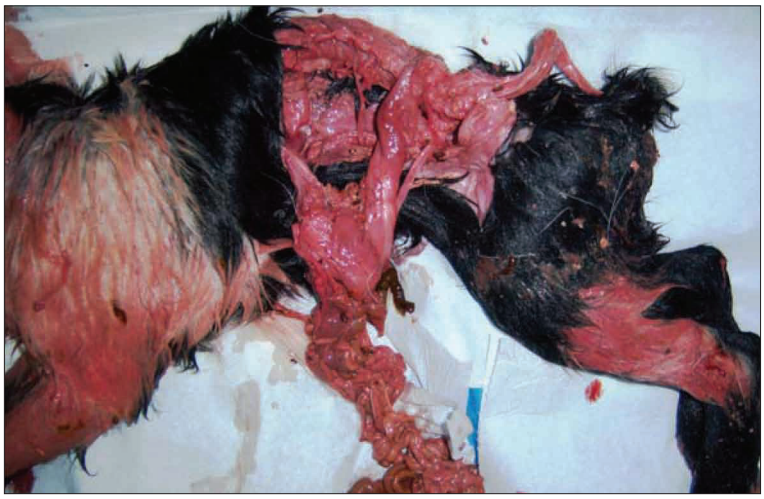

Fig 1. Clinical appearance of the case

Şekil 1. Olgunun klinik görünümü

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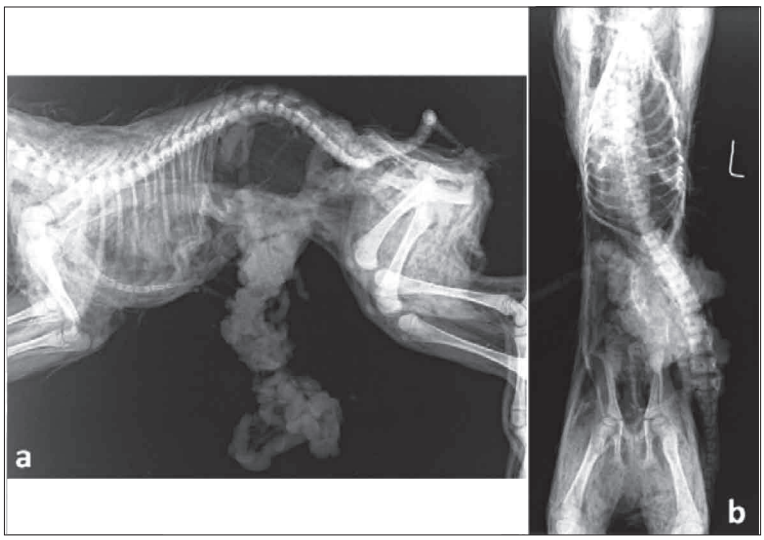

Fig 2. These radiographs demonstrate the intraabdominal organ protrusion **(a)**, absence of the continuity and integrity on the left abdominal muscles and bilateral sacroiliac joint separation **(b)**

Şekil 2. Bu radiograflar intraabdominal organ protrüzyonunu **(a)**, sol abdomen kaslarında bütünlük ve devamın olmadığını ve bilateral sakroiliak eklem ayrılmasını göstermektedir **(b)**

The lateral and ventrodorsal radiographs of the fetus were taken. Radiologically, there was no continuity and integrity on the left abdominal muscles. Intraabdominal organ protrusion and bilateral sacroiliac joint separation were corrected *(Fig. 2a-b)*.

Based on the macroscopic findings of the fetus, agenesis of the left abdominal wall and its results were diagnosed in this fetal yeanling.

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YAZIM KURALLARI

1- Yılda 6 (Altı) sayı olarak yayımlanan Kafkas Üniversitesi Veteriner Fakültesi Dergisi'nde (Kısaltılmış adı: Kafkas Univ Vet Fak Derg) Veteriner Hekimlik ve Hayvancılıkla ilgili (klinik ve paraklinik bilimler, hayvancılıkla ilgili biyolojik ve temel bilimler, zoonozlar ve halk sağlığı, hayvan besleme ve beslenme hastalıkları, hayvan yetiştiriciliği ve genetik, hayvansal orijinli gıda hijyeni ve teknolojisi, egzotik hayvan bilimi) orijinal araştırma, kısa bildiri, ön rapor, gözlem, editöre mektup, derleme ve çeviri türünde yazılar yayımlanır. Dergide yayımlanmak üzere gönderilen makaleler Türkçe, İngilizce veya Almanca dillerinden biri ile yazılmış olmalıdır.
2- Dergide yayımlanması istenen yazılar Times New Roman yazı tipi ve 12 punto ile A4 formatında, 1.5 satır aralıklı ve sayfa kenar boşlukları 2.5 cm olacak şekilde hazırlanmalı ve resim, tablo, grafik gibi şekillerin metin içindeki yerlerine Türkçe ve yabancı dilde adları ve gerekli açıklamaları mutlaka yazılmalıdır.
Dergiye gönderilecek makale ve ekleri (şekil vs) *http://vetdergi.kafkas.edu.tr* adresindeki online makale gönderme sistemi kullanılarak yapılmalıdır.
Başvuru sırasında yazarlar yazıda yer alacak şekilleri (13 X 18 cm boyutlarından büyük olmamalı) online makale gönderme sistemine yüklemelidirler. Yazının kabul edilmesi durumunda tüm yazarlarca imzalanmış *Telif Hakkı Devir Sözleşmesi* editörlüğe gönderilmelidir.
3- Yazarlar yayınlamak istedikleri makale ile ilgili olarak gerekli olan etik kurulu onayı aldıkları kurumu ve onay numarasını Materyal ve Metot bölümünde belirtmelidirler. Yayın kurulu gerekli gördüğünde etik kurul onay belgesini ayrıca isteyebilir.
4- ***Makale Türleri***
Orijinal Araştırma Makaleleri, yeterli bilimsel inceleme, gözlem ve deneylere dayanarak bir sonuca ulaşan orijinal ve özgün çalışmalardır.
Türkçe yazılmış makaleler Türkçe başlık, Türkçe özet ve anahtar sözcükler, yabancı dilde başlık, yabancı dilde özet ve anahtar sözcükler, giriş, materyal ve metot, bulgular, tartışma ve sonuç ile kaynaklar bölümlerinden oluşur ve toplam (metin, tablo, şekil vs dahil) 10 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 4 sayfayı aşmamalıdır. Ayrıca, en fazla 4 şekil veya tablo içermelidir.
Ön Rapor, kısmen tamamlanmış, yorumlanabilecek aşamaya gelmiş orijinal bir araştırmanın kısa (en çok 2 sayfa) anlatımıdır. Bunlar orijinal araştırma makalesi formatında yazılmalıdır.
Gözlem, uygulama, klinik veya laboratuar alanlarında ender olarak rastlanılan olguların sunulduğu makalelerdir. Bu yazıların başlık ve özetleri orijinal makale formatında yazılmalı, bundan sonraki bölümleri giriş, olgunun tanımı, tartışma ve sonuç ile kaynaklardan oluşmalı ve 4 sayfayı geçmemelidir.
Editöre Mektup, bilimsel veya pratik yararı olan bir konunun veya ilginç bir olgunun resimli ve kısa sunumudur ve 1 sayfayı geçmemelidir.
Derleme, güncel ve önemli bir konuyu, yazarın kendi görüş ve araştırmalarından elde ettiği bulguların da değerlendirildiği özgün yazılardır. Bu yazıların başlık ve özet bölümleri orijinal araştırma makalesi formatında yazılmalı, bundan sonraki bölümleri giriş, metin ve kaynaklardan oluşmalı ve 10 sayfayı geçmemelidir.
Çeviri, makalenin orijinal formatı dikkate alınarak hazırlanmalıdır.
Yazarla ilgili kişisel ve kuruma ait bilgiler ana metin dosyasına değil, on-line başvuru sırasında sistemdeki ilgili yerlere unvan belirtilmeksizin eklenmelidir.
5- Makale ile ilgili gerek görülen açıklayıcı bilgiler (tez, proje, destekleyen kuruluş vs) makale başlığının sonuna üst simge olarak işaret konularak makale başlığı altında italik yazıyla belirtilmelidir.
6- Kaynaklar, metin içinde ilk verilenden başlanarak numara almalı ve metin içindeki kaynağın atıf yapıldığı yerde parantez içinde yazılmalıdır.
Kaynak dergi ise, yazarların soyadları ve ilk adlarının başharfleri, makale adı, dergi adı (orijinal kısa ad), cilt ve sayı numarası, sayfa numarası ve yıl sıralamasına göre olmalı ve aşağıdaki örnekte belirtilen karakterler dikkate alınarak yazılmalıdır.
Örnek: **Gokce E, Erdogan HM:** An epidemiological study on neonatal lamb health. *Kafkas Univ Vet Fak Derg,* 15 (2): 225-236, 2009.
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Örnek: **McIlwraith CW:** Disease of joints, tendons, ligaments, and related structures. **In,** Stashak TS (Ed): Adam's Lameness in Horses. 4th ed. 339-447, Lea and Febiger, Philadelphia, 1988.
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