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Economic analysis of beekeeping enterprises in Aegean Region, Turkey*

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Summary: The aim of this study was to determine the economic analysis of beekeeping enterprises in Aegean Region of Turkey. The material of the current study was collected through face to face interviews from the total of 73 small, medium and large-scale enterprises determined by simple random sampling in Aegean region of Turkey (Aydın, Denizli, Mugla provinces). A backward regression model was developed to assess the input and output relationships in the enterprises. According to the results, the factors that affect the total profit, namely, marketing costs, sale price, unit cost, equipment and other expenses were found to be statistically significant ($p<0.05$). In the study, the lowest cost of producing honey was found to be in large-scale enterprises (1.82 US$/kg) and in the province of Aydın (1.64 US$/kg), whereas the highest cost of producing honey was found to be in small-scale enterprises (3.14 US$/kg) and in the province of Denizli (3.79 US$/kg). Net profit was calculated to be 41.16 US$/hive in small-scale, 28.75 US$/hive in medium-scale and 35.45 US$/hive in large-scale enterprises. In conclusion, considering that the major problem of beekeeping enterprises in Aegean Region is the marketing (64.3%) and the, study also suggested that some measures and supports actions should be put into practice including augmentation of the sale opportunities of the honey produced and the activation of structures of cooperatives so that the profitability of enterprises may be increased, and beekeeping activity may be carried out in a sustainable manner.

Keywords**:** Beekeeping enterprises, cost, economic analysis, honey, marketing.

Türkiye'de arıcılık işletmelerinin ekonomik analizi; Ege Bölgesi örneği

Özet: Bu araştırma Türkiye'de Ege bölgesindeki arıcılık işletmelerinin ekonomik analizinin gerçekleştirilmesi amacıyla yapılmıştır. Araştırma materyalini Ege bölgesinde (Aydın, Denizli, Muğla) tesadüfi örnekleme yöntemiyle belirlenen küçük, orta ve büyük olmak üzere üç farklı ölçekte toplam 73 adet işletmenin yüz yüze görüşme yöntemi ile elde edilen 2014-2015 yıllarına ait veriler oluşturmuştur. İşletmelerde girdi-çıktı ilişkilerinin değerlendirilmesi için backward regresyon modeli oluşturulmuş, toplam kâra etki eden unsurlardan; pazarlama masrafları, satış fiyatı, birim maliyet, ekipman giderleri ve diğer giderler istatistiksel olarak anlamlı ($p<0.05$) bulunmuştur. Araştırmada bal üretim maliyeti en düşük (1.82 US$) büyük ölçekli işletmelerde ve iller bazında Aydın'da (1.64 US$/kg) bulunurken, en yüksek üretim maliyeti (3.14 US$/kg) küçük ölçekli işletmelerde ve iller bazında Denizli'de (3.79 US$/kg) tespit edilmiştir. Kovan başına net kâr küçük ölçekli işletmelerde 41.16 US$, orta ölçekte 28.75 US$, büyük ölçekte 35.45 US$ olarak hesaplanmıştır. Sonuç olarak Ege Bölgesi'nde arıcılık işletmelerinin başlıca sorununun pazarlama olduğu (%64,3), işletmelerde kârlılığının artması ve sürdürülebilir bir arıcılık faaliyeti için, üretilen balın perakende satış imkânlarının çoğaltılması, kooperatif yapıların etkinleştirilmesi başta olmak üzere pazarlama alanında bir takım destek ve tedbirlerin hayata geçirilmesi büyük önem arz etmektedir.

Anahtar sözcükler**:** Arıcılık işletmesi, bal, ekonomik analiz, maliyet, pazarlama.

Introduction

Beekeeping has some advantages, of which can be carried out in conjunction with various plant and animal production activities and without depending on land and that it requires less capital and labour. In addition, it provides socio-economic functions by increasing the level of income of the farmers that do not have plenty of lands, while also preventing migration from rural to urban areas and, creating employment for the young population in rural areas (10, 16, 20).

Turkey ranks second following China in terms of both the number of hives and the annual amount of honey production in the world (9). Nevertheless, Turkish beekeeping sector has been facing technical and economic

* This manuscript is derived from the Ph.D. thesis of the first author. The study was presented as an oral presentation 45th APIMONDIA (International Apicultural Congress)

problems which include the low productivity per hive, bee diseases, pests, failure to increase the export capacity, difficulties encountered by beekeeping enterprises in marketing their products, insufficient of the level of industry organization, and unexpected migratory beekeeping. In addition to the sector's own problems, competition in the globalizing world has become a key factor that the Turkish beekeeping sector needs to consider (7, 21).

This study aimed to calculate the unit cost of producing per kg of honey in different provinces and enterprises and to figure out whether there is a statistically significant difference between these costs as well as between the sale prices of honey produced in Aydın, Denizli and Mugla provinces and enterprises. Furthermore, it also attempted to identify what sort of economic problems the enterprises faces in marketing honey by assessing the input and output relationships of the enterprises.

Materials and Methods

Beekeeping enterprises were categorised as small-scale (30-150 hives), medium-scale (151-300 hives) and large-scale (301 and more hives) enterprises. Taking into account the number of population hives of enterprises that had 30 or more hives among 1,057 enterprises in Aydın, 571 enterprises in Denizli and 3,568 enterprises in Mugla, the average number of hives of each stratum on the basis of three provinces and three stratums, the number of enterprises in each stratum and their standard deviation values were calculated. In determining the sample size of the study, "Stratified Random Sampling" was employed, which included the use of Neyman allocation $n=[\sum(Nh\times Sh)^2]/[N^2D^2+\sum(Nh\times(Sh))^2]$ [1] with a margin of error of 10% and within a confidence limit of 90%. First, the total sample size in each province was determined, and then they were distributed to the different stratum (17). The numbers of beekeeping enterprises included in the sample for each province and stratum are given in Table 1.

Table 1. Numbers of beekeeping enterprises in the sample by provinces and scales
Tablo 1. Örneklem kapsamındaki arıcılık işletmelerinin illere ve ölçeklere göre sayıları

Provinces	Number of sample	Scale	Number of sample
Aydın	24	Small	30
Denizli	16	Medium	23
Mugla	26	Large	13
Total number of sample	66		

Table 1 demonstrates that the minimum sample size of the study is 66, which includes 24 enterprises from Aydın, 16 from Denizli and 26 from Mugla. As to the distribution of this number by scales, it includes 30 small-scale, 23 medium-scale and 13 large-scale enterprises. Together with the reserve enterprises selected for the study, 80 beekeeping enterprises were interviewed between October 2014 and May 2015, and the data of 73 reliable enterprises were taken into account. The questionnaire drawn up for this purpose was distributed to the enterprises during the visits paid, and also additional information was acquired during the visits paid at intervals. The production costs of beekeeping enterprises taken into account in the study include feeding costs, labour costs, costs of auxiliary materials used in production, marketing expenses, hive transportation costs, loan interests, other expenses, overhead costs and building/equipment costs. Operating revenues include the revenues generated from the sale of honey, whereas the revenues generated from the sale of pollen, propolis, etc. were grouped as the additional income. The data collected in the study are intended to provide information on the use of inputs and marketing.

In analysing the data, one-way analysis of variance (ANOVA) was carried out to find out whether there was a significant difference between unit costs of producing 1 kg of honey in different provinces and enterprise scales as well as between the sale prices of honey. In the first two hypotheses, scales (small, medium and large) are the independent variables, whereas the cost of producing 1 kg of honey and sale price of 1 kg of honey in US dollars are the dependent variables. In the third and fourth hypotheses, provinces (Aydın, Denizli, and Mugla) are the independent variables, whereas the cost of producing 1 kg of honey and sale price of 1 kg of honey in US dollars are the dependent variables. It was found that there was no difference between the groups' average unit costs and sale prices of honey among the provinces in which the enterprises were located. Tukey and adjustment Bonferroni post-hoc tests were used to determine from which scale and province the difference between the groups arose (11, 19).

Multiple regression analysis was conducted in an effort to functionally assess honey production in the beekeeping enterprises. The regression model was checked for the assumptions for multiple linear regression analysis, namely, normality, linearity, zero-mean error terms, homoscedasticity, and no autocorrelation and no multicollinearity between independent variables. The variables were found to be not correlated with each other at an autocorrelation factor of 0.80 and above. In the regression analysis, all independent variables were included in the model and backward regression method

was applied (2, 11, 19). The model used in the regression analysis is as follows:

$$Y= b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5 + b_6X_6 + b_7X_7 + b_8X_8 + b_9X_9 + b_{10}X_{10} + b_{11}X_{11} + \varepsilon \quad [2]$$

In the model developed for this study, the dependent variable (Y) denotes the total profit generated by beekeeping enterprises from the sale of honey in US dollars.

Y= Enterprise's profit in US dollars;

The independent variables included in the model are as follows:

X_1= Cost of feeding bees; feeding costs of bees in US dollars, X_2= Labour costs; total cost of family and external labour in US dollars, X_3= Cost of auxiliary materials; cost of materials procured annually in US dollars, X_4= Marketing costs; packaging and marketing costs of honey in US dollars, X_5= Transportation costs; costs incurred for transportation of hives and leasing of land in US dollars, X_6= Other expenses; total cost of loan interest, veterinary health expenses, hive and vehicle insurance, water and lighting in the hive site, and shuttle for checking the hives in US dollars, X_7= Equipment cost; Maintenance, repair and depreciation costs of hives, machinery, equipment and tools used in beekeeping in US dollars, X_8= Sale price; sale price of 1 kg of honey in US dollars, X_9= Unit cost of production; production cost of 1 kg of honey in US dollars, X_{10}= $Scale_1$ (Small vs. Large), X_{11}= $Scale_2$ (Medium vs. Large), ε = Error term.

Excluding the scale, the independent variables in the model are quantitative. The scale variables X_{10} and X_{11} are qualitative and have three categories (small, medium and large). Since the qualitative independent variables with more than one category need to be included in the model as dummy variables, 2 dummy variables were included in the regression analysis for the scale variable with three categories (11). As some of the cost items such as loan interest and veterinary health expenses are zero for some enterprises and their percentage in the total costs of the enterprises is very low, than they were named as other expenses and considered together in the regression analysis.

In regression analysis, a high correlation between the dependent (total profit) and independent variables is an undesirable condition. If the coefficient of correlation between independent variables is 0.80 or above, this is considered an indication of the multicollinearity problem. Another criterion for the presence of autocorrelation in the model is the requirement that the Durbin-Watson statistic should be between 1.5 and 2.5 (5, 6). SPSS 18.0 was used to conduct the statistical analyses.

Results

Using the cost items, total honey production records and additional income information provided by the enterprises, the cost of producing 1 kg of honey in the beekeeping enterprises was calculated for each scale and province. Using the sale price of 1 kg of honey, net profit per hive was calculated for each scale and province. These figures are given in Table 2.

Table 2 shows that the lowest cost of producing 1 kg of honey was found to be in large-scale enterprises as 1.82 US$ and in Aydın as 1.64 US$. The highest cost of producing 1 kg of honey was found to be in small-scale enterprises as 3.14 US$ and in Denizli as 3.79 US$. As to the sale prices, the highest sale price of 1 kg of honey is in small-scale enterprises as 6.10 US$ and in Denizli as 6.47 US$. Net profit per hive was found to be the highest in small-scale enterprises as 41.16 US$ and in Aydın as 47.53 US$. The average cost of production, sale price and net profit per hive of all enterprises within the scope of the study are 2.49 US$, 4.93 US$ and 35.64 US$, respectively. The lowest net profit per hive was found to be in Mugla as 26.49 US$.

Variance analyses conducted and statistically significant differences are given in Table 3.

Table 3 shows that there is no statistically significant difference between the average unit costs of the groups of enterprise scales (p>0.05).

Table 2. Honey production costs, sale price of honey and net profit per hive of beekeeping enterprises by scales and provinces
Tablo 2. Arıcılık işletmelerinde ölçekler ve iller itibariyle ortalama bal üretim maliyetleri, satış fiyatları ve kovan başına net kâr miktarları

Criterion	Number of enterprises	Honey cost ($X \pm S_{\bar{X}}$) (US$/kg)	Honey sale price ($X \pm S_{\bar{X}}$) (US$/kg)	Net profit per hive (US$)
Small scale	33	3.14±1.20	6.10±1.21	41.16
Medium scale	26	2.01±0.74	4.00±0.73	28.75
Large scale	14	1.82±1.17	3.79±1.60	35.45
Aydın	24	1.64±1.52	5.07±1.07	47.53
Denizli	19	3.79±1.18	6.47±1.78	35.09
Mugla	30	2.34±0.49	3.82±0.89	26.49
Total	73	2.49±0.66	4.93±0.75	35.64

Table 3. Findings from variance analyses
Tablo 3. Varyans analizlerine ilişkin bulgular

Scales	Small	Medium	Large	F	p
Unit cost US$	3.14	2.01	1.82	3.121	0.0502
Sale price US$	6.10[a]	4.00[b]	3.79[b]	9.234	0.0002***
Provinces	Aydın	Denizli	Mugla	F	p
Unit cost US$	1.64[b]	3.79[a]	2.34[b]	6.579	0.002**
Sale price US$	5.07[a,b]	6.47[a]	3.82[b]	8.868	0.0003***

a,b: Values that bear different letters in the same row are statistically different.
*: p<0.05, **p<0.01 ***p<0.001

Table 4. Regression coefficients of the model
Tablo 4. Regresyon modeline ilişkin katsayılar

Parameters	Multiple linear regression model				Collinearity statistics	
	β	Std. Error	t	p	Tolerance	VIF
Constant	7609.922	5658.993	1.345	0.183	-	-
Marketing	24.711	3.063	8.067	0.001	0.596	1.677
Other Expenses	-2.635	0.891	-2.957	0.004	0.388	2.576
Equipment	2.510	0.833	3.015	0.004	0.347	2.881
Sale Price	1207.763	271.472	4.449	0.001	0.689	1.451
Unit Cost	-1463.276	306.914	-4.768	0.001	0.697	1.435
	R^2 0.747		Adjusted R^2 0.728		Durbin-Watson 1.969	

Table 5. Problems encountered by beekeeping enterprises in marketing their products and percentages of encountering
Tablo 5. Arıcılık işletmelerinin pazarlamada karşılaştıkları sorunlar ve karşılaşma oranları

The name of problems encountered by marketing	The number of enterprises encountered the problem	The percentage of enterprises encountered the problem (%)
Low selling price	40	54.8
Monopoly marketing structure	28	38.3
Insufficient cooperation and associations	23	31.5
Lack of quality-price relationship in Honey	17	23.2
Fake and smuggled honey in the market results in unfair competition	15	20.5
Glucose problem in honey	7	9.5
Residues problem in honey	2	2.7
Do not have any problem in marketing	16	21.9

The differences between the average sale prices of honey of the groups of enterprise scales were found to be statistically significant ($p<0.001$). The average sale price of honey among small-scale enterprises was found to be higher than the average sale price of honey among medium- and large-scale enterprises.

The differences between the average production costs in the provinces were found to be statistically significant ($p<0.01$). The lowest and highest unit cost of producing 1 kg of honey are in Aydın and Denizli, respectively. Production costs were found to be similar in Aydın and Mugla.

The average sale prices of honey of the groups of provinces were tested for a difference, and the sale price differences were found to be statistically significant ($p<0.001$). Based on the result, the sale prices were found to be higher than that in other provinces. The sale price difference in Aydın was found to be not statistically significant ($p>0.05$).

In the regression analysis conducted, the cost items which consists the total cost and the independent variables named scales were all included in the analysis, and each independent variable that contributed to the model the least and was not statistically significant was excluded

from the model in six steps, leaving the model with five independent variables that were important for explaining the total profit. The R^2 value, t statistics of independent variables and the regression coefficients derived from the result and findings of the analysis on the model developed with these independent variables are given in Table 4.

Table 4 shows that the adjusted coefficient of determination (R^2) of this model is 0.728. This means that 72.8% of the change in the dependent variable is explained by the independent variables included in the model. The Durbin-Watson statistic, which is used to ascertain whether there is autocorrelation in the model, is 1.969, indicating no autocorrelation in the model. The explanatory variables in the model were found to be statistically significant. Therefore, the model developed is as follows:

$$\text{“}Y = 7609.922 + 24.711X_4 - 2.635X_6 + 2.510X_7 + 1207.763X_8 - 1463.276X_9 + \varepsilon\text{”} \quad [3]$$

The analysis revealed that for each additional 1 US$ incurred for "marketing (X_4)", the total profit increases by 24.711 US$; for each additional 1 US$ incurred for "other expenses (X_6)", the total profit decreases by 2.635 US$; and for each additional 1 US$ incurred for "equipment (X_7)" used in beekeeping, the total profit increases by 2.51 US$. For each 1 US$ increase in the "sale price (X_8)", which is one of the independent variables, the total profit increases by 1207.763 US$, and for each 1 US$ increase in the "unit cost (X_9)", the total profit decreases by 1463.276 US$. Accordingly, the enterprises need to focus on marketing activities to increase their profitability. They need to enhance their retail sale opportunities to raise their sale prices and can reduce their unit costs by managing their operations successfully.

The 49.4% of beekeeping enterprises sell their honey to wholesalers/traders, 42.5% by retail, 6.8% to honey processing companies and 1.3% to a cooperative. While the average sale price is 6.63 US$/kg when enterprises market their honey by retail in small packages up to 5 kg, the price falls to 3.87 US$/kg when sold by retail in 27-kg large tins and to 2.79 US$/kg when sold wholesale. Attempts were made to find out in detail the problems encountered by the enterprises in marketing their honey. It was found that a significant portion of the enterprises faces more than one problem. While 47 of them (64.3%) report that they encounter more than one problem in marketing their products, 10 enterprises (13.8%) report that they face one problem and 16 enterprises (21.9%) report that they do not have any problem in marketing their products. It was found that the enterprises that did not encounter any problem marketed their products by retail without depending on any wholesaler or company.

The findings on the problems encountered in marketing are given in Table 5 along with the name of the problems and the percentage of encounters.

Table 5 shows that the problem that enterprises encounter the most in marketing their products is that they cannot sell their products at a price they are worth with a percentage of 54.8%. The second most encountered problem (38.3%) is that wholesalers and companies follow a monopolistic purchase price policy, and the third most encountered problem (31.5%) is that cooperatives and provincial associations are not effective in honey marketing channels. The percentage of enterprises reporting that fake and smuggled honey in the market results in unfair competition and this is a major problem for the marketing of their products is 20.5%. 9.5% of the enterprises are having problems due to the glucose in their honey and 2.7% of the enterprises are having problems due to the residues in their honey.

Discussion and Conclusion

The reason why the unit cost of production is the lowest in Aydın is associated with the fact that the production and sale of by-products such as pollen, propolis, etc. are higher than those in the other provinces. The unit cost of production is the highest in Denizli and the difference was found to be statistically significant ($p<0.01$). This resulted from the enterprises in Denizli do not have high amount in terms of the honey production and sale of by product and that the number of points to which migratory beekeepers go is less than that in the other provinces. As the scale of the enterprise increases, the unit cost decreases, and this can be accounted for by increasing returns to scale in terms of production costs.

Similar findings were obtained from two studies conducted in Adana, Turkey and Bosnia and Herzegovina. The cost of 1 kg of honey in Adana is 6.7 TRY (3.99 US$) in small-scale enterprises, 5.3 TRY (3.15 US$) in medium-scale enterprises and 4.7 TRY (2.80 US$) in large-scale enterprises (12). In a similar study conducted in Bosnia and Herzegovina, the cost of 1 kg of honey was found to be €1.71 for an enterprise with 100 hives and €1.51 for an enterprise with 300 hives (3). Based on these results, it is understood that beekeeping enterprises can reduce their production costs if they focus more on a production of by product such as pollen, propolis, etc., which have quite high sale prices, keep track of nectar flows in migratory beekeeping and increase their scale.

In a study in which the unit cost of producing 1 kg of honey is calculated, the unit cost is reported to be 2.67 TRY (2.00 US$) in Izmir, 2.19 TRY (1.63 US$) in Mugla and 2.29 TRY (1.70 US$) in average (15). In another study conducted throughout Turkey (7), the cost of producing 1 kg of honey was calculated to be 6.96 TRY (2.56 US$) which is close to 2.49 US$/kg calculated in this study. In

a study conducted in Albania, the cost of producing 1 kg of honey is reported to be 0.6-1.5 US$ (4).

In a study conducted in Izmir and Mugla, it is reported that 75% of the extracted honey is marketed to wholesalers/traders and 6.67% of it is marketed by retail (15). In another study conducted in the Aegean Region (13), 84% of the honey produced is reported to be marketed by wholesalers/traders. The percentage of sales to wholesalers/traders in this study, which is 49.4%, is lower than that in previous studies, and the percentage of retail sales, which is 42.5%, is higher than that in previous studies. It is clear that the percentage of retail sales in the Aegean Region and its provinces has an increasing trend. It was found that the producer's province of Adana in Turkey sells their products to wholesalers/traders (52.9%), cooperatives (29.42%) and processing companies (7.35%) (12). It is reported that 62.4% of the extracted honey Ordu province in Turkey is sold to wholesalers/traders and 35.3% by retail (14). In another study conducted throughout Turkey (7), the percentage of wholesale is reported to be 76%. The findings from studies conducted in different parts of Turkey indicate that the percentage of sales to wholesalers/traders is 50% and above.

Considering the studies on the sale prices of honey in Turkey, the average sale price of 1 kg of extracted honey was calculated to be 4.92 TRY in Adana (2.93 US$) and 13.6 TRY (5.00 US$) in average in Turkey, which is close to 4.93 US$ found in this study (7, 12). In the scope of the research, average sales price of honey in small scale enterprises was found to be higher than medium and large-scale enterprises. For this reason, small-scale enterprises may be able to market their honey in small retail packages in local markets and through personal connections.

Medium and large-scale enterprises, which make more mass production, sell wholesale in large packages (27kg box) in the oligopolistic market conditions. Due to the oligopolistic conditions of marketing, the sales prices of medium and large-scale enterprises are falling, which is also seen in the amount of profit per hive.

In a study carried out in Croatia, 59.12% of the beekeeping enterprises are reported to be selling their products by retail, 33.96% both wholesale and retail and only 6.92% wholesale (1). In the province of Tekirdağ in Turkey, producers are reported to prefer selling their products by retail (85%), to wholesalers/traders (11%), to processing companies (2%) and to the association (1%), but 70% of the producers are reported to have difficulties in marketing their products (18). In a study conducted province of Van in Turkey, it is reported that migratory beekeepers sell 26.67% of their honey by retail and regular beekeepers sell 88.23% of their products by retail, and that as the amount of production increases, the enterprises dealing with migratory beekeeping are having more difficulties in marketing their products (8). According to this study and the previous studies conducted in Turkey, it is obvious that producers predominantly market their honey wholesale. Since the honey marketing channels in Turkey are not effective from the viewpoint of producers, their marketing preferences necessarily tend to shift from retail to wholesale as the amount of production grows. This is in line with the results of other studies suggesting that as the scale of enterprises increases, they shift to wholesale and therefore, their profitability decreases. A low price of honey, which is the major problem of beekeeping enterprises selling their products wholesale, and the marketing problem, which is reported to be the primary problem of honey producers in a study conducted in Turkey (7), is in line with the results of this study.

To conclude, the problems experienced by beekeeping enterprises in marketing their products directly affect their profitability and indirectly affect the growth and development of the sector. Beekeeping enterprises in Turkey are facing oligopolistic market conditions. Despite the presence of numerous and unorganized beekeeping enterprises, there are a limited number of wholesalers and intermediary firms purchasing honey, which agree on the price of honey or exhibit similar behaviour and follow similar policies. Unless beekeeping enterprises enhance their level of the organization in the market, it seems difficult to reach a powerful position in which they market their products at reasonable prices. Therefore, the lack of effective marketing organization by beekeeping enterprises is a significant problem that should be addressed from the viewpoint of the sector. In addition, although the unit costs of large-scale enterprises are lower, small-scale enterprises facing higher unit costs have higher profitability, and it does not seem possible for beekeeping enterprises to enhance their production scale and level of organization under the current market/marketing conditions. In order to increase the scale of enterprises is necessary to solved the problems in marketing structure. To overcome the vicious circle of the Turkish beekeeping, the marketing problems experienced by beekeeping enterprises, particularly the low level of wholesale prices, should be resolved. In the case that the marketing problems are resolved, beekeeping will be more profitable, encouraging young and dynamic producers to enter the sector, which will result in the growth of enterprise scales and the desired level of increase in the amount of honey production in Turkey. To resolve the marketing problem, the cooperatives should be rendered more effective, the existing beekeeping associations should be provided with more opportunities for marketing, and the government should implement policies aimed at dealing with the technical, economic and financial challenges in this field. The sustainability of beekeeping and honey production seems to be highly associated with this.

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Milk yield and quality traits in different lactation stages of Damascus goats: Concentrate and pasture based feeding systems

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Summary: This study aimed to survey milk yield, lactation stages and milk quality traits of Damascus goats reared under different feeding systems. Goats were divided according to feeding systems as pasture based and concentrate based. Feeding systems were found to have significant effect on lactation milk yield but not on lactation duration. Although differences between feeding systems were not found significant on pH, milk composition factors (fat, lactose, dry matter), somatic cell count and malondialdehyde (except for early lactation stage) for each lactation stage, significant effects were detected on same parameters among lactation stages. Calcium levels at early lactation stage in milk differed either between feeding systems or among lactation stages. Also, other minerals were found to decrease with lactation. While most of the fatty acids were affected in any of the lactation stages by the feeding system, all of them were significantly altered by lactation stages. Pastured goats had a lower percentage of total saturated fatty acids, atherogenic index, thrombogenic index and odour index ratios. In accordance with milk fatty acid composition, compared to the milk obtained from goats fed with concentrate, the milk obtained from the goats that pastured was healthier and early lactation stage was found to produce healthier milk than late lactation stage.

Keywords: Damascus goat, feeding system, lactation stage, milk quality, milk yield.

Şam keçilerinde laktasyonun farklı dönemlerinde süt verimi ve süt kalitesi özellikleri: Konsantre ve meraya dayalı besleme sistemleri

Özet: Bu çalışma, farklı besleme sistemlerindeki Şam keçilerinin laktasyonun farklı dönemlerinde süt verimi ve süt kalite özelliklerinin araştırılması amacıyla yapılmıştır. Keçiler konsantre yeme ve meraya dayalı olarak iki farklı besleme sistemine ayrılmıştır. Besleme sistemlerinin, laktasyon süresi üzerine etkisi önemsiz olurken laktasyon süt verimi üzerinde önemli farklılığa sebep olmuştur. Besleme sistemleri arasında pH, süt kompozisyonu (yağ, laktoz, kuru madde), somatik hücre sayısı ve laktasyonun erken dönemleri hariç malondialdehit düzeyleri benzer olurken, aynı parametreler üzerinde laktasyon dönemlerinin etkisi önemli olmuştur. Sütteki Kalsiyum seviyeleri erken laktasyon dönemlerinde besleme sistemleri bakımından farklılık göstermiştir. Ayrıca laktasyon dönemleri arasında da farklılık meydana gelmiştir. Sütte bulunan diğer mineral maddelerin de laktasyonla beraber azaldığı tespit edilmiştir. Yağ asidi kompozisyonu besleme sistemlerinden büyük oranda etkilenirken, tüm yağ asitleri laktasyon dönemlerine göre önemli düzeyde farklılık göstermişlerdir. Meraya dayalı besleme sistemindeki keçilere ait sütler daha düşük toplam doymuş yağ asidi oranı, aterojenik indeks, trombojenik indeks ve koku indeks değerine sahip olmuştur. Süt yağ asidi kompozisyonu bakımından meraya dayalı beslenen keçilerden elde edilen süt konsantre yeme dayalı beslenen gruba göre daha sağlıklı bulunmuştur. Benzer şekilde erken laktasyon döneminde üretilen süt, geç laktasyon döneminde üretilene göre daha sağlıklı olmuştur.

Anahtar sözcükler: Besleme sistemi, laktasyon dönemi, süt kalitesi, süt verimi, Şam keçisi.

Introduction

Turkey goat population is approximately 11 million heads, only 2.5% of them is dairy. Most of dairy goats have been raised in Southern Turkey. Damascus goats are known as dairy and more adaptable to the environmental conditions. Guler et al (17) stated that lactation milk yield, duration, total solid and fat is 330.73 l, 244.5 days, 12.90% and 4.02%, respectively.

Compared to cow and sheep milk; goat milk and goat products have less allergenic components and they generate higher economic gain; therefore, they are significant in human nutrition (13, 37). Increase in the

number of goats in Turkey for the past years (43) has also shown that goat milk and its products are better valued and the value of goat milk has simultaneously increased as well. In the last years, quality parameters of milk have been taken into account as well as milk yield because quality parameters have been found important for consumer preference. Generally, in developing countries, high incidences of cardiovascular diseases are observed and clients demand top quality animal products with connatural flavor and taste (45). Similar to cow milk, goat milk delivers many nutrients with low energy content and it is advantageous for clients' well-being of throughout their lives. Therewithal, goat milk has ample benefits over cow and sheep milk since it can be used as an alimentary resource and remedial food for both infants and children (37).

Production systems or diet affects the composition of goat milk and goat milk products (14). Grazing environments of goats include a number of crucial metabolites that have advantages for human wellness and comprise alkaloids, fatty acids, tannins and flavonoids (13). On the other hand, milk composition may change during all lactation stages because of pasture or concentrate based feeding systems (23).

Community has grasped the significance of meadow-gazing goats. European countries lately decreased amounts of grazing goats due to hardships of finding shepherds and the activity of grazing, lack of meadows, diminished capacity of soils and abundance of goat breeds with low grazing ability (45). Thence, it is significant to compare pasture or concentrate based feeding systems.

While milk yield is one of the most important parameters in dairy goat systems, milk quality parameters (chemical composition, fatty acid composition, lipid oxidation capacity, mineral matter, etc.) are also substantial. Moreover, pricing is affected by milk quality levels such as milk fat content, dry matter, pH, etc.

This research intended to investigate milk yield-quality parameters and the changes observed during all lactation stages of Damascus goats under pasture based and concentrate based feeding systems.

Material and Methods

Ethical consideration: The actual research was approved by Animal Studies Ethic Committee of Mustafa Kemal University (Approval number: 2013/9-10).

Animal material and experiential procedures: The experiment was performed at a private goat farm in Hatay at south central region of Turkey. The animal materials of experiments were randomly selected from 400 heads flock. The goats and kids were medicated for the parasite with ivermectin/clorsulon and foksim and vaccinated against enterotoxaemia, foot and mouth diseases, ecthyma, agalaxia, mycoplasmosis, blue tongue and peste des pestits ruminants at physiologically suitable times. All goats were familiarized to the feed and pens for two weeks. All goats in the study were controlled against mastitis by using California Mastitis Test and mastitis negative goats were used in experiments and the goats never caught clinical mastitis during lactation. Experiments were carried out with concentrate-based feeding group (CBFG) (n=18) and pasture-based feeding group (PBFG) (n=18). Age of the goats in CBFG and PBFG were 4.70±0.28 (10 heads is 4≤ ages and 8 heads is 4> ages) and 4.38±0.24 (9 heads is 4≤ ages and 9 heads is 4> ages), respectively (P>0.05) and parturition type was the same in both pasture based and concentrate based groups (6 single, 12 twin). The goats (4 single and 8 twin) used for milk mineral matter and fatty acid composition were randomly selected from each group. Goats in both feeding groups were sheltered in fattening pens (4 m^2 of ground per goat) prepared for each group. While CBFG was housed all time in pen and fed 1.2 kg/goat/day concentrate feed and 1 kg/goat/day wheat straw, PBFG went to pasture one week later from parturition for grazing between 06:00-18:00 each day and was housed in pen after grazing. The PBFG goats consumed 0.6 kg/goat/day the concentrate feed (Table 1). The kids of CBFG stayed with their mothers until weaning (3 months old), but in milk control days, kids were separated from their mothers after last milking and separated kids were fed with a bottle during control days. Similarly, PBFG kids were kept in pen until they were 1 month old and later, they went to pasture together with their mothers but didn't go to pasture at milk control days. Like CBFG, PBFG kids also were weaned when they were 3 months old. Briefly, the kids in both groups were isolated from their mothers for 24 hours before milk control. Lactations were generally continued between January-February and August-September in both groups.

Milking and milk quality analyses: All goats used for milk yield and milk quality were milked with an interval of 14 days by hand milking methods during lactation and when daily milk yield decreased lower than 100 g (a criterion that was accepted to be the end of lactation for goats). Although milk control stages were in 14-day intervals, all milk quality values were assessed for early lactation stage (ELS), mid lactation stage (MLS) and late lactation stages (LLS). While the point on lactation curve between the start of lactation and maximum milk yield level was accepted as ELS, other lactation areas on lactation curve were divided into two parts as MLS and LLS. All milk yields were normalized for each goat by interpolation methods and lactation milk yields were calculated according to Trapez II methods (46).

Table 1. Chemical and physical composition of concentrate feeds
Tablo 1. Konsantre yemin kimyasal ve fiziksel kompozisyonu

Items contents	Proportions (%)	Proteins (%)	Metabolic energy (kcal/ kg)
Wheat	19.5	11.4	2800
Maize barn	20.6	19.5	2670
Corn	18.5	7.8	2880
Sunflower meal	15.5	28.0	2570
Cottonseed meal	10.0	31.0	2170
Barley	7.5	10.8	2750
Wheat barn	2.3	14.9	2320
Molasses	5.0	8.5	2890
Marble powdered (%38 Ca^{++})	0.3	-	-
NaCl	0.7	-	-
Premix*	0.1	-	-
Total	100.0	16.70	2649.28
Dry matter	88.91		
Crude ash	5.96		
Ether extract	2.58		
Crude protein	16.51		

Fatty acid composition (%)

C12:0	0.14	C18:1	21.14	C22:1	1.49
C14:0	0.24	C18:2 n6	53.48	C22:6 n3	0.18
C15:0	0.09	C18:3 n3	3.52	C24:0	0.32
C16:0	15.19	C20:0	0.41	∑SFA	18.96
C16:1	0.32	C20:1	0.91	∑MUFA	23.86
C18:0	2.22	C22:0	0.35	∑PUFA	57.18

*: Per 1.5 kg premix contains15 000 000 IU Vit A, 3 000 000 IU Vit D_3, 50 000 IU Vit E, 50 g manganese, 50 g ferrous, 50 g zinc, 10 g copper, 0.8 g iodine, 0.2 g cobalt, 0.3 g selenium.
*: Her 1.5 kg'lık premix 15 000 000 IU Vit A, 3 000 000 IU Vit D_3, 50 000 000 IU Vit E, 50 g Manganez, 50 g Demir, 50 g Çinko, 10 g Bakır, 0.8 g İyot, 0.2 g Kobalt, 0.3 g Selenyum içermektedir.

On control days, milk samples of approximately 200 ml were taken from each goat for milk quality analyses and these milk samples were swiftly transported to laboratory under ice bath. While pH and electric resistance were determined with a portative pH meter (HI83141, Hanna Ins.), milk color was colorimetrically tested as L* (lightness), a* (redness) and b* (yellowness) index values (Konica-Minolta CR-400). Before color analysis, reflection photometer was already calibrated with a blank and later, 20 ml of the samples were added to transparent plastic bags fitted to the photometer output. Readings were replicated three times per sample.

Forty ml milk samples with chemical tablets (Microtab II, Weber Sci.) were allocated for fat, protein, lactose, dry matter and somatic cell count (SCC) and later analyzed for milk chemical component (Combi 150, Bentley Ins.), SCC (Somacount 150, Bentley Ins.).

Malondialdehyde analysis (MDA) and milk mineral matter analyses were made from frozen milk samples that were kept at -24 ºC until analysis. While MDA levels were determined with UV-Spectrophotometers based on Esterbauer & Cheeseman (12), mineral matters were analyzed by Microwave Plasma Atomic Emission Spectroscopy (MP-AES 4100, Agilent Tech.). For mineral matters (n=12, 4 single and 8 twin parturition), milk samples were burned under nitric acid + perchloric acid first and later analyzed at suitable dilutions at the following device conditions: uptake time 30 s; rinse time 15 s; stabilization time 25 s; pump speed 15 rpm; wave length and nebulizer pressure for Ca, Mg, Fe, Cu, Zn were 393.366, 285.213, 371.993, 324.754, 213.857 nm and 120, 240, 120, 240, 140 kPa, respectively.

Two 10 ml milk samples from each goat (n=12, 4 single and 8 twin parturition) were centrifuged for 15 min, 3000 g at +4 ºC and milk cream from top of tube was gathered in 1,5 ml vials, frozen and frozen at -24 ºC until analysis. During analysis, approximately 500 µl milk cream was saponified with 2ml of 2N methanolic KOH for 2 min/ mixed at room temperature. Later, 4 ml n-Heptan was added and mixed for 1 min. Later, tubes were centrifuged at 200 g/ 3 min and allocated to separate organic phase. Fatty acid methyl esters (FAME) were collected from the top layer and transferred into vials. Separation of fatty acids was performed with HP Agilent 6890/5972 model GC-MS equipped with HP Innowax colon (60 m length, 0.25 mm i.d. x 0.25 µm film). While

injector temperature was set at 250 ºC, detector temperature was 270 ºC. Injection was splitless with a total injection volume of 1 µl and injector was washed three times with n-Heptan. Oven temperature was programmed initially at 120 ºC for 3 min and was increased to 250 ºC with a 3 ºC/min ramp rate. Helium was used as a carrier gas.

Statistical analyses: All statistical analyses were performed using the SPSS 14.0 statistical package (license number: 9869264). Effect of feeding systems, age and birth types (fixed factors) based on the comparisons (between pasture and concentrate based groups; between 4≤ and 4> ages; between single and twin birth type) on milk yield, lactation duration and milk quality traits were tested by Generalized Linear Models. On the other hand, effect of lactation stages on milk yield and milk quality characteristics was analyzed with repeated measurement One-Way ANOVA while Duncan's multiple range test was applied to evaluate the significance of the difference.

Results

While lactation curve for feeding systems is presented in Figure 1, mean values of lactation milk yield and lactation duration in groups are shown in Table 2. Lactation milk yield was 238.185 kg and it was higher in pasture-based group (P<0.001) and older age group (P<0.05) but no difference was found for lactation duration (P>0.05).

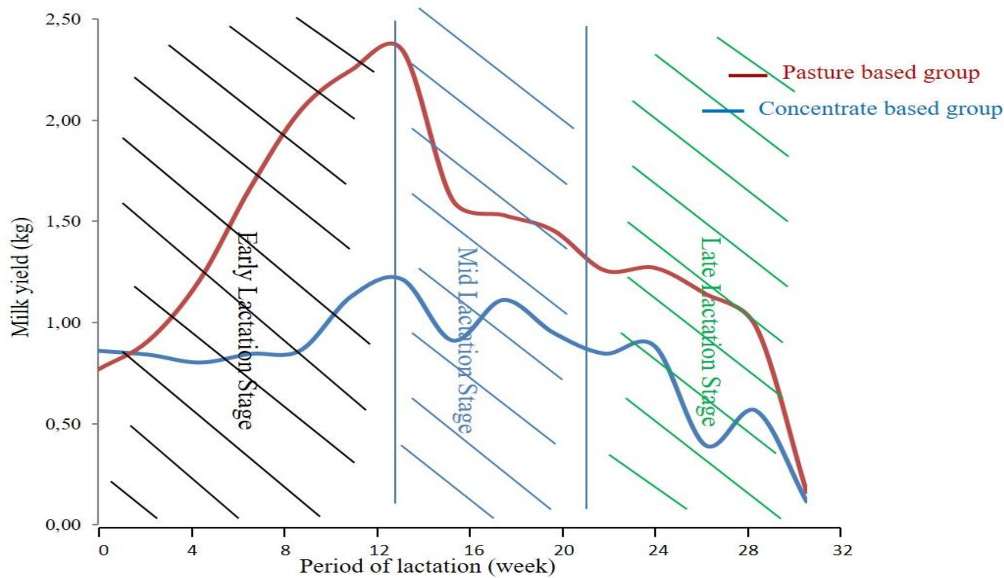

Figure 1. Lactation curves for feeding systems
Resim 1. Besleme sistemlerine göre laktasyon eğrileri

Table 2. Pooled Means±SE of milk yield and lactation duration for feeding systems, age and birth type
Tablo 2. Besleme sistemleri, yaş ve doğum tipi için süt verimi ve laktasyon süresine ait ortalamalar ve standart hatalar

Characters	Pooled Means± SE	P		
		Feeding systems (Means difference#)	**Age (Means difference $)**	**Birth type (Means difference&)**
Lactation milk yield (kg)	238.185±10.06	126.297***	-42.171*	-16.646-
Lactation duration (days)	210.487±4.57	16.184-	7.939-	-15.298-
ELS (kg/day)	1.259±0.07a	0.682***	-0.276*	-0.115-
MLS (kg/day)	1.239±0.06a	0.569***	-0.187-	-0.050-
LLS (kg/day)	0.713±0.04b	0.436***	-0.078-	0.027-
P	***			

ELS: Early lactation stage; MLS: Mid lactation stage; LLS: Late lactation stage
a, b Means with unlike letters in columns differ significantly (P<0.05).
a, b Aynı sütunda farklı harf taşıyan ortalamalar arası farklılıklar önemlidir (P<0.05)
-: P>0.05; *: P<0,05; ***: P<0.001
#: Means difference of pasture based from concentrate-based feeding systems; $: Means difference of 4≤ ages goats from 4> ages goats; &: Means difference of single from twin birth types

Table 3 presents the means of milk chemical composition and some milk quality parameters. Generally, although both milk chemical composition and some milk quality parameters were not affected from fixed factors (feeding systems, age and birth types), significant differences were detected among lactation (P<0.01; P<0.001). While L* values didn't display significant differences for fixed factors and lactation stages, a* and b* values were significantly different both in ELS for feeding systems (P<0.05; P<0.01) and among lactation stages (P<0.01; P<0.001) (Table 4).

Table 3. Pooled Means±SE of milk chemical composition and some milk quality traits for feeding systems, age and birth type
Tablo 3. Besleme sistemleri, yaş ve doğum tipi için sütün kimyasal kompozisyonu ve bazı süt kalite özelliklerine ait ortalamalar ve standart hatalar

Characters		Pooled Means±SE	P		
			Feeding systems (Means difference$^{\#}$)	**Age (Means difference$^{\$}$)**	**Birth type (Means difference$^{\&}$)**
pH	ELS	6.662±0.01^{a}	0.036^{-}	-0.003^{-}	-0.044*
	MLS	6.669±0.01^{a}	0.001^{-}	-0.032^{-}	0.034^{-}
	LLS	6.696±0.01^{b}	0.010^{-}	-0.005^{-}	-0.024^{-}
	P	**			
Electric resistance (mV)	ELS	17.008±0.576^{b}	2.217^{-}	-1.696^{-}	-0.336^{-}
	MLS	16.535±0.664^{b}	-1.801^{-}	-0.699^{-}	0.414^{-}
	LLS	15.036±0.738^{a}	-0.399^{-}	-0.239^{-}	1.218^{-}
	P	**			
Fat (%)	ELS	3.258±0.07^{a}	-0.069^{-}	-0.210^{-}	-0.094^{-}
	MLS	3.652±0.09^{b}	-0.119^{-}	0.149^{-}	-0.138^{-}
	LLS	3.461±0.10^{ab}	-0.196^{-}	0.251^{-}	-0.048^{-}
	P	***			
Protein (%)	ELS	2.837±0.04^{a}	0.195*	0.092^{-}	-0.023^{-}
	MLS	3.062±0.07^{b}	0.028^{-}	0.188^{-}	0.026^{-}
	LLS	3.296±0.08^{c}	-0.163^{-}	0.160^{-}	-0.018^{-}
	P	***			
Lactose (%)	ELS	5.044±0.02^{c}	0.039^{-}	0.034^{-}	0.112*
	MLS	4.813±0.07^{b}	0.011^{-}	0.007^{-}	0.165^{-}
	LLS	4.532±0.06^{a}	0.054^{-}	0.034^{-}	-0.067^{-}
	P	***			
Dry matter (%)	ELS	10.586±0.15^{a}	0.470^{-}	0.284^{-}	-0.154^{-}
	MLS	12.494±0.20^{b}	-0.161^{-}	0.367^{-}	0.062^{-}
	LLS	12.294±0.15^{b}	-0.362^{-}	0.470^{-}	-0.172^{-}
	P	***			
SCC (x10^{3})	ELS	599.600±76.51^{a}	65.449^{-}	-226.576^{-}	-258.500^{-}
	MLS	727.195±61.48^{a}	57.545^{-}	-174.313^{-}	-21.463^{-}
	LLS	1098.000±81.73^{b}	-36.892^{-}	101.348^{-}	113.827^{-}
	P	***			
MDA (nmol/ml)	ELS	8.406±0.49^{c}	2.808**	-0.086^{-}	1.018^{-}
	MLS	5.145±0.32^{b}	0.553^{-}	-0.917^{-}	0.037^{-}
	LLS	4.157±0.31^{a}	-0.716^{-}	0.051^{-}	0.513^{-}
	P	***			

$^{a, b, c}$ Means with unlike letters in columns differ significantly (P<0.05).
$^{a, b, c}$ Aynı sütunda farklı harf taşıyan ortalamalar arası farklılıklar önemlidir (P<0.05)
-: P>0.05; *: P<0,05; **: P<0,01; ***: P<0.001
$^{\#}$: Means difference of pasture based from concentrate-based feeding systems; $^{\$}$: Means difference of 4≤ ages goats from 4> ages goats; $^{\&}$: Means difference of single from twin birth types

Table 4. Pooled Means±SE of milk color for feeding systems, age and birth type
Tablo 4. Besleme sistemleri, yaş ve doğum tipi için süt rengine ait ortalama ve standart hatalar

Characters		**Pooled Means±SE**	**P**		
			Feeding systems (Means difference$^{\#}$)	**Age (Means difference$^{\$}$)**	**Birth type (Means difference$^{\&}$)**
L*	ELS	91.584±0.15	0.310^{-}	0.249^{-}	-0.543^{-}
	MLS	91.304±0.19	0.140^{-}	0.131^{-}	-0.707^{-}
	LLS	91.386±0.15	0.217^{-}	0.261^{-}	-0.525^{-}
	P	-			
a*	ELS	-3.385 ± 0.07^{b}	-0.308^{*}	0.013^{-}	-0.253^{-}
	MLS	-3.047 ± 0.08^{a}	-0.114^{-}	0.183^{-}	-0.325^{-}
	LLS	-3.024 ± 0.06^{a}	0.161^{-}	0.127^{-}	-0.200^{-}
	P	***			
b*	ELS	5.499 ± 0.22^{a}	1.175^{**}	-0.028^{-}	-0.130^{-}
	MLS	5.915 ± 0.22^{b}	0.555^{-}	-0.123^{-}	0.068^{-}
	LLS	6.130 ± 0.21^{b}	-0.168^{-}	0.109^{-}	0.441^{-}
	P	*			

$^{a, b}$ Means with unlike letters in columns differ significantly (P<0.05).
$^{a, b}$ Aynı sütunda farklı harf taşıyan ortalamalar arası farklılıklar önemlidir (P<0.05)
-: P>0.05; *: P<0,05; **: P<0,01; ***: P<0.001
$^{\#}$: Means difference of pasture based from concentrate-based feeding systems; $^{\$}$: Means difference of 4≤ ages goats from 4> ages goats; $^{\&}$: Means difference of single from twin birth types

Table 5. Pooled Means±SE of milk mineral matters for feeding systems, age and birth type
Tablo 5. Besleme sistemleri, yaş ve doğum tipi için sütteki mineral maddelere ait ortalamalar ve standart hatalar

Characters		**Pooled Means±SE**	**P**		
			Feeding systems (Means difference$^{\#}$)	**Age (Means difference$^{\$}$)**	**Birth type (Means difference$^{\&}$)**
Ca (mg/ml)	ELS	888.696 ± 30.33^{c}	135.333^{*}	52.522^{-}	-67.188^{-}
	MLS	590.380 ± 18.23^{a}	71.500^{-}	17.348^{-}	-46.348^{-}
	LLS	664.565 ± 21.75^{b}	34.000^{-}	44.174^{-}	-20.174^{-}
	P	***			
Mg (ng/ml)	ELS	94.194 ± 3.57^{b}	8.217^{-}	3.372^{-}	-3.439^{-}
	MLS	68.957 ± 2.45^{a}	8.800^{-}	1.717^{-}	-0.217^{-}
	LLS	73.125 ± 3.05^{a}	1.450^{-}	3.400^{-}	0.400^{-}
	P	***			
Fe (ng/ml)	ELS	2.441 ± 0.35^{c}	-0.217^{-}	0.557^{-}	-0.563^{-}
	MLS	1.447 ± 0.19^{b}	-0.820^{*}	0.281^{-}	-0.001^{-}
	LLS	0.907 ± 0.04^{a}	0.110^{-}	0.187^{*}	0.103^{-}
	P	***			
Zn (ng/ml)	ELS	4.315 ± 0.34^{b}	0.142^{-}	0.502^{-}	0.778^{-}
	MLS	2.690 ± 0.08^{a}	0.055^{-}	0.429^{*}	0.351^{*}
	LLS	2.868 ± 0.158^{a}	-0.205^{-}	0.618^{-}	0.422^{-}
	P	***			
Cu (ng/ml)	ELS	0.410 ± 0.03^{c}	-0.018^{-}	-0.062^{-}	-0.038^{-}
	MLS	0.248 ± 0.01^{b}	-0.015^{-}	-0.034^{-}	-0.026^{-}
	LLS	0.159 ± 0.01^{a}	0.020^{-}	-0.003^{-}	-0.017^{-}
	P	***			

$^{a, b, c}$ Means with unlike letters in columns differ significantly (P<0.05).
$^{a, b, c}$ Aynı sütunda farklı harf taşıyan ortalamalar arası farklılıklar önemlidir (P<0.05)
-: P>0.05; *: P<0,05; **: P<0,01; ***: P<0.001
$^{\#}$: Means difference of pasture based from concentrate-based feeding systems; $^{\$}$: Means difference of 4≤ ages goats from 4> ages goats; $^{\&}$: Means difference of single from twin birth types

All minerals significantly decreased during lactation (P<0.05; P<0.01; P<0.001) and Ca levels were higher in PBFG compared to CBFG at ELS (P<0.05). Also, Fe levels in LLS and Zn levels in MLS were higher 4≤ ages than 4> ages goats (P<0.05) (Table 5).

Values for fatty acid composition of milk cream are given in Table 6. The main fatty acids were palmitic acid (C16:0) and oleic acid (C18:1) followed by stearic acid (C18:0) in all experimental groups. All fatty acids with a few exceptions were affected by lactation stages (P<0.05; P<0.01; P<0.001) and on the other hand, short chain fatty acid (eight carbons or shorter) levels were different except butyric acid in LLS for feeding system effect (P<0.05, P<0.001). The best part of medium chain fatty acids (between fourteen and eighteen carbons) was not different for feeding systems but some of them were different. Table 7 presents the sums and ratios and index values obtained from fatty acids. Significant differences in the all total percentage of fatty acids (Saturated Fatty Acids (SFA), Monounsaturated Fatty Acid (MUFA), Polyunsaturated Fatty Acid (PUFA) etc.) were detected in lactation stages (P<0.05; P<0.001), but same differentiation in the all total percentages of fatty acids was not detected for feeding systems. The all sum and ratio based on milk fatty acids were not statistically different for ages (P>0.05). The odour index (OI) was different between lactation stages (P<0.001) and MLS and LLS were different for feeding systems (P<0.05; P<0.001) but not affected from ages and birth types.

Table 6. Pooled Means±SE of milk fatty acid composition for feeding systems, age and birth type
Tablo 6. Besleme sistemleri, yaş ve doğum tipi için süt yağ asidi kompoziyonuna ait ortalamalar ve standart hatalar

Characters		Pooled Means±SE	P		
			Feeding systems (Means difference[#])	**Age (Means difference[$])**	**Birth type (Means difference[&])**
C4:0 (%)	ELS	0.241±0.01^{a}	-0.131***	-0.051*	0.001^{-}
	MLS	0.262±0.02^{a}	-0.248***	-0.007^{-}	-0.011^{-}
	LLS	0.432±0.04^{b}	0.069^{-}	-0.060^{-}	0.039^{-}
	P	***			
C6:0 (%)	ELS	0.590±0.03^{a}	-0.261***	-0.100^{-}	-0.055^{-}
	MLS	0.589±0.03^{a}	-0.647***	-0.053^{-}	-0.063^{-}
	LLS	0.880±0.04^{b}	-0.203**	0.035^{-}	0.107^{-}
	P	***			
C8:0 (%)	ELS	1.246±0.06^{b}	-0.283*	-0.142^{-}	-0.104^{-}
	MLS	1.129±0.06^{a}	-1.234***	-0.111^{-}	-0.140^{-}
	LLS	1.639±0.07^{c}	-0.388*	0.113^{-}	0.224^{-}
	P	***			
C10:0 (%)	ELS	5.873±0.22^{a}	-0.164^{-}	-0.540^{-}	-0.642^{-}
	MLS	5.349±0.26^{a}	-5.133***	-0.397^{-}	-0.771^{-}
	LLS	8.226±0.33^{b}	-1.593*	0.671^{-}	0.780^{-}
	P	***			
C12:0 (%)	ELS	3.047±0.12^{a}	0.146^{-}	-0.146^{-}	-0.342^{-}
	MLS	2.906±0.19^{a}	-2.272***	-0.284^{-}	-0.539^{-}
	LLS	4.281±0.20^{b}	-0.755^{-}	0.503^{-}	0.108^{-}
	P	***			
C14:0 (%)	ELS	8.387±0.19^{a}	0.242^{-}	-0.340^{-}	-0.267^{-}
	MLS	8.562±0.27^{a}	-2.505***	0.016^{-}	-0.798^{-}
	LLS	12.239±0.29^{b}	0.086^{-}	0.093^{-}	0.420^{-}
	P	***			
C14:1 (%)	ELS	0.191±0.01	0.009^{-}	-0.024^{-}	0.006^{-}
	MLS	0.195±0.02	-0.079^{-}	0.047^{-}	-0.007^{-}
	LLS	0.238±0.01	0.016^{-}	0.001^{-}	0.005^{-}
	P	-			
C15:0 (%)	ELS	0.832±0.03	0.057^{-}	0.050^{-}	0.011^{-}
	MLS	0.941±0.04	0.102^{-}	-0.071^{-}	0.018^{-}
	LLS	0.951±0.09	-0.026^{-}	0.319^{-}	-0.001^{-}
	P	-			
C15:1 (%)	ELS	0.243±0.01	-0.038^{-}	0.007^{-}	0.013^{-}
	MLS	0.212±0.01	-0.043^{-}	0.030^{-}	0.028^{-}
	LLS	0.301±0.02	ND	0.074^{-}	0.094^{-}
	P	-			

Table 6. Pooled Means±SE of milk fatty acid composition for feeding systems, age and birth type (continued)
Tablo 6. Besleme sistemleri, yaş ve doğum tipi için süt yağ asidi kompoziyonuna ait ortalamalar ve standart hatalar (devam)

Characters		Pooled Means±SE	P		
			Feeding systems (Means difference#)	**Age (Means difference$)**	**Birth type (Means difference&)**
C16:0 (%)	ELS	26.939±0.46^{a}	-0.277^{-}	0.311^{-}	-0.159^{-}
	MLS	30.225±0.96^{b}	-0.982^{-}	0.680^{-}	-0.590^{-}
	LLS	37.408±1.31^{c}	2.203^{-}	-4.372^{-}	-2.777^{-}
	P	***			
C16:1 (%)	ELS	0.693±0.03^{a}	-0.071^{-}	0.050^{-}	0.001^{-}
	MLS	0.727±0.02^{a}	0.021^{-}	-0.064^{-}	-0.020^{-}
	LLS	0.878±0.06^{b}	-0.175^{-}	-0.002^{-}	-0.045^{-}
	P	*			
C17:0 (%)	ELS	0.845±0.02^{b}	0.014^{-}	0.077^{-}	-0.031^{-}
	MLS	0.758±0.02^{a}	0.249***	0.014^{-}	-0.003^{-}
	LLS	0.730±0.04^{a}	0.145*	0.060^{-}	-0.021^{-}
	P	**			
C17:1 (%)	ELS	0.243±0.01	-0.091**	0.039^{-}	0.024^{-}
	MLS	0.218±0.02	-0.043^{-}	0.060^{-}	0.003^{-}
	LLS	0.256±0.02	0.051^{-}	-0.010^{-}	-0.016^{-}
	P	-			
C18:0 (%)	ELS	16.908±0.50^{b}	1.349^{-}	0.129^{-}	0.443^{-}
	MLS	16.350±0.81^{b}	5.304**	0.108^{-}	0.508^{-}
	LLS	8.290±0.56^{a}	1.465^{-}	0.983^{-}	0.445^{-}
	P	***			
C18:1 (%)	ELS	27.227±0.531^{b}	-1.167^{-}	0.602^{-}	1.360^{-}
	MLS	25.388±0.76^{b}	6.443***	0.450^{-}	2.333^{-}
	LLS	20.016±0.68^{a}	-0.614^{-}	1.208^{-}	0.490^{-}
	P	***			
C18:2 n6 (%)	ELS	3.730±0.10^{c}	0.135^{-}	-0.321^{-}	-0.612**
	MLS	3.117±0.12^{b}	-0.255^{-}	-0.202^{-}	0.032^{-}
	LLS	2.162±0.06^{a}	-0.508***	0.436**	0.233^{-}
	P	***			
C18:3 n6 (%)	ELS	1.017±0.09^{b}	0.566**	-0.131^{-}	-0.359^{-}
	MLS	1.194±0.12^{b}	0.006^{-}	-0.204^{-}	-0.160^{-}
	LLS	0.631±0.08^{a}	0.125^{-}	-0.092^{-}	-0.010^{-}
	P	*			
C18:3 n3 (%)	ELS	0.863±0.06^{b}	0.270*	0.081^{-}	0.124^{-}
	MLS	0.824±0.06^{b}	0.138^{-}	-0.014^{-}	0.060^{-}
	LLS	0.488±0.04^{a}	-0.042^{-}	0.052^{-}	0.087^{-}
	P	***			
C20:0 (%)	ELS	0.333±0.02^{a}	0.005^{-}	-0.037^{-}	-0.032^{-}
	MLS	0.728±0.04^{b}	0.682***	-0.195*	0.033^{-}
	LLS	0.350±0.02^{a}	0.117**	-0.015^{-}	-0.022^{-}
	P	***			
C20:4 n6 (%)	ELS	0.167±0.01^{a}	-0.026*	-0.008^{-}	-0.019^{-}
	MLS	0.186±0.02^{a}	-0.004^{-}	-0.016^{-}	-0.063*
	LLS	0.247±0.01^{b}	ND	0.040^{-}	0.038^{-}
	P	*			
C22:0 (%)	ELS	0.113±0.01	0.024^{-}	0.007^{-}	-0.011^{-}
	MLS	0.230±0.03	0.264***	-0.056^{-}	0.012^{-}
	LLS	ND	ND	ND	ND
	P	-			
C24:0 (%)	ELS	0.200±0.01	0.031*	-0.009^{-}	-0.005^{-}
	MLS	0.222±0.02	0.162**	-0.029^{-}	-0.016^{-}
	LLS	ND	ND	ND	ND
	P	-			

$^{a, b, c}$ Means with unlike letters in columns differ significantly (P<0.05).
$^{a, b, c}$ Aynı sütunda farklı harf taşıyan ortalamalar arası farklılıklar önemlidir (P<0.05)
-: P>0.05; *: P<0,05; **: P<0,01; ***: P<0.001
#: Means difference of pasture based from concentrate-based feeding systems; $: Means difference of 4≤ ages goats from 4> ages goats; &: Means difference of single from twin birth types; ND: This value can't be determined in at least one of the groups.

Table 7. Pooled Means±SE of sum and ratio based on milk fatty acids for feeding systems, age and birth type
Tablo 7. Besleme sistemleri, yaş ve doğum tipi için süt yağ asidi oranlarına ait ortalamalar ve standart hatalar

Characters		Pooled Means±SE	P		
			Feeding systems (Means difference[#])	Age (Means difference[$])	Birth type (Means difference[&])
SFA (%)	ELS	66.675±0.62[a]	0.379[-]	-0.329[-]	-0.566[-]
	MLS	68.909±0.89[b]	-7.471[***]	0.723[-]	0.358[-]
	LLS	75.997±0.97[c]	0.532[-]	-1.339[-]	0.389[-]
	P	***			
MUFA (%)	ELS	28.561±0.55[b]	-1.347[-]	0.689[-]	1.418[-]
	MLS	26.332±1.00[b]	5.997[**]	0.863[-]	-0.450[-]
	LLS	20.841±0.86[a]	-0.419[-]	1.118[-]	-0.339[-]
	P	***			
PUFA (%)	ELS	5.764±0.19[c]	0.968[*]	-0.360[-]	-0.852[*]
	MLS	4.711±0.14[b]	0.970[**]	-0.069[-]	-0.190[-]
	LLS	3.159±0.15[a]	-0.112[-]	0.213[-]	-0.042[-]
	P	***			
UFA (%)	ELS	34.325±0.62[c]	-0.379[-]	0.329[-]	0.566[-]
	MLS	31.121±1.05[b]	6.829[**]	0.624[-]	-0.754[-]
	LLS	24.001±0.97[a]	-0.532[-]	1.332[-]	-0.381[-]
	P	***			
PUFA/SFA (%)	ELS	0.088±0.01[c]	0.014[*]	-0.004[-]	-0.012[-]
	MLS	0.068±0.01[b]	0.023[**]	0.002[-]	0.007[-]
	LLS	0.042±0.01[a]	-0.002[-]	0.003[-]	-0.001[-]
	P	***			
UFA/SFA (%)	ELS	0.529±0.02[b]	-0.015[-]	0.006[-]	0.009[-]
	MLS	0.452±0.03[b]	0.152[*]	0.051[-]	-0.006[-]
	LLS	0.321±0.02[a]	-0.013[-]	0.020[-]	-0.011[-]
	P	***			
n6	ELS	4.909±0.16[c]	0.684[*]	-0.460[-]	-0.990[**]
	MLS	3.955±0.12[b]	0.714[**]	0.026[-]	-0.119[-]
	LLS	2.721±0.12[a]	-0.143[-]	0.234[-]	-0.039[-]
	P	***			
n3	ELS	0.863±0.06[b]	0.269[*]	0.082[-]	0.125[-]
	MLS	0.754±0.06[b]	0.238[*]	0.028[-]	-0.020[-]
	LLS	0.506±0.03[a]	-0.095[-]	0.047[-]	0.094[-]
	P	***			
n6/n3	ELS	6.745±0.25[b]	-0.644[-]	-0.925[-]	-1.646[**]
	MLS	6.010±0.40[a]	-0.836[-]	-0.630[-]	0.390[-]
	LLS	6.745±0.30[b]	-0.882[-]	0.511[-]	-0.296[-]
	P	*			
NV	ELS	1.751±0.07[c]	-0.136[-]	0.137[-]	0.247[-]
	MLS	1.329±0.09[b]	0.626[**]	0.062[-]	0.048[-]
	LLS	0.771±0.06[a]	-0.024[-]	0.079[-]	-0.011[-]
	P	***			
AI	ELS	1.597±0.05[a]	0.076[-]	-0.061[-]	-0.079[-]
	MLS	1.927±0.09[b]	-0.907[***]	-0.152[-]	-0.128[-]
	LLS	2.704±0.14[c]	-0.089[-]	-0.063[-]	0.021[-]
	P	***			
TI	ELS	1.607±0.05[a]	0.042[-]	-0.042[-]	-0.100[-]
	MLS	2.101±0.10[b]	-0.768[**]	-0.188[-]	-0.072[-]
	LLS	2.675±0.14[c]	0.085[-]	-0.214[-]	-0.127[-]
	P	***			
OI	ELS	7.488±0.43[a]	-0.731[-]	-0.643[-]	0.430[-]
	MLS	6.461±0.36[a]	-5.891[***]	0.115[-]	-0.571[-]
	LLS	10.609±0.51[b]	-2.566[*]	1.244[-]	1.376[-]
	P	***			

[a, b, c] Means with unlike letters in columns differ significantly (P<0.05).
[a, b, c] Aynı sütunda farklı harf taşıyan ortalamalar arası farklılıklar önemlidir (P<0.05)
-: P>0.05; *: P<0,05; **: P<0,01; ***: P<0.001
[#]: Means difference of pasture based from concentrate-based feeding systems; [$]: Means difference of 4≤ ages goats from 4> ages goats; [&]: Means difference of single from twin birth types

Discussion and Conclusion

Lactation Duration and Milk Yield: Longer lactation is generally desired because length of lactation is one of most important factors for lactation milk yield (14). This duration can change based on breed which is determined according to dairy, meat type or indigenous breeds. Dairy goats have a long lactation stage between 7-10 months (2). In this research, lactation durations were 210.48 days and it was not affected from fixed factors (feeding systems, age and birth types), but lactation milk yield was 238.185 kg and it was higher in PBFG compared to CBFG (126 kg means difference) (P<0.001), higher in older goats than younger (42 kg means difference) (P<0.05). Classical knowledge says that birth types is important on milk yield, but this study result were not compatible with this classical knowledge because there was no difference between single and twin birth type for lactation milk yield. Both lactation length and lactation milk yield were lower compared to previous analysis on Damascus breed (17) because the experimental groups of this study were not selected as herd but reared for milk yield under local breeding conditions (16). But, detected lactation length and milk yield in this study proved that lactation was longer and milk yield was higher compared to other indigenous local goat breeds (11) or similar to Turkish Saanen goats (25). On the other hand, it is generally expected that lactation milk yield of goats with diets rich in concentrates may be higher than goats fed with pasture-based feeding systems due to higher energy intakes in concentrate-based feeding systems (23), but in this study findings were higher in PBFG compared to CBFG (126 kg means difference). This finding was interpreted that PBFG obtained sufficient energy levels from pasture grazing. Also, in terms of carcass and meat quality, Yakan et al. (45) stated that that higher production in intensive systems is not expected to be the same in extensive or semi-intensive production systems for goats because goats intrinsically need to graze more than other ruminant species (cow and sheep etc). When lactation stages are examined, although ELS and MLS showed similar milk yield in both experimental groups, LLS milk yields were lower than first two lactation stages (P<0.001). This decrease in milk yield in LLS might be caused by oestrus and due to pregnancy of goats in this period because it is well known that oestrogen and progesterone hormones are suppressor factors for lactation (4). The fact that the goats were detected to pregnancy from flock owner records.

Milk quality traits: pH is a very important parameter for determining milk quality because it converts milk to cheese via coagulation of proteins. pH values in this study were compatible with the findings of Sampelayo et al. (34) for Granada goats fed different concentrate feed. Although pH values were similar at all lactation stages for feeding systems, ages and birth types (except ELS), there were significant differences among lactation stages (P<0.01). Other important effects were not observed for feeding systems on the electric resistance, fat, protein (except ELS), lactose, dry matter, SCC and MDA (except ELS) and milk composition (fat, protein, lactose and dry matter) was generally consistent with numerous authors (3, 30, 31) but, all the above parameters were significantly affected from lactation stages (P<0.01; P<0.001). Milk fat and dry matter levels increased with lactation stage as consistent with lactation physiology and lactose levels decreased. Similar findings were indicated for Beetal and their crossbreed of goats by Prasad et al. (30). It is thought that the reason for the increase in milk fat ratio was due to both decreases in milk yield as a result of lactation and increases in roughage intake in LLS. Intake of cellulose volume is the reason of high milk fat ratio (10) and LLS was lengthened due to drying grass during the study period.

SCC forms the basis of abnormal milk control programs and the legal limit in USA was established by Food and Drug Administration for goats is $1000x10^3$ /ml (26). Although results from the data in this study indicate that SCC was increased with stage of lactation, the highest SCC was observed in LLS, the values were just at the legal limit. Cell counts for uninfected mammary glands have been shown to increase with period of lactation (19). While SCC in this study was not affected by fixed factors, it was found to be significantly different among lactation stages (P<0.001). Detected SCC in this work was generally compatible with findings of Orman et al. (25) and Gomes et al. (15). According to these SCC results, it can be argued that SCC increased as lactation went by, regardless of the presence of mastitis. Also, Hinckley and Williams (18) stated that no significant correlation was revealed between SCC and the leucocyte count. On the other hand, while SCC increased with lactation periods, milk yields decreased. This means that as SCC increases, milk yield decreases in goats. This finding is consistent with the findings of Raynal-Ljutovac et al. (32) and Orman et al. (25). Electric resistance of milk depends on the cations and anions levels in milk (6). While electric resistance was not affected from fixed factors, it significantly decreased with lactation stages (P<0.01). That finding is compatible with the study by Das and Singh (6).

Milk MDA level is the most generally preferred indicator to evaluate peroxidation status (24). MDA levels were significantly higher in PBFG in ELS compared to CBFG (P<0.01). Experimental goats were in shelter during the last 3 months of pregnancy period. After PBFG goats gave birth, they directly went to pasture during daytime and probably walked about 8-10 km/day in pasture. This walking effect could be the reason to

oxidative stress and that MDA levels might be higher in PBFG compared to CBFG. Later, this difference in MDA levels disappeared in mid and LLS since goats might have been accustomed to pasture. On the other hand, MDA levels decreased in time by lactation while SCC increased by lactation. Some authors (40, 47) reported that MDA and SCC were related to mastitis in cows. This result was not confirmed by our findings. On the contrary, it is shown that MDA level is not indicator of mastitis in goats, similar to SCC. Some authors (40, 47) investigated milk MDA levels and mastitis relationship but no study found MDA levels in all lactation stages and in different feeding systems in goats.

The color of butterfat contributes to the color of milk just like other milk components such as proteins and volatile compound. Reflectance spectra for whole milk showed that there was no effect in L* for both fixed factors and lactation stages. b* index was significantly differ for feeding system in ELS, but this effect disappeared in MLS and LLS. In the study area, while the pasture was green in ELS, it dried in MLS and LLS. Higher b* values presumably depended on consumed green grass which contained flavonoids. Solah et al., (38) reported that b* values are affected by cows' feeding systems such as pasture, silage and grain feed etc. It is shown that similar effect is valid for goats. a* index had negative values for all groups and stages and this finding is compatible with the results of Rufian-Henares et al., (33) but PBFG came close to zero ($P<0.001$).

The concentration of milk macro-minerals does not alter much, but they depend on the breed, diet, lactation stage and mammary health of goats. Important changes in contents of the macro-minerals in goat milk were detected during lactation (27). Total calcium is the most abundant mineral in milk (32) and was affected by ELS ($P<0.05$) and lactation stages ($P<0.001$). Calcium values were lower for different goat breeds (3, 29, 31). The reason of this difference was probably caused by breed effect. Other minerals in milk, Mg, Fe, Zn and Cu, also significantly decreased during lactation ($P<0.001$). This change of milk mineral concentrations is consistent with some studies (1, 28).

Feed is a very important parameter in milk fatty acid composition (29). Twenty-two fatty acids were detected from butyric acid (C4:0) to lignoceric acid (C24:0) for both feeding systems. While most of them, particularly short-chain fatty acids (from C4:0 to C10:0), were significantly different for feeding systems, there were some exceptions for lactation stages ($P<0.05$; $P<0.01$; $P<0.001$). Kondyli and Katsiari (20) stated that variations in milk fatty acids were generally caused by seasonal variations and appeared to be diet-related. The three most important fatty acids in quantitative terms, palmitic (C16:0), stearic (C18:0) and oleic acid (C18:1) accounted for approximately > 75%. This ratio was consistent with the findings of some authors who studied Damascus goats (17) and other goat breeds (9, 41, 42). These major milk fatty acids may be affected by stage of lactation (9, 41). Similar to this finding, while palmitic acid increased ($P<0.001$) with ongoing lactation, stearic acid decreased ($P<0.001$) by LLS. But decreasing of oleic acid with lactation wasn't consistent with the reports of Pakistan and Norwegian goats (9, 41). Short chain fatty acids (from C4:0 to C10:0) were responsible for goat odours and were defined as rancid and tart (9, 39) and it was postulated that short chain fatty acids occurred from depletion of body fats (14). Our findings showed that short chain fatty acids increased with lactation duration because depletion of body fats probably increased and short chain fatty acids broke free. On the other hand, some breeders state that source of goat odour is oestrus. This opinion can explain why oestrus generally comes up at the last trimester of lactation and increases the depletion of body fats. Several previous studies on different goat breeds (7, 41, 42) show that milk fat contains twenty-two carbon fatty acids as the longest chain (C22:0 and C22:6). Also, fatty acids with about twenty-four carbons (C24:0) were detected in Damascus goat milk in this study. The reason for this may be related to gas chromatography conditions and breed effect.

Sums and ratios based on fatty acids were generally shaken via feeding systems and it were not altered by age and birth types. Varied feed resources, especially browses and meadow plants, generate unequable ratios of unsaturated fatty acids (UFA) in milk in so far as increased UFA ratios or due to dissimilarity way of feed is finished in the rumen (35). The shape of rumen process for varied feed resources are different because of the different lipolysis procedures put accounted by rumen enzymes (21, 29). The detected SFA and UFA values were similar with numerous reports for different breeds (3, 7, 35, 41). SFA ratio in PBFG was lower than CBFG ($P<0.001$) and UFA ratio was higher in MLS ($P<0.01$). These results that depended on feeding systems in this study showed consistence with some earlier reports (14, 35). But the transition from SFA to UFA didn't occur in ELS and LLS. Results show that the effect of feeding systems on fatty acid ratios may be different among stages of lactation. Another index used to access the important value of fat is PUFA/SFA and the recommended value for the diet is 0.45. High levels of PUFA/SFA are desired because this may induce an increase in low blood cholesterol levels (8, 36). In the present research, this percentage differs importantly among lactation stages ($P<0.001$). The values were found to be lower than recommended levels. The effect of fatty acids on sanitation is not exactly stated by the rates a forenamed. Thus, it is suggested that NV, AI and TI should be ciphered. NV refers the wellness of the

feed in virtue of its lipid composition. AI values higher than 1 point to atherosclerosis risk and TI indicates potential aggregation of blood platelets; both are recommended for a healthy diet (44). The NV, AI and TI of PBFG yielded better values than CBFG in MLS (P<0.01; P<0.001) because NV increased while AI and TI decreased. Although values of AI and TI in ELS and MLS were lower than the values found by Chiofalo et al. (5) for ewes, they were compatible with their indications in LLS. Goat odour in milk is a very important parameter for consumption of milk. If milk has goat odour, it may not be preferred by consumers. In the present study, OI was calculated first time for goat milk and results indicated that PBFG had significantly lower values than CBFG in MLS (5.891 means difference) (P<0.001) and LLS (2.566 means difference) (P<0.05). When the sums and ratios based on fatty acids were evaluated in terms of lactation stages, it was shown that all values significantly differed among stages of lactation. It was noted that all values deteriorated as lactation progressed.

In conclusion, pasture-based feeding system may be favored over concentrate based feeding systems for more milk production and better milk quality traits, but it should be noted that starting of pasture period after parturition should be acclimatized to exercise. On the other hand, it was shown that lactation duration was not affected by feeding systems. It was detected that milk SCC was not related to mastitis although it could tend to increase during lactation stages. As milk mineral matters decreased with ongoing lactation, it is advised that more mineral should be supplemented according to lactation periods for goat health and healthy milk production. The increase of short chain fatty acids towards the end of lactation showed the destruction of depot fats and hence goats should be fed a more balanced diet in this period. Also, it says that age and birth types generally were not important on milk quality parameters. According to these results, it can be argued that milk quality is generally better in ELS than LLS. Finally, feeding system based on pasture and the obtained milk in ELS may be more preferable compared to concentrate based feeding systems and LLS. Feeding programs should be organized according to lactation stages to ensure good milk quality parameters.

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Osmanlı Devleti'nde Avrupa'ya gönderilen veteriner hekimliği öğrencileri*

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Özet: Osmanlı Devleti'nde on sekizinci yüzyılda başlayan iyileştirme çalışmaları ile Avrupa'da ivme kazanan bilimsel gelişmelerin takip edilmesine ve bu gelişmelerin devlet yapılarına uyarlanmasına çalışılmıştır. Bu kapsamda, Avrupa'dan örnek alınarak açılan okulların öğretim üyesi ihtiyacını karşılamak için önce yurt dışından uzmanlar getirtilmiş, daha sonra devamlı bir öğretim kadrosu yetiştirmek amacıyla Türk öğrencilerin Avrupa'ya gönderilmesi uygulaması başlatılmıştır. Askeri, mesleki ve teknik alanlarda eğitim almak üzere Avrupa ülkelerine gönderilen bu öğrenciler arasında veteriner öğrenciler de bulunmaktadır. Veteriner hekimliği öğrenimi görmek amacıyla Sivil Tıp Okulundan mezun hekimlerin ve Askeri Veteriner Okulu öğrencilerinin Avrupa'ya gönderilmesiyle başlayan bu uygulama, izleyen yıllarda veteriner hekimliğin çeşitli alanlarında uzmanlık eğitimi almak için veteriner hekimlerin gönderilmesiyle devam etmiştir. Öğrenimlerini başarıyla tamamlayan bu veteriner hekimler İstanbul'a döndüklerinde veteriner okullarının öğretim kadrosuna alınmış veya çeşitli devlet kademelerinde istihdam edilmiştir. Bu kişilerin büyük bir bölümü Türkiye'de veteriner hekimliği eğitim öğretimi ve örgütlenmesinde önemli hizmetlerde bulunmuşlar, veteriner hekimliğin akademik alanlarının gelişimine temel oluşturmuşlardır. Çalışmada Osmanlı Devleti'nde Avrupa'ya öğrenim görmek üzere gönderilen veteriner öğrenciler hakkında saptanabilen yeni bilgileri gün ışığına çıkarmak ve konuyu veteriner hekimliği tarihi açısından değerlendirmek amaçlanmıştır.

Anahtar sözcükler: Osmanlı Devleti, veteriner hekimliği öğretimi, veteriner hekimliği tarihi, yurt dışında öğrenim.

Veterinary students sent to Europe by the Ottoman State

Summary: In the Ottoman State, with the initiation of the reformation movement in the eighteenth century, it was aimed to both keep up with the scientific developments that had gained momentum in Europe at that time, and to apply these novelties to the state institutions. Within this scope, in order to establish the teaching staff of the schools that had been newly founded in view of the European model, firstly it was resorted to the tutorship of European experts. Thereafter, with an aim to establish permanent academic staff for these schools, Turkish students were started to be sent to Europe to be trained. Veterinary students were also included among these students, who visited European countries for military, professional and technical training. The practice of sending doctors, who were graduates of the Civil Medical School, and students of the Military Veterinary School to Europe for veterinary medical education was continued in the following years by sending veterinarians to European countries for specialisation training in various branches of veterinary medicine. These veterinarians, who successfully completed their education, were either appointed as teaching staff at the veterinary schools or employed at various public institutions, upon their return to İstanbul. Many of these students made significant contributions to veterinary education as well as to occupational organisations and established the basis of the development of the academic fields of veterinary medicine in Turkey. This study both reveals new information gathered on veterinary students sent by the Ottoman State to Europe for training and provides an assessment of the implications of this practice in view of the history of veterinary medicine.

Keywords: Foreign education, Ottoman State, veterinary education, veterinary history.

Giriş

Osmanlı Devleti'nin gerileme döneminde (1699-1792) yaşadığı askeri yenilgiler sonrasında Avrupa'yı kendisine örnek almaya başlaması, devletin temel dinamiklerinde yapılan bazı reformları da beraberinde getirmiştir. On sekizinci yüzyılın ilk çeyreğinde başlayan bu iyileştirme çalışmaları, Osmanlı Devleti'nin Avrupa'da meydana gelen gelişmeleri takip etmesi ve bu gelişmeleri devlet yapılarına uyarlaması ile gerçekleştirilmiştir. Böylelikle, bir yandan devletin Avrupa'ya yavaş yavaş uyum sağlaması beklenirken, diğer yandan geleneksel Osmanlı askeri ve idari yapısı değişime uğramıştır (9, 12). Gerçekleştirilen düzenlemeler kapsamında Avrupa

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tarzında yeni okullar açılmış, bu okullara gerekli ders araç ve gereçleri tedarik edilmiştir. Ancak okulların en büyük ihtiyaçlarından biri dönemin bilim ve teknolojisine uygun eğitim-öğretim yaptıracak bir öğretim kadrosu olmuştur. Bu sorunu aşmak amacıyla, öncelikle Avrupa'dan uzmanlar ve öğretim üyeleri getirtilmiştir. İzleyen yıllarda, çağdaş birer öğretim kurumu olmasına özen gösterilen bu okullarda görevlendirilen yabancı öğretim üyelerinin yerine bilimsel bilgilere sahip Türklerden oluşan devamlı bir öğretim kadrosuna ihtiyaç duyulmuştur. Bunun üzerine Türk öğrencilerin Avrupa'ya gönderilerek buradaki okullarda eğitim almaları düşünülmüş, ilk kez II. Mahmut döneminde (1808-1839) bu uygulamaya başlanmıştır (6, 15).

Batı örnekli düzenlemelerin içinde Avrupa'ya öğrenci gönderilmesi, Osmanlı eğitim sisteminde olduğu kadar çeşitli devlet kurumlarının iyileştirilmesi açısından da önemli bir yer tutmuştur. Osmanlı Devleti'nin tüm tebaasından gönderilen bu öğrencilerin yönetim ve denetimi Avrupa'da bulunan Osmanlı elçilikleri ile yürütülmeye çalışılmış, on dokuzuncu yüzyıl sonundan itibaren yürürlüğe konulan nizamnameler ile hangi esaslara tâbi olacakları belirlenmiştir (6, 9, 20). Bu uygulama memuriyete benzer bir yol izlemiş, öğrenciler mevkilerine göre maaş almışlar, hastalık, ölüm veya başarısızlık gibi sebeplerden dolayı öğrencinin eksilmesi durumunda yerine başka bir öğrenci gönderilmiştir (9). Askeri, mesleki ve teknik alanlarda eğitim almak üzere Avrupa ülkelerine gönderilen bu öğrenciler arasında veteriner hekimliği öğrencileri de bulunmaktadır. Söz konusu öğrencilerin on dokuzuncu yüzyıl sonu ve yirminci yüzyıl başında Fransa ve Almanya'ya gönderildikleri bildirilmiştir (10). Bu kapsamda gönderilen ilk öğrenciler veteriner hekimliği öğrenimi görmüş, daha sonraki yıllarda gönderilenlerin ise veteriner hekimliğin çeşitli bilim dallarında uzmanlık eğitimi almaları mümkün olmuştur.

Osmanlı Devleti'nde veteriner hekimliği öğrenimi görmek üzere Avrupa'ya gönderilen öğrenciler ile ilgili bilgi veren bazı yayınlar (3, 4, 10, 18, 19) bulunmaktadır. Ancak konu özelinde ayrıntılı bilgi veren herhangi bir çalışmaya rastlanmamıştır. Makalede, Osmanlı Devleti'nde Avrupa'ya öğrenim görmek üzere gönderilen veteriner öğrenciler hakkında saptanabilen yeni bilgileri gün ışığına çıkarmak ve bir devlet politikası haline gelen bu uygulamayı veteriner hekimliği tarihi açısından değerlendirmek amaçlanmıştır.

Materyal ve Metot

Çalışmanın ana materyalini, Başbakanlık Devlet Arşivleri Genel Müdürlüğünün Osmanlı Arşivinde saptanan orijinal belgeler ile sözü edilen dönemde yayımlanan Sabah, Tanin, Tasvir-i Efkâr ve İkdam adlı günlük gazetelerde yer alan konuya ilişkin duyuru ve yazılar oluşturmaktadır. Bununla birlikte, konu bütünlüğünü sağlayabilmek üzere kitap, makale ve diğer yayınlardan da yararlanıldı. Arşiv belgelerinin ve gazete yazılarının metin içerisinde kullanımında Osmanlıcadan transkripsiyonu yapıldıktan sonra günümüz Türkçesine uygun sadeleştirmeleri yapıldı. Orijinal belgeler ve gazete yazılarının künyeleri ile birlikte açıklayıcı ek bilgiler dipnotlarda gösterildi.

Yirminci yüzyıl başında birbirinin peşi sıra gelen Balkan, I. Dünya ve Kurtuluş Savaşları sırasında eğitim-öğretim faaliyetleri kesintiye uğradığı için çalışmanın kapsamı, veteriner hekimliği öğrenimi amacıyla öğrencilerin ilk kez Avrupa'ya gönderildiği 1889 yılı ile I. Balkan Savaşı'nın başladığı 1912 yılı arasında sınırlı tutuldu. Çalışma materyali, tarih araştırmalarında uygulanan analiz ve sentez yöntemleriyle değerlendirildi ve konu kronolojik olarak yazıya aktarıldı.

Bulgular

Osmanlı Devleti'nde veteriner hekimliği öğrenimi amacıyla yurt dışına öğrenci gönderilmesi, Sivil Veteriner Okulunun 1889 yılında faaliyete başlaması ile gündeme gelmiştir. Sivil Veteriner Okulunun kuruluş sürecinde yaşanan yer ve bütçe sıkıntısından dolayı eğitim-öğretimin ilk iki yıl Ahırkapı'da bulunan Sivil Tıp Okulu'nda, son iki yıl ise Halkalı'da ziraat okulu olarak kurulması planlanan binalarda yapılmasına karar verilmiş, okulun adı "*Halkalı Ziraat ve Baytar Mektebi*" olarak değiştirilmiştir.[1] Hazırlanan öğretim programına göre, veteriner hekimliği öğrencilerinin fizik, kimya, botanik, zooloji gibi ortak dersleri tıp öğrencileriyle beraber Sivil Tıp Okulunun öğretim üyelerinden, anatomi ve fizyoloji derslerini bu okulda konu ile ilgili görevlendirilecek bir öğretim üyesi tarafından almaları öngörülmüş, üçüncü sınıftan itibaren klinik uygulamaların ağırlıkta olduğu müfredatı ise Halkalı Ziraat ve Baytar Mektebinde görmeleri kararlaştırılmıştır (14). Veteriner hekimliği öğrencilerinin ilk iki yıl süresince alacakları temel derslerin Sivil Tıp Okulunun derslerine uygun olması sebebiyle öğretim açısından herhangi bir sorun yaşanmazken, Halkalı'da bulunan okula geldiklerinde veteriner hekimliği eğitim-öğretimini devam ettirecek öğretim üyesi ihtiyacı ortaya çıkmıştır. Bu ihtiyacı karşılamak amacıyla Avrupa'dan öğretim üyelerinin getirtilmesinin hem daha masraflı olacağı hem de bu öğretim üyelerinin Türkçeyi bilmemelerinden dolayı tam anlamıyla verimli bir eğitimin yapılamayacağı göz önüne alınarak, Türk öğrencilerin bu amaçla yetiştirilmeleri için

[1] BOA, MF.MKT., Dosya No: 102, Gömlek No: 93, Tarih: 23/S/1306 (29.10.1888); MF.MKT., Dosya No: 107, Gömlek No: 27, Tarih: 09/B/1306 (11.03.1889); İ.HUS., Dosya No: 17, Gömlek No: 020, Tarih: 27/04/1311 (06.11.1893).

yurt dışına gönderilmesine karar verilmiştir. Tıp eğitimi gören kişilerin, veteriner hekimliği eğitimini kolaylıkla tamamlayacağı ve bu suretle "*az masrafla mükemmel hocaların yetişeceği*" düşünülerek Avrupa'ya gönderilecek bu kişilerin Sivil Tıp Okulu'ndan mezun olan hekimler arasından seçilmesi uygun görülmüştür.[2] Bunun üzerine, Dr. Abdülbaki, Dr. Galib, Dr. Emin İzzet ve Dr. Ahmed Abdullah Beylerden oluşan ilk öğrenciler, dört yıl süresince veteriner hekimliği öğrenimi görmek üzere 1889 yılında Fransa'da bulunan Alfort Veteriner Okuluna kaydolmuştur. Ancak Fransa'ya gittiklerinde büyük ölçüde yabancı dil sorunu yaşayan bu öğrencilerin öncelikle Fransızca dersi almaları gerektiği için öğrenim sürelerinin bir yıl daha uzatılmasına karar verilmiştir. Konuyla ilgili olarak Ticaret ve Nafia Nezaretinden yazılan bir belgede sonraki dönemlerde gönderilecek öğrencilerin iyi derecede Fransızca bilenler arasından seçilmesi tavsiye edilmiştir.[3] Bu öğrenciler arasında yer alan Ahmed Abdullah (Şekil 1) ve Abdülbaki Beyler, 1894 yılının Temmuz ayında mezun olmuş, Türkiye'ye döndükten hemen sonra Sivil Veteriner Okulunun öğretim kadrosuna alınmışlardır.[4] Bu okulda Abdülbaki Bey "*fenn-i eşkal-i feres*" (at eşkali); Ahmed Abdullah Bey "*emraz-ı dahiliye*" (iç hastalıklar) ve "*teftiş-i lühum*" (et muayenesi) derslerini vermekle görevlendirilmiştir.[5] Devamsızlık yaptıkları için Alfort Veteriner Okulundan ilişikleri kesilen Galib ve Emin İzzet Beylerin ise öğrenimleri için yapılan tüm masrafların kendilerinden tahsil edilmesine karar verilmiştir.[6] Benzer bir durumun tekrar yaşanmaması için 1894 yılında, Osmanlı Hükümeti Maarif Nezareti tarafından, yurt dışına gönderilen öğrencilerin tabii tutulacağı bir nizamname layihası hazırlanmıştır.[7] Nizamname layihasında yer verilen esaslar şu şekildedir;

Şekil 1. Ahmed Abdullah Bey
Figure 1. Ahmed Abdullah Bey

"*Birinci madde: Bir öğrencinin Avrupa'ya gönderilmesi gerektiğinde, o öğrencinin öğrenim geçmişi bağlı bulunduğu kurum tarafından göz önüne alınacaktır. Bu nedenle, siyasi bilimler ve hukuk için Mülkiye ve Hukuk Mektepleri, tıp bilimleri için Mülkiye Tıp Mektebi, diğer alanlar için devlet tarafından uygun görülen okullardan mezun kişiler seçilecektir.*

İkinci Madde: Bağlı olduğu kurum tarafından seçilecek öğrencilerin bilimsel yeterlikleri ve müsabaka sınavları, Maarif Nezareti tarafından oluşturulan bir komisyonda icra edilecek ve padişah emrinin hazırlanması için ilgili kurum tarafından Bab-ı Âliye ulaştırılacaktır.

Üçüncü Madde: Seçilen öğrencinin öncelikle Osmanlı Devleti tebaasından olması, yaşının 20'den az 26'dan çok olmaması, öğrenim görmeye ve devlete hizmet etmeye engel bir kusurunun olmaması, cinayetten mahkûm ve kötü halinin olmaması, iyi derecede Türkçe, okuyup yazabilecek derecede Fransızca bilmesi şarttır.

Dördüncü Madde: Öğrenim süresi boyunca öğrenciye gerekli harcamalar ve diğer masraflar dâhil olmak üzere 250-300 Fransız Frangı maaş bağlanır, ayrıca okulun bulunduğu yerin uzaklığına bağlı olarak gidiş-dönüş masrafları için uygun bir harcırah öğrencinin ilişikli olduğu kurum bütçesinden karşılanacaktır.

Beşinci Madde: Öğrenci öğrenimi için kabul edilen süre boyunca maaş almaya devam eder, bu süreyi aşması durumunda ise maaşı kesilir. Ancak öğrencilerin dâhil oldukları okulun öğrenim dilini bilmemeleri halinde o dili öğrenmeleri için iki seneyi aşmamak kaydıyla uygun görülen süre boyunca maaşları verilecektir.

Altıncı Madde: Öğrencinin okul içindeki mesaileri okul dışındaki tavır ve hareketleri, Osmanlı elçilikleri ve konsoloslukları tarafından teftiş edilecek, altı ayda bir okul müdürü ve hocaları tarafından yazılan bir rapor öğrencinin bağlı bulunduğu kuruma gönderilecektir.

Yedinci Madde: Öğrencilerin yurt dışında memur oldukları okula gitmeyip, boşladıkları, kaydolduktan sonra okuldan kaçmaları, bulundukları okulun kuralları gereğince okuldan uzaklaştırılmaları halinde, kötü ahlakları ve bağlılıklarına aykırı hareketler göstermelerinden dolayı iadeleri gerektiğinde, Avrupa'ya gönderilme amaçlarının dışında davrandığında ve sekizinci maddede bildirilen şartlara uymamaları

[2] BOA, İ.DH., Dosya No: 1146, Gömlek No: 89334, Tarih: 15/Za/1306 (13.07.1889)
[3] BOA, İ.TNF., Dosya No: 1, Gömlek No: 32, Tarih: 04/B/1310 (22.01.1893)
[4] BOA, İ.OM., Dosya No: 1, Gömlek No: 27, Tarih: 17/L/1311 (23.04.1894); İ.OM., Dosya No:2, Gömlek No: 18, Tarih: 17/R/1312 (17.10.1894)
[5] Salname-i Devlet-i Âliye-i Osmaniye, 1312, İstanbul Matbaa-i Amire.
[6] BOA, BEO., Dosya No: 204, Gömlek No: 15292, Tarih: 06/Za/1310 (22.05.1893); MF.MKT., Dosya No: 192, Gömlek No: 79, Tarih: 07/B/1311 (14.01.1894)
[7] BOA, MF.MKT., Dosya No: 206, Gömlek No: 45, Tarih: 06/Za/1311 (11.05.1894); ŞD., Dosya No: 213, Gömlek No: 17, Tarih: 21/M/1312 (25.07.1894).

durumunda kendilerine yapılan masrafın ödenmesi için yola çıkmalarından ve harcırahlarının verilmesinden önce her öğrenci için bir kefil alınacaktır.

Sekizinci Madde: Öğrenim süresini tamamlayan öğrenciler gerekmedikçe bulundukları yerde kalmayarak geri dönecek ve 10 seneden az olmamak üzere devlet tarafından teklif edilen hizmeti kabul edecektir. Karşı gelmeleri halinde yedinci madde gereğince masrafların tamamı kefillerinden alınacaktır.

Dokuzuncu Madde: Öğrencilerin bağlı bulunduğu kurumların nazır ve başkanları bu nizamnamenin uygulanması ile görevlendirilir."

Diğer taraftan 1890 yılında yurt dışına gönderilen öğrencilerin tamamı Askeri Veteriner Okulu öğrencileri arasından seçilmiştir. Bu amaçla söz konusu Okulun birinci ve ikinci sınıf öğrencileri arasında açılan sınavı kazanan Âdil Mustafa, Mehmet Nuri (Ural), Hayrettin Arif (Şekil 2-4) ve Ahmet Beyler beş yıl süreyle yine Alfort Veteriner Okuluna gönderilmiştir (10, 19). Erk ve Dinçer (10), Ahmet Bey'in Fransa'da ölmesi üzerine yerine İsmail Hakkı (Çelebi) Bey'in (Şekil 5) gönderildiğini bildirmiştir. Adı geçen öğrencilerin 20 Ekim 1892 tarihinde kaleme aldıkları bir mektup (Şekil 6) ile Avrupa'daki çalışmaları hakkında padişaha bilgi verdikleri, teşekkür ve minnetlerini ifade ettikleri tespit edilmiştir.[8] Bununla birlikte, öğrenimlerine başarıyla devam eden bu kişilerin 1894 ve 1895 yıllarında padişah tarafından taltif edildiği, Âdil Mustafa, Hayrettin Arif ve Mehmet Nuri (Ural) Beylere yüzbaşılık rütbesinin, İsmail Hakkı (Çelebi) Bey'e ise üsteğmenlik rütbesinin verildiği belirlenmiş,[9] Âdil Mustafa Bey ve Mehmet Nuri (Ural) Bey'in okuldaki çalışmalarından dolayı ayrıca Fransa Hükümeti tarafından gümüş madalya ile ödüllendirildiği bildirilmiştir (2, 18). Öğrenimlerini tamamladıktan sonra 1895 yılında yurda dönen bu veteriner hekimler Askeri Veteriner Okulunun öğretim kadrosuna alınmış, Mehmet Nuri (Ural) Bey "*emraz-ı dahiliye*" (iç hastalıklar) ve "*seririyat*" (klinik), Hayrettin Arif Bey "*emraz-ı hariciye*" (dış hastalıklar) ve "*ameliyat-ı cerrahiye*" (cerrahi ameliyatlar), İsmail Hakkı (Çelebi) Bey zootekni ve "*hayvanat-ı tıbbiye*" (tıbbi zooloji) derslerini vermekle görevlendirilmiş, Âdil Mustafa Bey ise "*emraz-ı sariye*" (bulaşıcı hastalıklar) ve "*teftiş-i lühum*" (et muayenesi) derslerini vererek insan ve hayvan hastalıklarına yönelik aşı ve serum ihtiyacını karşılayan bir araştırma merkezi olan "*Bakteriyolojihane-i Şahane*" kadrosuna girmiştir (2, 10, 11, 21).

Şekil 2. Âdil Mustafa Bey
Figure 2. Âdil Mustafa Bey

Şekil 4. Hayrettin Arif Bey
Figure 4. Hayrettin Arif Bey

Şekil 3. Mehmet Nuri (Ural) Bey
Figure 3. Mehmet Nuri (Ural) Bey

Şekil 5. İsmail Hakkı (Çelebi) Bey
Figure 5. İsmail Hakkı (Çelebi) Bey

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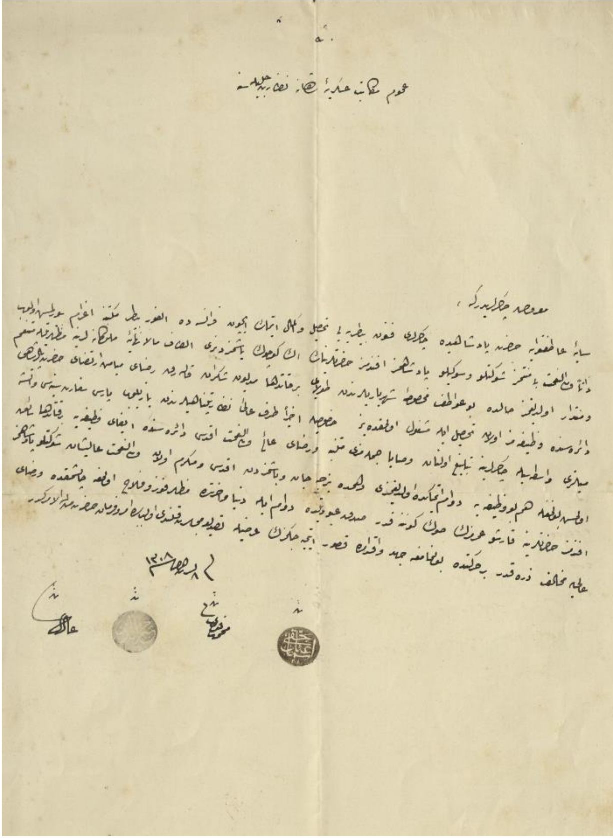
[8] BOA, Y.PRK.ASK., Dosya No: 86, Gömlek No: 58, Tarih: 11/R/1310 (02.11.1892)

[9] BOA, HR.TH., Dosya No: 142, Gömlek No: 38, Tarih: 03.06.1894; HR.TH., Dosya No: 161, Gömlek No: 39, Tarih: 18.08.1895; HR.TH., Dosya No: 166, Gömlek No: 103, Tarih: 07.12.1895



Şekil 6. Avrupa'ya gönderilen askeri veteriner öğrenciler tarafından 20 Ekim 1892 tarihinde Padişah'a yazılan mektup
Figure 6. The letter from military veterinary students sent to Europe to the Sultan on October 20, 1892

Şekil 7. Nikolaki (Mavroğlu) Bey
Figure 7. Nikolaki (Mavroğlu) Bey

On dokuzuncu yüzyılın sonundan itibaren veteriner hekimlik öğrenimi yerine veteriner hekimliğin çeşitli bilim dallarında uzmanlık öğrenimi görmek amacıyla Avrupa'ya veteriner hekimler gönderilmeye başlanmıştır. Bu amaçla, Sivil Veteriner Okulu mezunlarından Halil Vehbi Bey klinik derslerine devam etmek, Nikolaki (Mavroğlu) Bey (Şekil 7) ise Edmond Nocard'la birlikte bulaşıcı hastalıklar laboratuvarında çalışmak üzere 1895 yılında Alfort Veteriner Okuluna kaydolmuştur. Konu ile ilgili olarak ertesi yıl iki veteriner hekimin daha gönderilmesine karar verildiği bildirilmiştir (1).[10] Ancak 1896 yılında Sultan II. Abdülhamit yönetimine muhalif olan ve yurt dışına kaçan öğrencilerin ülkeye giriş çıkışını kontrol altında tutmak için (12), Avrupa'da bulunan Türk öğrencilerin geri dönmeleri kararı çıkarılmıştır. Bu karar üzerine öğrenimlerini yarım bırakarak yurda dönen Vehbi ve Nikolaki Beyler Sivil Veteriner Okulunun öğretim kadrosuna alınmışlardır.[11]

II. Meşrutiyetin ilanından sonra Sultan II. Abdülhamit döneminde başlatılan yasaklar kaldırılmış, Avrupa'ya öğrenci gönderme uygulaması tekrar hükümetin gündemine girmiştir. Meşrutiyetin öngördüğü temelleri atabilmek için öğretim kadrosundaki iyileştirme çabalarına öncelik verilmiş, bu amaçla ilgili bakanlıklar tarafından alanlarında uzman kişilerin yetiştirilmesi için Avrupa'ya öğrenci gönderilmesi kararı alınmıştır. Bu dönem, çeşitli gazete yazılarında Osmanlı öğrencilerinin Avrupa'ya gönderilmesini teşvik eden ve bu öğrencilerin ne surette öğrenim göreceğini ele alan yazılara rastlanmıştır.[12]

Maarif Nezareti tarafından Avrupa'ya gönderilecek öğrencilerin taşımaları gereken özellikler ve kabul etmeleri beklenen koşullar, 17 Ağustos 1909 tarihli Tanin Gazetesi'nde genel ve özel şartlar olarak iki başlık altında ilan edilmiştir. Bildirilen genel şartlar arasında "*öğrencilerin 17-25 yaşları arasında olmaları, bulaşıcı hastalığının bulunmadığının tıbbi muayene ile belirlenmesi ve öğrenimlerini etkileyecek herhangi bir bedensel engelinin olmaması, öğrencinin çalışma gayretinin, iyi hal ve davranışlarının mezun olduğu okul tarafından onaylanması ve bu onayın Maarif Nezareti tarafından araştırılması, öğrencinin öğrenimini tamamladıktan sonra İstanbul'a döndüğünde devlet tarafından uygun görülen yerlerde ve koşullarda en az beş yıl süresince istihdam etmeyi kabul etmesi, yapması gereken hizmeti yerine getirmediği takdirde öğrenimde bulunduğu süre boyunca kendisine tahsis edilen maaş ve yol masraflarının geri ödeneceğini bir kefil ile taahhüt etmesi, Almanca, İngilizce veya Fransızca dillerinden en az birine vâkıf olması, öğrenimlerine gerekli özeni göstermedikleri, sınavlarda başarısız oldukları veya kötü hal ve davranışlarda bulundukları tespit edildiğinde yapılan harcamaların geri ödenmesi*" yer almış, özel şartlarda ise seçilecek öğrencilerin mezun oldukları okullar, öğrenim durumları ve öğrenim görecekleri alana göre taşımaları gereken şartlar değerlendirilmiştir.[13]

[10] BOA, BEO., Dosya No: 596, Gömlek No: 44671, Tarih: 13/L/1312 (09.04.1895)
[11] BOA, BEO., Dosya No: 811, Gömlek No: 60820, Tarih: 07/S/1314 (18.07.1896)
[12] "Ne Duruyorsunuz?", İkdam, 7 Ekim 1908, Numara: 5162, s.3; Hüseyin Cahit."Avrupa'ya Talebe İzamı", Tanin Gazetesi, 13 Ağustos 1909, Numara: 340, s.1; "Avrupa'ya Talebe İzamı", Tanin, 17 Ağustos 1909, Numara:344, s.4; Süleyman Nazif, "Avrupa'ya Talebe İ'zâmı", Tasvir-i Efkâr, 22 Ağustos 1909, Numara: 83, s.1
[13] "Avrupa'ya talebe i'zamı", Tanin,17 Ağustos 1909, numara:344, s.4.

Şekil 8. Fazlı Faik (Yeğül) Bey
Figure 8. Fazlı Faik (Yeğül) Bey

Şekil 9. Hüseyin Sabri (Okutman) Bey
Figure 9. Hüseyin Sabri (Okutman) Bey

Şekil 10. Samoel (Aysoy) Bey
Figure 10. Samoel (Aysoy) Bey

Tanin Gazetesi'nde 30 Ağustos 1909 tarihinde yayımlanan diğer bir yazıya göre çeşitli alanlarda öğrenim görmek için Avrupa'ya gönderilecek öğrencilerin belirlenmesi amacıyla "*kabul*" ve "*müsabaka*" olmak üzere iki imtihanın yapıldığı bildirilmiştir. Kabul imtihanlarında öğrencilerin yabancı dil bilgisi sorgulanırken müsabaka imtihanlarında alanlarıyla ilgili bilgilerinin yeterlik düzeyi tespit edilmiştir. İmtihanların yapılacağı yer, saat bilgileri ile bu imtihanları kazanan öğrencilerin adlarının da yine günlük gazeteler aracılığıyla duyurulduğu belirlenmiştir.[14]

"*Orman, Maadin ve Ziraat Nezareti*"[15] tarafından 1909 yılında 18 öğrencinin Avrupa'ya gönderilmesi kabul edilmesine rağmen, "*Meclis-i Mebusan*"[16] tarafından bu sayının iki katına çıkarıldığı bildirilmiştir.[17] Bu öğrenciler arasında yer alan Fazlı Faik (Yeğül) (Şekil 8), Takfor, Hüseyin Sabri (Okutman) (Şekil 9), Samoel (Aysoy) (Şekil 10), Santor ve Yorgi Beylerden oluşan 6 sivil veteriner hekimin de uzmanlık eğitimi almak üzere yurt dışına gönderilmeleri kabul edilmiştir. Nezaret tarafından hazırlanan program gereğince Fazlı Faik (Yeğül) Bey'in kimya alanında uzmanlaşmak üzere Berlin Üniversitesine; Takfor Bey'in mikrobiyoloji alanında uzmanlaşmak üzere Pasteur Enstitüsüne; Hüseyin Sabri (Okutman), Samoel (Aysoy), Santor ve Yorgi Beylerin ise sırasıyla fizyoloji, iç hastalıklar, dış hastalıklar ve zootekni alanlarında uzmanlık öğrenimi görmek için Alfort Veteriner Okuluna gönderilmeleri kararlaştırılmış, bu kişiler 6 Ekim 1909 tarihinde Fransız Pacquet vapuru ile İstanbul'dan ayrılmışlardır (1).[18] Öngörülen öğretim programı ile ilgili çıkabilecek aksaklıkların giderilmesi ve gerekli yardımlarda bulunmak amacıyla Ziraat Genel Müdürü İstrati Bey'in bizzat kendisinin, önce Berlin'e daha sonra ise Paris'e giderek öğrencileri yerleştirdiği saptanmıştır.[18]

II. Meşrutiyet'in ilanının ardından uzmanlaşma eğitimi almaya hak kazanan veteriner hekimlerden oluşan ikinci grubun Avrupa'ya gönderilmesi yaklaşık bir yıl sonra mümkün olmuştur. Bu amaçla, Sivil Veteriner Okulu mezunlarından Şefik (Kolaylı) Bey (Şekil 11), mikrobiyoloji alanında uzmanlaşmak üzere Pasteur Enstitüsünde Doktor Maurice Nicolle'ün laboratuvarına, Hilmi (Dilgimen) Bey (Şekil 12) anatomi, Salih Zeki (Berker) Bey (Şekil 13) cerrahi, Sadık (Sözeri) Bey zootekni ve Armenak Bey ise patoloji alanında uzmanlık öğrenimi almak için Alfort Veteriner Okuluna kabul edilmiştir (4, 10).[19, 20] Ziraat Genel Müdür Yardımcısı Vahan Surniyan Efendinin ikinci grupta bulunan bu öğrencilerin yerleşmelerini sağlamak üzere görevlendirildiği ve Fransa Hükümetinin bu konuda çok yardımcı olduğu belirlenmiştir.[19] Başağaç ve Özkul (4), Maurice Nicolle'ün laboratuvarında Ekim 1910 – Nisan 1911 tarihleri arasında teorik derslere ve pratik uygulamalara katılan Şefik Kolaylı'nın aynı dönem içinde Hall Centrale Laboratuvarında gıdaların kokuşması konusunda yaklaşık dört ay kadar (1 Ocak – 19 Nisan1911) çalıştığını, 11 Haziran 1911'den 1912 Haziran ayına kadar Lyon

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14 "Avrupa'ya izam olunacak talebe hakkında", Tanin, 30 Ağustos 1909, Numara:357 s.4; "Avrupa'ya izam olunacak talebe hakkında", Tanin, 8 Eylül 1909, Numara:366, s.3.

15 Osmanlı Devleti'nde 1846 yılında kurulan "Ziraat Nezareti" (Tarım Bakanlığı) sonraki yıllarda pek çok kez isim değiştirmiş, 1891ve 1911 yılları arasında "Orman ve Maadin ve Ziraat Nezareti" adını almıştır.

16 Osmanlı Devleti'nde üyeleri halk tarafından seçilmiş olan millet meclisi

17 Hüseyin Cahit. "Avrupa'ya Talebe İzamı", Tanin Gazetesi, 13 Ağustos 1909, Numara:340, s.1

18 "Avrupa'ya giden efendiler", Tanin, 7 Ekim 1909, Numara:395, s.2.

19 "Fransa'da talebe-i Osmaniye", Sabah, 3 Şubat 1911. Numara: 7675, s.2.

20 Dipnot 18'de künyesi belirtilen gazete yazısında bu grupta toplam 6 veteriner hekimin uzmanlık amacıyla Avrupa'ya gönderildiği bildirilmiş ancak 6. kişinin ismi tespit edilememiştir.

Şekil 11. Şefik (Kolaylı) Bey
Figure 11. Şefik (Kolaylı) Bey

Şekil 12. Hilmi (Dilgimen) Bey
Figure 12. Hilmi (Dilgimen) Bey

Şekil 13. Salih Zeki (Berker) Bey
Figure 13. Salih Zeki (Berker) Bey

Veteriner Okulunda Prof. Penisset'in yanında kurs gördüğünü, Temmuz - Ağustos aylarında ise Bükreş'te bulunan veteriner okuluna bağlı Serum ve Aşı Enstitüsünde çalışmalar yaptığını bildirmiştir. Uzmanlığını tamamladıktan sonra yurda dönen Şefik (Kolaylı) Bey Bakteriyolojihane-i Baytari'ye, Sadık (Sözeri) Bey dönemin Tarım Bakanlığı bünyesinde genel müfettişliğe atanmış, diğerleri ise Sivil Veteriner Okulunun öğretim kadrosuna alınmıştır. İzleyen yıllarda Balkan, Birinci Dünya ve Kurtuluş Savaşlarının birbirinin peşi sıra gelmesi nedeniyle eğitim-öğretim faaliyetlerine aralıklarla devam edilmiş, savaş yıllarında yurt dışına öğrenci gönderilmesi kesintiye uğramıştır (4, 12).

Tartışma ve Sonuç

Osmanlı modernleşmesinin bir yapı taşı olarak kabul edilen yurt dışına öğrenci gönderme uygulamasının, II. Mahmut döneminde (1808-1839) topçuluk, mimarlık, mühendislik gibi alanlarda başladığı (6, 9) göz önüne alınırsa veteriner hekimliği alanında daha geç gündeme geldiği görülmektedir. Bununla birlikte, Sivil Veteriner Okulunun kurulması kararının, on dokuzuncu yüzyıl Avrupa'sında gelişen modern bilimin ışığında eğitim gören çağdaş veteriner hekimlerden oluşan bir öğretim kadrosuna duyulan ihtiyacı ortaya çıkardığı söylenebilir. Bu ihtiyacı karşılamak üzere 1889 ve 1890 yıllarında Fransa'ya gönderilen ve Türkiye'ye döndükten sonra sivil ve askeri veteriner okullarının kadrosuna alınan veteriner hekimler ile veteriner hekimliği eğitim-öğretiminde yapılan iyileştirme çalışmalarının genel olarak başarılı sonuçlar verdiği ileri sürülebilir. Aynı yıllarda Sivil Veteriner Okulu için hazırlanan ders programlarında gerek ders sayısı gerekse ders saati bakımından yapılan düzenlemeler (16) bu çalışmaların somut örneklerinden biri olarak kabul edilebilir. Melikoğlu Gölcü ve Erer'e (16) göre, 1896 yılında yayımlanan bir ders programında veteriner hekimliği öğretimi süresince öğrencilere verilmesi kararlaştırılan 39 dersin içeriği ayrıntılı bir şekilde hazırlanmış ve veteriner hekimliği müfredatının ana hatları çizilmiştir. Bu bilgilere uygun olarak Devlet salnamelerinde[21] Sivil Veteriner Okulunun ilk yıllarında sadece 6 öğretim üyesinin görevlendirildiği belirlenmiş, yurtdışına gönderilen veteriner hekimlerin okul kadrosuna alınmalarından sonra bu sayının 22'ye ulaştığı tespit edilmiştir. Ayrıca, Subhi Edhem (19) ile Erk ve Dinçer'in (10), bu kişilerin Türkiye'de modern bilim anlayışı ile ders programlarını ve eğitim şeklini Fransız ekolüne uydurarak veteriner hekimliği öğretiminde köklü değişiklikler yaptıklarını bildirmesi, bu kanıyı desteklemektedir.

Erdoğan (9), Osmanlı okullarının öğretim kadrosu açısından yetkin hale gelmesiyle sadece ihtisas veya staj yapmak üzere öğrencilerin Avrupa'ya gönderildiklerini, ayrıca devlet bütçesi için oldukça ağır bir yük haline gelen yurt dışına öğrenci gönderme uygulamasına zamanla son verilmek istendiği için bu uygulamanın sonradan sınırlandırıldığını bildirmiştir. Benzer şekilde, veteriner hekimliği öğrenimi görmek amacıyla Sivil Tıp Okulundan mezun hekimlerin ve Askeri Veteriner Okulu öğrencilerinin Fransa'ya gönderilmesiyle başlayan bu uygulamanın sonraki yıllarda veteriner hekimliğin çeşitli alanlarında uzmanlık eğitimi almak için veteriner hekimlerin gönderilmesiyle devam ettiği dikkati çekmiştir. Bu nedenle, diğer okullarda olduğu gibi veteriner hekimliğinde de öğretim kadrolarına ait ihtiyacın belli ölçüde giderilmesinin ardından sadece mezuniyet sonrası öğretim programları için yurt dışına öğrenci gönderme uygulamasından yararlanıldığını söylemek yanlış olmayacaktır.

Avrupa'ya gönderilen veteriner hekimliği öğrencilerinin Fransa'ya gönderilmesi Osmanlı Devleti'nin dış ilişkilerine bağlı (8, 9) bir tutum olarak düşünülebilir. Veteriner hekimliği dışında diğer alanlardan gönderilen

[21] Salname-i Devlet-i Âliye-i Osmaniye, 1311 ve 1313

öğrencilerin de öğrenim kaynağının ağırlıklı olarak Fransa olması (8, 9, 20) bunun bir göstergesi olarak kabul edilebilir. Bununla birlikte, Fransa'da ilk veteriner okullarının kurulması (4, 19) ile Louis Pasteur ve ekibinin tüm dünyaya ilan ettiği mikrobiyoloji alanındaki başarılı çalışmalarının da (21) bu seçimde rol oynadığı ileri sürülebilir. Nitekim 13 Temmuz 1889 tarihli arşiv belgesinde[2] yer alan "*Fransa'da Alfort Veteriner Okulunun bilimsel gelişmeler ve keşifler açısından Avrupa'daki diğer veteriner okullarından üstün bulunduğu*" ifadesi bu konudaki düşünceleri gözler önüne sermiştir. Benzer şekilde II. Meşrutiyet'in ilanının ardından bir veteriner hekimin Berlin Üniversitesine gönderilmesi, on dokuzuncu yüzyıl sonundan itibaren Osmanlı Devleti ile Almanya arasında artan yakınlaşmanın (17) bir sonucu olarak değerlendirilebilir. Diğer taraftan, veteriner hekimliği öğrenimi görmek üzere Alfort Veteriner Okuluna kaydedilen Dr. Galib ve Dr. Emin İzzet Beylerin okuldan ilişiklerinin kesilmesi üzerine hazırlanan nizamname layihasının[7] yedinci maddesi ile kefaletname uygulamasının yasal güvence altına alındığı görülmektedir. Devlet bütçesinin zarara uğramasını engellemek amacıyla eklenen bu maddenin yurtdışına öğrenci gönderilmesinde şart koşulan kefil uygulamasının ilk örneklerinden olduğu söylenebilir. Cumhuriyet'in ilanından sonra "*Ecnebi Memleketlere Gönderilecek Talebe Hakkında Kanun*"[22] ile hukuki dayanağına kavuşan bu uygulama günümüzde de devam etmektedir.

Osmanlı Devleti'nin yurt dışına öğrenci göndermesi uygulamasında dil, din, ırk farkının gözetilmemesine ilişkin tutumunun (20) veteriner hekimliği alanında da değişmediği görülmektedir. Bu kişiler arasında yer alan Santor, Yorgi ve Armenak'ın Türkiye'ye döndükten sonra veteriner okullarında istihdam edildiği tespit edilmiştir. Ancak bu kişilerin özellikle Osmanlı Devleti'nin son yıllarında faaliyetlerine ilişkin bir bilgiye rastlanmamıştır. Bu durum, Lozan Barış Anlaşması kapsamında hayata geçirilen ve Türkiye ile Yunanistan arasında gerçekleşen nüfus mübadelesinin (7) bir sonucu olarak değerlendirilebilir. Nitekim, Nikolaki'nin anılarında[23] 1920 yılından itibaren "*harici muallimlerin*" okulla ilişiğinin kesildiğini bildirmesi bu kanıyı güçlendirmektedir. Diğer taraftan öğretim kadrosunda kalanlar ise Cumhuriyet'in ilanıyla yeniden yapılandırılan veteriner hekimliği öğretimi ve örgütlenmesinde önemli hizmetlerde bulunmuşlar, Türkiye'de veteriner hekimliğin akademik alanlarının gelişimine temel oluşturmuşlardır (3, 4, 5, 10, 18). Bu kişiler arasında yer alan Âdil Mustafa, Maurice Nicolle ile yaptığı çalışmalar sonucunda Türkiye'de ilk yerli serum olan difteri antitoksin serumunun üretilmesini sağlamış, 1899 yılında sığır vebası etkeninin filtreleri geçen bir virus olduğunu keşfederek klasik literatürlere geçmeyi başarmış (18); Şefik (Kolaylı) ve Nikolaki (Mavroğlu) Beyler, dönemin tek hayvan hastalıkları araştırma merkezi olarak faaliyet gösteren Bakteriyolojihane-i Baytari'de pek çok çalışmaya imza atmış, savaşlar sırasında özellikle hayvan sağlığı ve hayvansal kaynaklı gıdaların tedariki ile ilgili önemli hizmetler vermiş (3, 10, 21); Fazlı Faik (Yeğül), Samoel (Aysoy), Salih Zeki (Berker) ve Hilmi (Dilgimen) Beyler Türk veteriner hekimliği tarihinde ordinaryüs profesörlük unvanı almaya hak kazanan 6 kişi arasında yer almış (13), İsmail Hakkı Bey ise veteriner okulları ve Tıp Fakültesindeki çalışmaları ile Türkiye'de modern parazitolojinin kurucularından biri olarak kabul edilmiştir (5).[24]

Sonuç olarak gerek veteriner hekimliği öğrenimi gerekse veteriner hekimliğin çeşitli alanlarında uzmanlık eğitimi için Avrupa'ya gönderilen bu kişilerin, Türkiye'de veteriner hekimliğin kurumsallaşması ve öğretiminde büyük rol oynadığı ve Türk veteriner hekimliğin gelişimine yön verdiği söylenebilir.

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[24] Fazlı Faik (Yeğül), Samoel (Aysoy), Salih Zeki (Berker) ve Hilmi (Dilgimen) Beyler veteriner hekimliği alanında gerçekleştirdiği çalışmaları ile 1944 yılında; İsmail Hakkı (Çelebi) Bey ise 1909 yılında görev almaya başladığı Tıp Fakültesindeki çalışmalarından dolayı 1933 yılında ordinaryüs profesörlüğe hak kazanmıştır

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Organisational analysis of agricultural development cooperatives engaged in social support projects in rural areas for livestock production purposes

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Summary: The present study evaluates the extent to which the livestock production subsidies extended to Agricultural Development Cooperatives, whose members were, by means of Social Assistance and Solidarity Foundations, found to be in economic deprivation in rural areas, are implemented successfully in social and economic terms, and analyses the organisational structures of the cooperatives that benefit from the subsidies. The material of the study consists of the qualitative and quantitative data of eight agricultural development cooperatives with a total of 612 members that benefit from the livestock production subsidies under the Project for Social Support in Rural Areas (KASDEP) in the province of Elazığ. A SWOT analysis was conducted to identify the cooperatives' capability to survive under the current production and market conditions. 2.135.554,07 US$ of a total subsidy amount of 3.876.851,75 US$ extended to Elazığ Agricultural Development Subsidies between 2004 and 2007 was repaid according to the data for 2015, which means a collection rate of 55.08%, and the remaining 44.92% is expected to be repaid through debt restructuring. The number of families that benefit from the subsidies as members of the cooperatives and continue production is 142, constituting 23.20% of the total number of members.

Keywords: Agricultural development cooperative, livestock production, rural poverty, rural support project, SWOT analysis

Hayvansal üretim amacıyla kırsal alanda sosyal destek projesi yürüten tarımsal kalkınma kooperatiflerinin örgütsel analizi

Özet: Bu çalışmada, üyeleri kırsal alanda ekonomik yoksunluk içinde oldukları Sosyal Yardımlaşma ve Dayanışma Vakıfları aracılığıyla belirlenen, Tarımsal Kalkınma Kooperatiflerine sağlanan hayvansal üretim desteklerinin uygulama başarılarının sosyal ve ekonomik açıdan ne düzeyde olduğu değerlendirilerek, destekleri kullanan kooperatiflerin örgütsel yapıları analiz edilmiştir. Çalışmanın materyali, Elazığ ilinde Kırsal Alanda Sosyal Destek Projesi (KASDEP) kapsamında hayvansal üretim desteği kullanan 612 üyeli sekiz Tarımsal kalkınma kooperatifinin nitel ve nicel verilerinden oluşmuştur. Kooperatiflerin mevcut üretim ve pazar koşullarında varlıklarını sürdürebilme yeteneklerini belirlemek amacıyla SWOT analizi yapılmıştır. Elde edilen verilere göre 2004-2007 yılları arasında Elazığ Tarımsal Kalkınma kooperatiflerinin kullandığı toplam 5.798.801 TL desteğin (3.876.851,75 US$) 3.194.255 TL'si (2.135.554,07 US$) 2015 yıl sonu verilerine göre ödenmiş olup, geri dönüşüm %55.08 oranında gerçekleşmekle birlikte geri kalan %44.92'lik kısmın yeniden yapılandırılan alacaklarla geri dönüşümü beklenmektedir. Kooperatiflerin üyesi olarak desteklerden yararlanan ve üretime devam eden aile sayısı 142 olup, üye varlığının %23.20'sini oluşturmaktadır.

Anahtar sözcükler: Hayvansal üretim, kırsal destek proje, kırsal yoksulluk, SWOT analizi, tarımsal kalkınma kooperatifi

Introduction

The social aspects and threatening widespread effects of poverty urge public authorities and non-governmental organizations to address the fight against poverty as a primary objective. While its scope and content have undergone major changes, poverty reduction still remains to be the common problem of humanity (1). Countries implement programmes to fight poverty in rural areas with their policies and existing resources and attempt to ensure the development of the segments of their population that face the risk of poverty.

The main purpose of rural development strategies is to fight underdevelopment and ensure the agricultural, economic and social improvement of backward rural communities by helping them make more rational use of existing resources (24). Efforts have been made to close the social and economic gap between urban and rural areas by means of numerous models aimed at ensuring the development of rural areas, such as urban villages, central villages, rural area projects, and attractive village projects (6).

One of the poverty reduction policies of Turkey is the project support. The Project for Social Support in Rural Areas (KASDEP) is intended to raise, through the most suitable organisations, the income of individuals and families living in economic and social deprivation in rural areas, to increase employment and to ensure that the livestock products produced are sold on-site, that the necessary material and technical support for marketing is provided in a timely manner, and that these people are involved in production on a continuous basis. The target group of the project is the citizens living in rural areas, who lack the resources necessary to be engaged in livestock production, wish to earn income from dairy farming and sheep breeding via cooperative organization and fall under the scope of the Law no. 3294 (4, 10).

With this project implemented in Turkey between 2003-2010, rural development cooperatives (with no less than 50 and no more than 120 members) whose members were, by means of Social Assistance and Solidarity Foundations (SYDV), found to be in economic deprivation were granted livestock farming subsidies. In line with these goals, a total of 322.544.630,23 US$ was transferred to 74,062 families in a total of 994 projects under KASDEP in Turkey between 2003-2010 (19). While 29.52% of the population were living in rural areas of Turkey in 2007, this figure dropped to 22.72% in 2012. Although the total population increased by 7.14% between 2007-2012, the rural population fell by 17.56% in the same period. While 27.99% of the population in the province of Elazığ were living in rural areas in 2007, it decreased to 25.54% in 2012 although the provincial population increased by 7.50% between 2007-2012, the rural population in the province decreased by 5.13% (21).

Cooperatives operating in the field of dairy cattle, especially where competition is more intense nowadays, are more needed in rural areas (15). Cooperatives contribute to profitability increases through providing low-cost inputs relative to enterprises (14). Cooperatives, provide information on new production methods, effective organization and personnel management to producers (27), as well as they are also beneficial to producers in adopting and implementing effective production methods (16). Therefore, cooperatives support producers for a higher and more stable income (23).

To date, no studies have been found on the extent to which livestock production subsidies granted to the members of the Agricultural Development Cooperatives through SYDV have been assessed. Therefore, the aim of this study is to analyse the extent to which livestock production subsidies are given to members of the Agricultural Development Cooperatives through SYDV and the organizational structures of the beneficiary cooperatives.

Materials and Methods

The material of this study consists of the qualitative and quantitative data of eight rural development cooperatives with a total of 612 members that benefit from the income-generating livestock production project subsidies under the Project for Social Support in Rural Areas (KASDEP) in the province of Elazığ. The data were obtained through face-to-face interviews conducted with the officials of the Social Assistance Foundation and presidents of the cooperatives operating in the sub-provinces of Baskil and Arıcak in Elazığ as well as with the president of the higher association to which the cooperatives were subordinate. In-depth interview technique, a qualitative research method, was employed in these interviews. The purpose of the research for the participants was explained and written informed consent was obtained from those who agreed to participate in the survey.

The cooperative presidents were asked what the procurement, production and marketing conditions they faced and the economic outputs they could obtain were, and a SWOT analysis was conducted in relation to the current status and future expectations of the cooperatives (5). This analysis helped to make an assessment of the internal aspects of the cooperatives and reveal their strong and weak aspects. Then, an external factors analysis was carried out to identify the position of the organizations relative to the rival companies as well as the opportunities and threats in the market.

Results

The below tables show the place of establishment, area of operation, scale of production of the cooperatives that borrowed livestock production loans under KASDEP in Elazığ as well as the breeds used in livestock production and the number of families that received subsidies (Table 1); details of the means of production acquired with the loans granted under KASDEP to the agricultural development cooperatives for livestock production purposes (Table 2); the year of allocation of the loans, loan amounts by years, amounts of the loans that have been repaid and the repayment percentages (Table 3); and the number of families that benefited from the loan subsidies and continue production within agricultural development cooperatives (Table 4).

The area of activity of the cooperatives is dairy farming. The total number of their members is 612, and the initial scale of production is 1,224 head. Since members receiving project subsidies are required to have a stable with a capacity of 10 head, the total capacity of the enterprises registered in the agricultural development cooperatives in Elazığ is as high as 6,120 head and only 10% of this capacity can be used actively.

Table 1. Place of the establishment, the area of activity, livestock assets, livestock breeds of the cooperatives under KASDEP in Elazığ and number of beneficiary families.
Tablo 1. Elazığ ili KASDEP kapsamında kooperatiflerin kuruluş yeri, faaliyet konusu, hayvan varlığı, ırkı ve faydalanan aile sayısı.

Place of establishment	Location	Area of activity	Livestock assets	Livestock breeds	Number of beneficiary families
Harmantepe	Town	Dairy cattle	200	Brown Swiss	100
Tadım	Town	Dairy cattle	200	Brown Swiss	100
Kızıluşağı	Baskil District	Dairy cattle	166	Brown Swiss	83
Sarıkamış	Town	Dairy cattle	200	Brown Swiss	100
Arıcak	Arıcak District	Dairy cattle	128	Brown Swiss	64
Karaali	Baskil District	Dairy cattle	108	Simmental	54
Kavaktepe	Town	Dairy cattle	110	Holstein	55
Muratcık	Town	Dairy cattle	112	Simmental	56
Total			1.224		612

Since the scope of activity of the cooperatives specified in Table 1 encompasses multiple villages, only the name of the residential area where their administration building is located is given in the tables.

Table 2. Means of production of the agricultural development cooperatives in Elazığ.
Tablo 2. Elazığ ili tarımsal kalkınma kooperatiflerinin üretim imkânları.

Place of establishment	Cooperative buildings (pcs.)	Enterprises capacity (head)	Cooling tank (pcs.)	Transportation vehicles (pcs.)	Dairies
Harmantepe	1	1.000	1	1	1
Tadım	1	1.000	1	-	1
Kızıluşağı	1	830	1	1	-
Sarıkamış	1	1.000	1	-	-
Arıcak	1	640	1	-	-
Karaali	1	540	1	-	-
Kavaktepe	1	550	1	-	-
Muratcık	1	560	1	-	-
Total	8	6.120	8	2	2

Table 3. Amount of loans granted to the agricultural development cooperatives in Elazığ by years, amount of loans repaid, and repayment percentage.
Tablo 3. Elazığ ili tarımsal kalkınma kooperatiflerine yıllara göre aktarılan kredi miktarı, kredilerin geri ödenen miktarı ve geri dönüşüm oranı.

Place of establishment	Year of loan allocation	Year of loan allocation (US$)	Amount of loan repaid (US$)	Repayment percentage (%)
Harmantepe	2004	525.744,94	291.675,75	55.48
Tadım	2004	528.654,52	187.865,62	35.54
Kızıluşağı	2004	477.376,57	421.902,72	88.38
Sarıkamış	2005	585.360,52	317.022,23	54.16
Arıcak	2005	413.256,89	404.625,10	97.91
Karaali	2006	460.795,59	278.188,87	60.37
Kavaktepe	2007	389.951,53	93.598,53	24,00
Muratcık	2007	495.711,18	140.675,25	28.38
Total		3.876.851,75	2.135.554,07	55.08

Table 4. Number of families benefiting from loan subsidies and continuing production within the agricultural development cooperatives in Elazığ.
Tablo 4. Elazığ ili tarımsal kalkınma kooperatiflerinde kredi desteklerinden yararlanan ve üretime devam eden aile sayısı.

Place of establishment	Number of beneficiaries families under KASDEP	Number of families continuing production under KASDEP	Percentage (%)
Harmantepe	100	20	20.00
Tadım	100	27	27.00
Kızıluşağı	83	25	30.12
Sarıkamış	100	15	15.00
Arıcak	64	3	4.69
Karaali	54	14	25.93
Kavaktepe	55	15	27.27
Muratcık	56	23	41.07
Total	612	142	23.20

Table 5. Assessment of the organizational factors internal and external to the agricultural development cooperatives in Elazığ.
Tablo 5. Elazığ ili tarımsal kalkınma kooperatiflerinde örgütsel yapıların iç ve dış durum değerlendirmesi.

Internal factors	
Strengths	Weaknesses
- Presence of the members willing to continue production despite the problems that have been experienced since the establishment of the cooperatives; - Cooperative assets that may be utilized actively; - Livestock production experiences of the members living in rural areas; - Rural labor potential.	- Poor level of confidence among the members; - Strong urge to act individually; - Poor entrepreneurship qualities of the members; - Distributed residential areas of the members; - Reluctance of the members to attain the common goals; - Poor level of productivity of the livestock purchased on credit; - Inadequate meadows and pasture lands; - Inadequate care and feeding conditions due to high feed costs; - Limited means of carrying out irrigated farming to cultivate forage plants; - Poor leadership qualities that ensure the capability to engage in common action; - A strong perception among the members that the government will not take back the social benefits; - The inadequate economic potential of the members.
External factors	
Opportunities	Threats
- Repayment conditions of loan subsidies allowing for no repayment for two years and no interest for six years; - Provision of administration building, stable and feed support prior to commencement of production; - Ease of access to town and city centers; - All cooperatives have milk cooling tanks; - Products can be sold at local markets; - Increased demand for natural products produced by traditional methods and perceived as specific to a region; - Socioeconomic relationship between urban and rural areas; - Repetition of the subsidy policies implemented in rural areas.	- Irrigation canal projects across Elazığ may not be put into practice; - Livestock deaths after procurement of livestock; - Difficulties encountered in repayment of cooperative debts; - Weakness in demand due to insufficiency of milk processing facilities; - Delays and difficulties in insurance payments for livestock losses; - Difficulties in procurement of feed due to seasonal drought; - Seasonal fluctuations in product prices; - Difficulties in being adapted to the market conditions.

The share of Elazığ's agricultural development cooperatives that have received livestock production loan subsidies under KASDEP in the total subsidies is 1.20%. It was found that 55.08% of the loans granted to the cooperatives for dairy farming activities in Elazığ was repaid. The percentage of the families in the cooperatives that previously borrowed loans and currently continue production is 23.20%, while the percentage of those that do not continue production is 76.80%.

The findings of the evaluation of internal and external factors of cooperatives operating under KASDEP in Elazığ are given in Table 5.

Discussion and Conclusion

Today, poverty has become a common problem in the entire world in parallel to globalization. Thus, poverty reduction strategies are included in the agenda of not only underdeveloped or developing countries but also developed ones (2).

Some of the studies conducted to explore the effects of agricultural investments on poverty found that agricultural investments had effects on reduction of poverty (11), that rural development subsidies granted to small producers and enterprises positively affected employment (8), that the growth in the agricultural sector had a higher effect on economic growth and poverty reduction compared to the industrial sector (13), and that the first step in breaking the poverty cycle was increasing agricultural productivity (20).

Being based on a cooperation model that combines and strengthens distributed and irregular means in rural areas, KASDEP aims to ensure that producers that were previously unable to sell their milk collectively in bulk amounts can sell their milk at higher prices to large enterprises under the umbrella of a cooperative, thereby earning regular income and using modern techniques in livestock farming (10).

Some of the comments expressed in some studies exploring the effects of social subsidies and microcredits granted to livestock farmers to reduce rural poverty in Turkey include that the projects have achieved the expected success (9, 12), that they have not succeeded in economic terms despite the social gains (7), and that they have not attained the expected goals in general (17, 25, 1).

When the economic and social impact of producer organizations examined in the animal production, it is obviously seen that, collaboration and organization are the safer ways in terms of sustainability and profitability between producers (3). However, it decreases the risks faced by the cooperative members (26), along with an increase in the marketing, purchasing and bargaining power of small-scale enterprises (22). On the other hand, it was found that cooperative members marketed more milk than non-member enterprises while non-member enterprise administrators reported gaining lower income despite their higher entrepreneurial skills (18).

It was found that in the eight cooperatives established with 612 members under KASDEP, 76.80% of the members had withdrawn from production over time due to various reasons, most notably economic problems as well as reluctance of the members to attain the common goals, individual behaviour, low level of trust among members, and insufficiency of the members' entrepreneurial qualifications, whereas 23.20% of the members were found to be willing to continue production despite all the problems experienced so far. It was also found that 55.08% of the loans allocated to the cooperatives had been repaid and 45% was expected to be repaid through the continuous restructuring of the debts. The major reasons why the borrowers did not repay the loans include the failure of the cooperatives in the establishment, procurement, production, and marketing activities as well as the perception of the members that the government will not ask for repayment of the social benefits and the insufficiency of the members' economic power.

A study conducted in 16 dairy farming cooperatives established under KASDEP in Sanliurfa reports that all of the cooperatives terminated their operations and faced execution and attachment proceedings, and that all cooperative members make a living from state benefits and do not have the cash capital and minimum land required for the care of dairy cattle (17).

In a study exploring the economic and social effects of the subsidies granted for improvement of dairy farming to 14 cooperatives by the Ministry of Agriculture and Rural Affairs and to six cooperatives under KASDEP in Adana between 1990-2006, it is underlined that the number and productivity of the cattle distributed under the project were insufficient, that producers sold their milk at prices below the cost of production due to the high costs of feed, and that the project could not achieve the expected success due to the inefficiency of the cooperatives (25).

A study in which a dairy farming project carried out by a total of 21 cooperatives in Adana between 1990-2006 is assessed from the viewpoint of the cooperatives and their members reports that the project could not achieve the expected economic success due to the insufficiency of the number and productivity of the cattle distributed to the raisers, high costs of feed, sale of milk at a loss by the producers and inefficiency of the cooperatives (7).

In a study exploring the effectiveness of microcredits in Diyarbakir in 2005, it was found that the loans granted had been used to relieve the daily lives of people and get their previous business activities back on track, rather than creating new job opportunities (1).

In this study researching the activities of KASDEP in Elazığ between 2003-2010, carried out in an attempt to increase the level of income of people living in economic and social deprivation in rural areas, the results of the project were found to be unsuccessful.

While 23% of the families benefited from KASDEP and continue production, 77% terminated their operations. There are numerous internal and external factors that cause the failure of the cooperative organizations.

The merger under the umbrella of cooperatives did not arise from the demands of the members, but as a prerequisite to receiving the subsidies. Since the number of participants, which was required to be no less than 50 and no more than 100 members, was not sufficient, members from neighbouring villages were included, and no sufficient communication, coordination, trust and common goals could be established among them. Most of

the members do not know the objectives, activities and legal obligations of the cooperatives, and have the perception that the subsidies granted from the social aid fund are social benefits and they will not have any obligation if they do not repay.

During the cooperatives' process of obtaining live material, serious problems were encountered in accessing sufficient supply of pregnant heifers of desired qualities. They had to settle for what they could obtain, rather than desired qualities. The support necessary for the care and feeding of the livestock could not be provided, and the necessary care and treatment activities could not be carried out due to the members' low level of income. Delays and difficulties were experienced receiving the insurance payments for livestock losses. Inadequate pasture lands and agricultural irrigation problems in the region were factors that restricted the capability to meet the forage needs of the livestock. Only two of the eight cooperatives attempted to carry out production and marketing activities, and the remaining cooperatives that could not survive in the market from the viewpoint of costs, prices, income, and competition terminated their functional activities although they legally continue to exist.

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Bacteriocinogenic bacteria isolated from Civil, Kashar and White cheeses in Erzurum, Turkey

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Summary: This study was carried out in order to identify the bacteriocinogenic lactic acid bacteria (LAB) isolated from civil, kashar and white cheeses produced by traditional methods in Erzurum, Turkey. LAB were isolated from 80 samples of cheese collected from the markets of Erzurum. Antimicrobial activities of the isolates were determined using agar spot and well diffusion methods. LAB that showing antimicrobial activity were characterized phenotypically and genotypically, and the bacteriocin-producing strains were determined. The susceptibilities of bacteriocins to different temperatures, pH and enzymes were tested. While 48.29% of the 381 LAB isolated from cheese samples had antimicrobial activity, only 4.35% of them were determined producing bacteriocin. While 168 of 184 isolates which were showing antimicrobial activity were identified by phenotypical methods at a genus level, and 11 of were at a species level, 135 of 184 isolates were identified by genotypical methods at a genus level, and 26 of were at a species level. *Lb. plantarum* (24.36%) and *Lb. brevis* (23.08%) in lactobacilli, *E. faecium* (38.89%) and *E. durans* (20.37%) in enterococci, and *Lc. lactis subsp. lactis* (100%) in lactococci were identified as the dominant species. All bacteriocin producing exhibited antimicrobial activity against *Micrococcus luteus*. It has been determined that bacteriocin producing *Lc. lactis* subsp. *lactis* and *Lb. pentosus* strains have inhibition impact on *Staphylococcus aureus* and *Listeria monocytogenes.* It has been concluded that the bacteriocin-producing isolates, due to not losing their activities in a wide range of pH and different temperature-time values, could be used as a bio-protective culture in food production and storage.

Keywords: Bacteriocin, Civil cheese, Kashar cheese, VITEK 2, 16S rDNA.

Erzurum ilindeki Civil, Kaşar ve Beyaz peynirlerden izole edilen bakteriyosinogenik bakteriler

Özet: Bu çalışma, Erzurum ilinde geleneksel yöntemlerle üretilen beyaz, civil ve kaşar peynirlerinden izole edilen bakteriyosinogenik laktik asit bakterilerinin (LAB) belirlemesi amacıyla yapıldı. Erzurum piyasasından toplanan 80 peynir örneğinden LAB izole edildi. İzolatların antimikrobiyal aktiviteleri agar spot ve kuyu difüzyon yöntemleri kullanılarak belirlendi. Antimikrobiyel aktivite gösteren LAB fenotipik ve genotipik olarak tanımlandı ve bakteriyosin üreten suşlar tespit edildi. Bakteriyosinlerin farklı sıcaklık, pH ve enzimlere karşı duyarlılıkları test edildi. Peynir örneklerinden izole edilen 381 izolatın %48.29'u antimikrobiyal etkiye sahipken bunlardan yanlızca %4.35'inin bakteriyosin ürettiği tespit edildi. Antimikrobiyal aktivite gösteren 184 izolattan 168 tanesi cins ve 11 tanesi tür düzeyinde fenotipik yöntemlerle tanımlanırken, 184 izolattan 135 tanesi cins ve 26 tanesi tür düzeyinde genotipik yöntemle belirlenmiştir. Laktobasillerde *Lb. plantarum* (%24.36) ve *Lb. brevis* (%23.08), enterokoklarda *E. faecium* (%38.89) ve *E. durans* (%20.37), laktokoklarda ise *Lc. lactis subsp. lactis* (%100) baskın türler olarak tespit edildi. Bakteriyosin üreten izolatların tamamı *M. luteus*'a karşı antimikrobiyel aktivite gösterdi. Bakteriyosin üreten *Lc. lactis subsp. lactis* ve *Lb. pentosus* suşlarının *S. aureus* ve *L. monocytogenes* üzerine inhibe edici etkisinin olduğu belirlendi. Bakteriyosin üreten izolatların geniş bir pH aralığında ve farklı ısı-zaman değerlerinde aktivitelerini kaybetmemelerinden dolayı gıda üretimi ve muhafazasında biyokoruyucu kültür olarak kullanılabileceği sonucuna varıldı.

Anahtar sözcükler: Bakteriyosin, Civil peynir, Kaşar peyniri, VITEK 2, 16S rDNA.

Introduction

LAB are Gram positive, non-spore forming, catalase negative, aerotolerant, acid-tolerant, and strictly fermentative rod or cocci, producing lactic acid as a major catabolic end product from glucose (23). Owing to their widespread existence in nature and playing a role in the production and ripening of some foods, these bacteria are very crucial for food technology. LAB are used to produce new foods by fermentation, provide desired characteristics, and add more durable structure to fermented foods (6, 10, 11). LAB, which are naturally found in many foods or used as a starting culture, exhibit antagonistic activity against saprophytes and pathogen microorganisms as a food preservative. The protective effect of these bacteria is mainly due to the organic acids they produce, which it shows itself by the decrease in pH. Likewise, antimicrobial compounds such as hydrogen peroxide, carbon dioxide, diacetyl, acetaldehyde, D-

isomer amino acids, and bacteriocin, produced during LAB fermentation, also contribute to flavor and aroma development, texture and shelf life of fermented foods (4, 29, 38).

This study is conducted in order i) to isolate the LAB from civil, kashar and white cheeses produced using traditional methods in the province of Erzurum, Turkey, ii) to define antimicrobial activities of the isolates, iii) to identification of the isolates showing antimicrobial activity, and to define whether the antimicrobial substances produced exist in the structure of bacteriocin.

Materials and Methods

Cheese samples and indicator bacteria: In this study, 30 civil cheese, 25 kashar and 25 white cheese samples produced by traditional methods in small-scale dairies and family enterprises in Erzurum, Turkey were used. Indicator bacteria (*Listeria monocytogenes* ATCC 19115, *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC, *Micrococcus luteus* RSK1123, *Enterococcus faecalis* ATCC 29212, *Lactobacillus plantarum* DSM 2601) obtained from Refik Saydam Hygiene Institute Culture Collection were used in determining antimicrobial activities of the isolates obtained from cheese samples.

***Isolation of LAB*:** Man Rogosa Sharpe (MRS-Merck 1.10660) MRS, M17 (Merck 1.15108) and Chromocult Enterococci (CE-Merck 100950) agar medium were used to increase the chance of isolation of LAB strains from the cheese samples. 0.1 mL of the appropriate dilutions were transferred to MRS, M17 and CE agar medium and plated by spread technique. At the end of the incubation, as far as possible morphologically different colonies were selected. Gram positive, catalase, and oxidase-negative, cocci or rod-shaped bacteria were determined as pure cultures for identification purposes (22, 30).

***Determination of antimicrobial activities of isolates*:** Agar spot (1, 31, 37) and agar well diffusion (8, 21, 27) methods were used in determining the antimicrobial activities of the isolates.

Phenotypic identification of isolates: In addition to biochemical and physiological tests at the identification of isolates, VITEK 2 compact system and VITEK 2 GP ID cards were used to identify phenotypically cocci-shaped isolates, and API 50 CHL test kit was used to identify phenotypically basil-shaped isolates.

***Genotypic identification of isolates*:** 16S rDNA regions on isolated genomic DNA; was bred in PCR device using 16S forward (5'-CCG TCA ATT CCT TTG AGT TT -3') and 16S reverse (3'-AGA GTT TGA TCC TGG CTC AG -5') primers (3). For this purpose, 25 µl molecular sterile water, 20 µl PCR master mix (Fermentas K0171), 1 µl 16S forward and 1 µl 16S reverse primer and 3 µl templete DNA were added to give a total volume of 50 µl. PCR protocol; 30 cycles consisting one cycle denaturation (double chain opening) at 94ºC for 30 s, after one cycle of initial denaturation step (pre-denaturation) for 120 s at 94ºC, 60 s at 55ºC primer binding and 90 s elongation steps at 72ºC and lastly one cycle at 72ºC and 10 min at the last elongation stages were composed (15). The amplified 16S rDNA PCR fragments' electrophoresis was made at a gel prepared in 1% agarose and the size of the fragment was calculated using a DNA ladder (Fermantas SM 321) marker of 100 bp. DNA sequence analysis of the PCR products was carried out at the Pendik Veterinary Control and Research Institute using automatic gene sequencing device (Perkin Elmer). 16S rDNA sequence similarity was determined using the National Center for Biotechnology Information (NCBI) BLAST program.

***Determining bacteriocin-producing isolates*:** For the determination of the presence of bacteriocin, 18 h of active cultures of isolates at MRS broth grown at 30°C, were centrifuged at 10,000 rpm for 15 min at 4°C. Neutralized supernatants were added catalase enzyme and then, were incubated for 2 h at 37°C in order to decompose possible hydrogen peroxide. At the end of this period, the enzyme effect was inhibited by standing in a water bath at 60ºC for 10 min (32). Then proteolytic enzyme (proteinase K) was applied to determine whether the substances produced by the isolates were in the protein structure. Samples with no added enzyme were used as controls. The antimicrobial activity of the neutralized supernatants with and without the enzyme was determined using the well diffusion method (35). The disappearance of antimicrobial activity as a result of proteolytic enzyme application showed that this effect originated from a substance having protein nature.

***The effect of enzyme, pH and temperature applications on bacteriocin activity*:** The effect of different pH, enzyme and temperature applications on the stability of bacteriocins produced by isolates in various conditions was examined (17, 35). In susceptibility tests, *M. luteus* was used as an indicator test bacteria.

Results

In this study, 450 isolates were obtained from 80 cheese samples collected from the markets of Erzurum, Turkey. Of these, 381 isolates with Gram positive, catalase and oxidase negative were selected as a probable LAB. From these, 184 (48.29%) isolates were found to have antimicrobial activity on all or a few of the indicator bacteria. Only eight (4.35%) of 184 isolates with antimicrobial activity were found to have an antimicrobial substances in protein structure.

While 168 of 184 isolates showing antimicrobial activity were identified by phenotypical methods at a genus level, and 11 of were at a species level, 135 of 184 isolates were identified with genotypical methods at a genus level, and 26 of were at a species level. As shown in Table 1., 77 of 102 cocci-shaped isolates, identified by phenotypic tests and the VITEK 2 system, and could have been verified genotypically. However, 52 of 77 rop-shaped isolates, identified by phenotypic tests and the API 50 CHL kit, and could have been verified genotypically (Table 2.).

In the white cheese samples *Lc. lactis subsp. lactis, E. faecium, Lb. plantarum* and *Lb. brevis,* in the civil cheese samples *E. faecium* and in the kashar cheese samples *Lb. paracasei* subsp. *paracasei* and *Lb. brevis* were determined as the most common types. While in white and civil cheese the dominant species were *Lactobacillus* spp. and *Enterococcus* spp., in kashar cheese the dominant species were *Lactobacillus* spp.

In this study 3 of strains producing bacteriocin were *Lc. lactis subsp. lactis*, 3 of strains producing bacteriocin were *Lb. pentosus*, others were defined as *Leu. lactis* and *Lb. paracasei subsp. paracasei*. The activity results of 8 bacteriocin producing isolates against indicator test bacteria are given in Table 3.

In Table 4, susceptibility test results of strains producing bacteriocin were given. It was determined that the isolates' bacteriocin activities stayed stable against *M. luteus* at different pH. As a result of different temperature and enzyme applications, it was observed that some of the isolates' bacteriocin activity was stabilized and did not lose their effects, but some of them has lost their effects.

Table 1. 16S rDNA sequence analysis verification of cocci-shaped isolates identified by VITEK 2.
Tablo 1. VITEK 2 ile tanımlanan kokların 16S rDNA dizi analizi ile doğrulanması.

Types of bacteria	VITEK 2	16S rDNA	%
Enterococcus durans	11	11	100
Enterococcus faecalis	7	6	85.71
Enterococcus faecium	21	21	100
Enterococcus lactis	7	4	57.14
Enterococcus spp.	11	8	72.72
Lactococcus lactis subsp. *lactis*	21	16	76.19
Lactococcus raffinolactis	1	0	0
Pediococcus pentosaceus	12	0	0
Leuconostoc mesenteroides	3	3	100
Leuconostoc mesenteroides subsp. *mesenteroides*	3	3	100
Leuconostoc pseudomesenteroides	1	1	100
Leuconostoc lactis	4	4	100
Total	102	77	75.49

Table 2. 16S rDNA sequence analysis verification of rod- shaped isolates Identified with API 50 CHL.
Tablo 2. API 50 CHL ile tanımlanan basillerin 16S rDNA dizi analizi ile doğrulanması.

Types of bacteria	API CH50	16S rDNA	%
Lactobacillus plantarum	16	14	87.50
Lactobacillus plantarum subsp. *plantarum*	3	1	33.33
Lactobacillus brevis	18	18	100
Lactobacillus casei	8	4	50
Lactobacillus paracasei	6	4	66.67
Lactobacillus paracasei subsp. *paracasei*	10	9	90
Lactobacillus curvatus spp. *curvatus*	4	0	0
Lactobacillus pentosus	7	4	57.14
Lactobacillus fermentum	5	0	0
Total	77	54	70.13

Table 3. Bacteriocinogenic effect of isolates against various pathogens.
Tablo 3. Çeşitli patojenlere karşı izolatların bakteriyosinojenik etkisi.

Indicator test bacteria	Bacteriocinogenic isolates							
	Lc. lactis* subsp. *lactis (B10)	***Lc. lactis* subsp. *lactis*** (B11)	***Lb. pentosus*** (C52	***Lb. pentosus*** (C56)	***Lc. lactis* subsp. *lactis*** (B47)	***Leu. lactis*** (K33)	***Lb. pentosus*** (B55)	***Lb. paracasei* subsp. *paracasei*** (C59)
L. monocytogenes	+	+	+	+	-	-	-	-
M. luteus	+	+	+	+	+	+	+	+
B. subtilis	+	+	+	+	+	+	-	-
E. coli O157:H7	+	+	+	+	-	-	-	-
S. aureus	+	+	+	+	+	-	-	-
E. faecalis	+	+	+	+	-	-	+	+
Lb. plantarum	-	-	-	-	-	-	-	-

(+):Bacteriocinogenic effect positive; (-):Bacteriocinogenic effect negative
B10, B11, B47, B55: White cheese isolates; C52, C59: Civil cheese isolates; K33: Kashar isolate

Table 4. The effect of pH, temperature/time and enzyme applications on bacteriocin activity.
Tablo 4. Bakteriyosin aktivitesi üzerine pH, sıcaklık/zaman ve enzimlerin etkisi.

	Lc. lactis* subsp. *lactis (B10)	***Lc. lactis* subsp. *lactis*** (B11)	***Lb. pentosus*** (C52	***Lb. pentosus*** (C56)	***Lc. lactis* subsp. *lactis*** (B47)	***Leu. lactis*** (K33)	***Lb. pentosus*** (B55)	***Lb. paracasei* subsp. *paracasei*** (C59)
pH								
2	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+
9	+	+	+	+	+	+	+	+
11	+	+	+	+	+	+	+	+
Temperature/time								
100ºC/5 min	+	+	+	+	+	+	-	-
100ºC/10 min	+	+	+	+	+	-	-	-
65ºC/30 min	+	+	+	-	+	+	-	+
121ºC/15 min	+	+	+	-	-	-	-	-
4ºC/7 day	+	+	+	+	+	+	+	+
Enzyme								
α-chymotrypsin	-	-	-	-	-	-	-	+
Protease	-	-	-	-	-	+	+	+
Trypsin	-	-	-	-	-	-	+	+
Lipase	-	-	-	-	-	+	+	-
Pepsin	-	-	-	+	+	+	+	+
Catalase	-	-	-	-	-	-	+	-
α-amylase	+	-	-	+	-	+	+	+
Proteinase-K	-	-	-	-	-	-	-	-

(+): Insensitive to; (-): Sensitive to
B10, B11, B47, B55: White cheese isolates; C52, C59: Civil cheese isolates; K33: Kashar isolate

Discussion and Conclusion

The transformation of milk to cheese is very complex and occurs in a dynamic microbial ecosystem. The most important part of this microbial system is constituted by LAB. While 48.29% of the 381 isolates, isolated from cheese samples, has shown to have antimicrobial activity, only 4.35% of that produced bacteriocin. 95.65% of the 184 isolates showing antimicrobial activity were found to have an antimicrobial effect due to low pH and organic acids. There are many studies that indicate that the protective effect of LAB is due to the organic acids they produce (18, 28, 32, 34).

In general, phenotypic methods that allow the identification of LAB at the genus-species level are based on the morphological, physiological, metabolic and biochemical characteristics of the bacteria (24, 25, 33). However, some species of bacteria do not show similar characteristics. Some lactococcal strains are known to tolerate 6.5% NaCl and/or 45°C temperature, as enterococci (12). In this study, it was determined that 3 lactococci isolates showed different characteristics. It was determined that one of them was tolerant to both salt and heat, while the second one tolerant to salt and the last one was tolerant to heat. This indicated that some LAB genus were difficult to distinguish from each other due to their similar phenotypic characteristics. Therefore, it was concluded that phenotypic descriptions based solely on morphological and biochemical characteristics were not reliable by themselves.

The 16S rRNA regions of LAB contain highly conserved sequences. Therefore, sequences of rRNA encoding genes (rDNA) are used to identify the taxonomic group of that organism. All types involved in any bacterial species can be precisely identified by determining sequence analyzes of variable regions in 16S rDNA (24). In this study, only 16 of 20 LAB species determined by phenotypic methods, could have been confirmed by genotypic methods (16S rDNA sequence analysis). It is thought that these differences between phenotypic and genotypic methods may be due to gene changes between strains in the natural microflora of the cheese. Gunay-Esiyok et al. (19) suggest that the formation of moderate prophylactic, linear plasmids and repeat regions that can accommodate chromosomes by horizontal gene transfers among strains found in the natural microflora of food may cause these differences. Molecular methods that give more precise and certain results should be preferred in the studies for the identification of LAB.

In our study, it is determined that 10.87% of the LAB isolated from cheese samples were *Lactococcus* spp., while 44.56% of them were *Lactobacillus* spp., 28.26% *Enterecoccus* spp. and 12.49% *Leuconostoc* spp. According to these results, the dominant flora in cheese samples was LAB not as the starter (*Lactobacillus* spp., *Enterecoccus* spp., *Pediococcus* spp., *Leuconostoc* spp.). These bacteria have also been reported in many studies (5, 7, 36) that affect proteolysis and lipolysis during cheese ripening and contribute to the taste and flavor of the cheese.

It is stated that the enterococci which were known to be very resistant to environmental conditions cause pathogenicity in humans as well as the positive effects for traditional cheeses on maturation and aroma development (20, 26). In our study, the second dominant flora in cheeses was enterococci. *E. faecium, E. durans, E. faecalis* and *E. lactis* are the most abundant species in cheese samples. *E. lactis* isolated from civil cheese samples in our study was also determined in a study by Morandi et al. (26) as a new enterococcus species.

Although the practice of many bacteriocins identified up to now is limited due to their narrow inhibitor spectra, bacteriocins produced by LAB are of interest in food preservation. Only the 8 LAB isolated from cheese samples were found to produce bacteriocin in our study. While all of the antimicrobial substances produced by these strains are effective against *M. luteus*, they did not affect *Lb. plantarum*. On the other hand, it was detected that they had inhibitory effects on some important foodborne pathogens. The inhibitory effect of *Lc. lactis subsp. lactis* and *Lb. pentosus* strains on *S. aureus* and *L. monocytogenes* was determined.

Although the antimicrobial effect of LAB is usually against Gram positive bacteria, we have identified 4 strains that have bactericidal activity against Gram negative bacteria namely *E. coli* O157: H7. Two of them are described as *Lc. lactis* subsp. *lactis* and 2 of them as *Lb. pentosus.* In a study conducted by Caridi (9), the cheese was isoleted *Lb. paracasei* subsp. *paracasei and Lb. curvatus* of which are reported to exhibit antimicrobial activity against *E. coli*.

The main reasons for the use of bacteriocins, produced by LAB, as food preservatives are that they are tolerant to pH and temperature (13). All of the bacteriocin-producing strains isolated from the cheese samples were found to retain antimicrobial activity at pH values between 2 and 11. In addition, many isolates found to maintain their antimicrobial activity in different temperature-time applications, demonstrating that bacteriocins produced by these isolates may maintain their stability, especially at pasteurization and sterilization temperatures.

Consequently, the identification of bacteriocin-producing LAB genus to improve quality and reliable dairy products is of utmost importance for human health as far as the milk industry. In this study 3 of strains producing bacteriocin were *Lc. lactis* subsp. *lactis*, 3 of them were *Lb. pentosus*, and others were defined as *Leu. lactis* and *Lb. paracasei* subsp. *paracasei*. Bacteriocin-producing *Lc. lactis* subsp. *lactis* and *Lb. pentosus* strains

were found to have inhibitory effects on *S. aureus* and *L. monocytogenes*. It has been concluded that the bacteriocin-producing isolates, due to not losing their activities in a wide range of pH and different temperature-time values, can be used as a bio-protective culture in food production and storage. It is concluded that these bacteria and the bacteriocins produced by them could be used in the production of fermented dairy products to prevent the development of pathogen or saprophyte microorganisms and to contribute as a food preservative to the dairy industry.

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Postmortem findings on a group of *Pica pica* (Passeriformes: Corvidae)

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Summary: Common magpies (Corvidae: *Pica pica*) distribute through rural and urban areas of Turkey. Because of their distribution in urbanised regions, magpies may have some potential infectious agents which may relate to domestic animals and humans. In this study, eight common magpies brought to the animal hospital in need of medical intervention were examined for endo-parasites and bacteria in a one-year period. Additionally, histopathologic examinations with related organs were carried out along with endo-parasitical, cytological and microbial examination the following necropsy. As results of the necropsies, three parasite species including two helminths and one protozoan (*Passerilepis sp.*, *Brachylaima sp.*, *Isospora rochalimai*, respectively) were identified, while *Staphylococcus xylosus*, *S. sciuri*, *Escherichia coli, Klebsiella pneumonia, Salmonella spp.* were isolated after microbiological examination. Histopathology revealed that subacute focal mycotic pneumonia, chronic nonpurulent granulomatous gastroenteritis, verminous enteritis, and the presence of paratyphoid nodules in liver. Both of the parasites and bacteria are the first records for Turkey's helminth/bacterial fauna in wild birds.

Keywords: Bacteria, endoparasites, histopathology, magpie, *Pica pica*

Bir grup *Pica pica*'da (Passeriformes: Corvidae) posmortem bulgular

Özet: Saksağan (Corvidae: *Pica pica*), Türkiye'nin kırsal ve şehirleşmiş alanlarında yaygın olarak bulunan bir kuş türüdür. Bu kuşlar şehirleşmiş alanlarda bulunduklarından dolayı, evcil hayvanlar ve insanlarla ilişkili bazı enfeksiyöz etkenleri taşıma potansiyeline sahip olabilmektedir. Bu durumu belirlemek için, bir yıl süresince tıbbi bakıma muhtaç bir halde hayvan hastanesine getirilen sekiz adet saksağan, iç parazitler ve bakteriler yönünden muayene edilmiştir. Yapılan nekropside iç parazitik, sitolojik ve mikrobiyal muayeneyi takiben, ilave olarak ilgili organlarda histopatolojik muayeneler de yapılmıştır. Nekropsiler sonucunda, iki helmint ve bir protozoondan oluşan üç tür parazit (sırasıyla *Passerilepis sp.*, *Brachylaima sp.* ve *Isospora rochalimai*) teşhis edilmiş ve beş tür bakteri izole edilmiştir (*Staphylococcus xylosus*, *S. sciuri*, *Escherichia coli, Klebsiella pneumonia, Salmonella spp.*). Histopatolojide subakut fokal mikotik pnömoni, kronik nonpurulent granulomatöz gastroenteritis, verminöz enteritis ve karaciğerde paratifoid nodüllerin varlığı gözlenmiştir. Belirlenen bu enfeksiyöz etkenler, diğer hayvanlar ve insanlar için potansiyel bir kontaminasyon kaynağı teşkil edebilmektedir. Tespit edilen tüm parazit ve bakteriler, Türkiye'nin yabani kuşlarının helmint ve bakteri faunası için ilk defa bildirilmektedir.

Anahtar sözcükler: Bakteri, histopatoloji, iç parazitler, *Pica pica*, saksağan

Introduction

Common Magpie, *Pica pica*, is a resident breeding bird throughout Europe, much of Asia, northwestern of North America and northwest Africa. They are common in suburban areas and can live towards with human (15).

Magpies are omnivorous, eating young birds and eggs, insects, scraps and carrion, acorns, grain and other vegetable substances. As a result of these dietary habits, it is likely that they are frequently exposed to larval or adult parasites. Several fatal/nonfatal parasitological and microbial infections can sometimes affect wild birds. Coccidiosis is probably one of the most important parasitic diseases of birds both in wild birds, poultry and free-ranging birds. There are numerous species of Isospora for which their entire life cycle is restricted to the intestinal epithelium of their avian hosts and most of the species of Isospora are host specific (13). Approximately 140 species of enteric Isospora have been reported from a wide variety of avian families (10). While most helminths infect wild birds without causing much damage, massive infections can result in reduced performance and increased mortality (18). Helminth infections are generally detected

during the necropsy of wild birds that die of other diseases, illegal hunting or starvation under severe climate conditions.

Microbial infections are common in free-living wild animals and might act as a reservoir and represent a potential risk for human health. Birds of prey were considered to be a natural reservoir of *Staphylococcus aureus* and coagulase-negative staphylococci (21, 30). Methicillin-resistant *Staphylococcus aureus* (MRSA) is getting a considerable challenge in livestock and domestic animal species (1).

This study aimed to determine the parasitic/infectious agents according to post-mortem findings of eight Eurasian magpies.

Materials and Methods

This study was conducted at the Animal Hospital of Uludag University in Bursa, Turkey between May 2015 and May 2016. Bursa is a mountainous province with a surface area of 10,891 km² that is covered with natural forest. This region is also generally quite humid (average humidity of 69%) due to the proximity of the Marmara Sea (2).

Ethical committee approval (No: 2015 – 06 / 03) for applications and investigation (Date: 29-06-2015, No: 138216) on birds has been obtained from Uludag University Local Ethical Committee and Ministry of Forestry and Water Affairs of Turkey, respectively.

All of the birds examined in this study were wounded or sick when they reached the hospital, but they died in spite of the medical interventions in the clinics.

In total, eight dead Eurasian magpies (*Pica pica*) (Figure 3A) were examined for endo-parasites, microbial infections and pathological findings.

Oral cavity, oesophagus, larynx and trachea were examined by making longitudinal incision following gross body inspection. A transverse skin incision across the middle of the abdomen was performed and the thoracoabdominal cavity was opened. The trachea, oesophagus, proventriculus, small intestine, large intestine of each bird were extracted separately for endoparasites. The contents of each organ were sieved through a 100 µm aperture sieve, and the residue was transferred to Petri dishes and examined under a stereo microscope. Additionally, the mucosa of the gastrointestinal organs was examined with a stereomicroscope to determine for the presence of helminths. All of the helminths obtained from the intestines were counted. Cestodes and trematodes were fixed in 70% ethanol, regressively stained with hematoxylin and mounted in balsam for examination. All helminths were identified under a light microscope according to the figures and descriptions presented by Bray *et al.* (5), Gibson *et al.* (11), Schmidt (26), Tolgay (33) and Yamaguti (36, 37). Finally, representative helminth specimens were deposited in the helminth collection of Uludag University Science Faculty, Bursa, Turkey.

Faecal examination detected the presence of eggs or oocysts based on flotation from samples obtained directly from the intestines. Oocyst positive stool samples were allowed to sporulate and were speciated morphologically. To facilitate sporulation, filtrates were mixed with potassium dichromate ($K_2Cr_2O_7$) to a final concentration of 2% and kept at room temperature for a week. To ensure good oxygenation during sporulation, the oocyst suspension was never more than 50 ml, and the containers were agitated daily. Differentiation of species was based on specific morphological features of the sporulated oocyst (size, shape, colour, presence or absence of micropylar cap), the shape of sporocysts and disposition of sporozoites in the sporocysts (3, 35).

Samples of brain, trachea, oesophagus, lung, heart, liver, spleen, proventriculus, ventriculus, kidney, small and large intestine were collected and fixed in 10% phosphate-buffered formalin for pathologic examination. The tissues were embedded in paraffin and they were sectioned at 5 µm thickness and stained with hematoxylin and eosin (H&E) and Grocott's methenamine silver stains. In the gross examination, some magpies had hyperaemic/haemorrhagic intestinal mucosa, touch imprint samples and a smear of intestinal content were prepared from different parts of small intestines and stained with Hemacolor staining kit (Merck) and examined with a light microscope.

The microbiological investigation was designed for the routine diagnosis of the specimens. All freshly collected internal organs (lung, liver, heart and spleen) taken from magpie were pooled together in 50 ml sterile vial within 10 ml of PBS. The internal organs were homogenized with an Ultra-Turrax Micra RT-D9 (ART Prozess & Labortechnik). The pooled suspension was inoculated on to Columbia agar (COS 43041; bioMérieux) with 7 per cent defibrinated sheep blood, Mac Conkey's Agar (CM115; Oxoid) and Levine Eosine Methylene Blue Agar (CM0069B; Oxoid) for routine diagnosis. Subsequently, all samples were inoculated directly on to Sabouraud Dextrose Agar for fungi (CM0041B; Oxoid), incubated at 25°C and 37°C in the dark for a minimum of three weeks, and examined weekly for evidence of fungal growth (22, 23). All samples were also streaked onto *Mycoplasma* agar base (CM0401B; Oxoid) containing *Mycoplasma* supplement G (SR0059C; Oxoid), were incubated for seven days at 37°C in a humidified atmosphere with 5 percent CO_2. Mycoplasma plates were examined after the seventh day of the incubation under 35 × magnifications for the typical 'fried egg' appearance.

An enrichment procedure was implemented according to De Boer *et al.* (7) without second step

enrichment procedure for the detection of *S. aureus*. Swabs were then placed into a Mueller Hinton Broth (Oxoid CM0405) containing 6.5% NaCl (Merck K37303004 -721) individually and incubated aerobically at 37°C for 24 h. A loopful of culture was then streaked onto Baired Parker Agar (Oxoid CM 961) containing 5% Egg Yolk Tellurite Emulsion (Oxoid - SR0054C) and incubated for 48 h in aerobic conditions. Suspected colonies were evaluated by API-Staph® and API-20E® (Biomérieux, Lyon, France) commercial test panels and the results received by API-Web® according to the manufacturer's guideline.

Results

Two helminthic species were identified, including one Cestoda species *Passerilepis* sp. (6 adults) on one bird (12.5%) and one Trematode species *Brachylaima* sp. (5 adults) (Fig.1) on one bird (12.5%) (Table 1). Helminths were free in the intestinal lumen when they were detected during necropsy. Both of these helminths are the first record for Turkey's helminth fauna.

Sporulated oocysts obtained from the sporulation assay measured 20.35 µm × 22.22 µm (ranging from 18.76 — 21.59 µm × 21.69 — 25.24 µm) and a shape index (length/width) of 1.08 (1.03 – 1.16). Coccidial infection was detected on one bird (12.5%) and identified as *Isospora rochalimai* (Fig. 2). This coccidian species is also recorded for the first time in Turkey.

At necropsy, a 2-mm-diameter inflammatory focus located on lung parenchyma was observed in magpie 1. In Magpie 3, dehydration, emaciation, splenomegaly, and hyperemia of the duodenum were observed (Fig. 3B). The pectoral muscle was weak and miliary necrosis foci on the liver were seen in magpie 6. At the cytological exam, many oocysts and coccobacilli were present in intestinal smears from magpie 3 (Fig. 3C). No oocyst was found in smears from magpie 1, 2 and 6. No significant macroscopic findings were observed in magpies 2, 4, 5, 7 and 8. In histopathologic examination, a granuloma is characterized by central necrosis surrounded by degenerate heterophil leucocytes, large number of macrophages and lymphocytes in magpie 1. Some alveoli are filled with heterophils. There are positive staining of fungal hyphae in green background in Grocott's methenamine silver staining of the lung. Furthermore, diffuse mononuclear inflammatory cells were observed in lamina propria of the small intestine in magpie 1. One cestoda (*Passerilepis* sp.) was observed in proventriculus lumen in magpie 2, microscopically. An intestinal granuloma characterising by centrally located necrosis, foreign body giant cells and mononuclear inflammatory cells were present in magpie 2 and 6. There were necrotic villi, numerous, ovoid shaped oocysts and mild mononuclear inflammatory cell infiltrations in propria mucosa of the small intestines of magpie 3 (Fig. 3D). In the liver, multifocal paratyphoid nodules, formed by multifocal hepatocyte necrosis surrounded by degenerate heterophils and macrophages, were observed in magpie 6. Besides, perivascular cuffings with gliosis were detected in the brain.

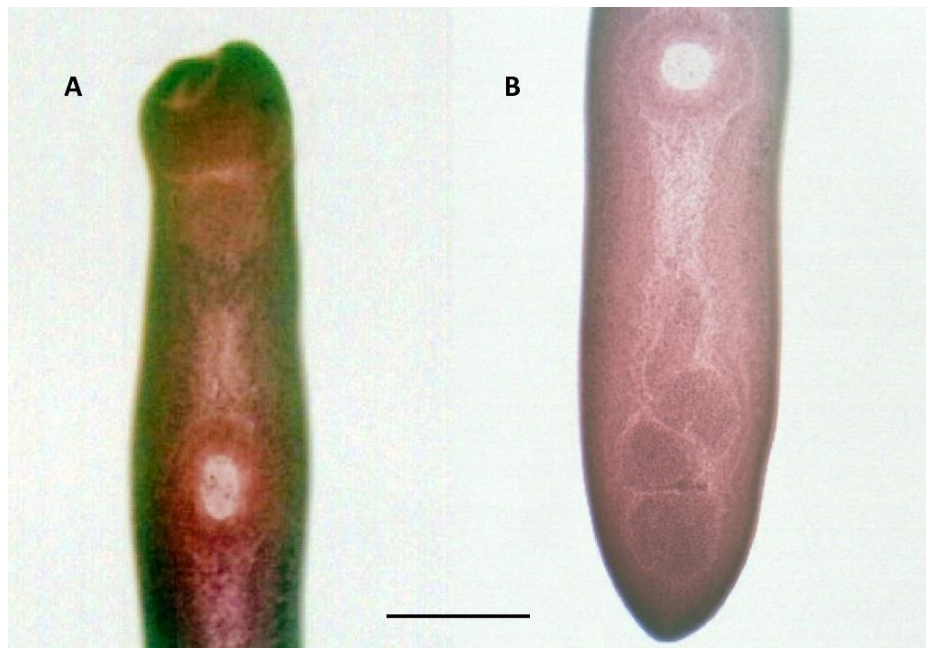

Figure 1. *Brachylaima* sp. collected from magpie 3. A) Anterior part, B) Posterior part. Bar line shows 0.4 mm.
Şekil 1. Saksağan 3'ten toplanan *Brachylaima* sp. A) Anterior kısmı, B) Posterior kısmı. Bar çizgisi 0,4 mm'yi göstermektedir.

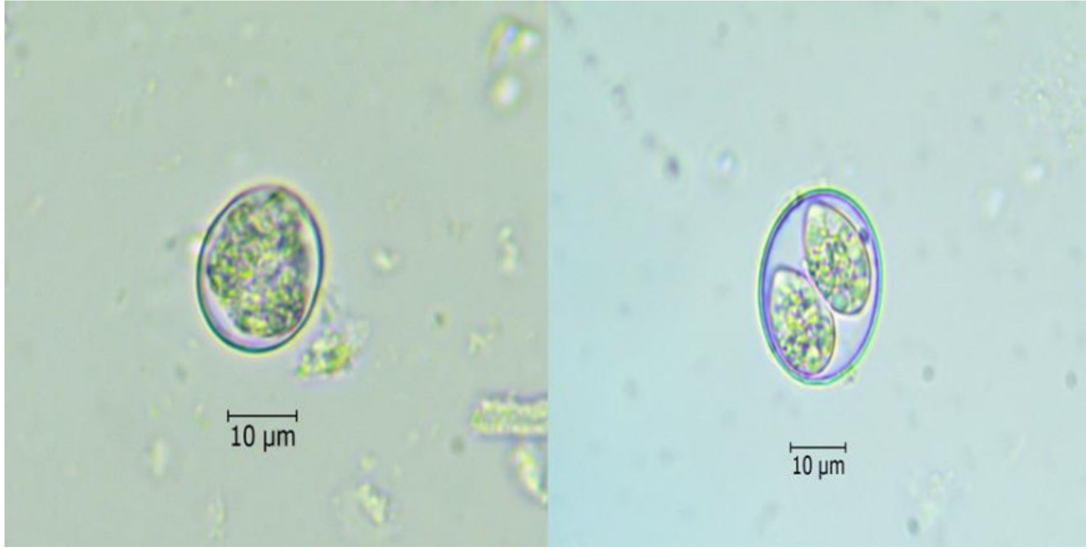

Figure 2. Unsporulated and sporulated (7th day) oocytes of *Isospora rochalimai*
Şekil 2. *Isospora rochalimai*'nin sporsuz ve sporlu (7. Gün) ookistleri

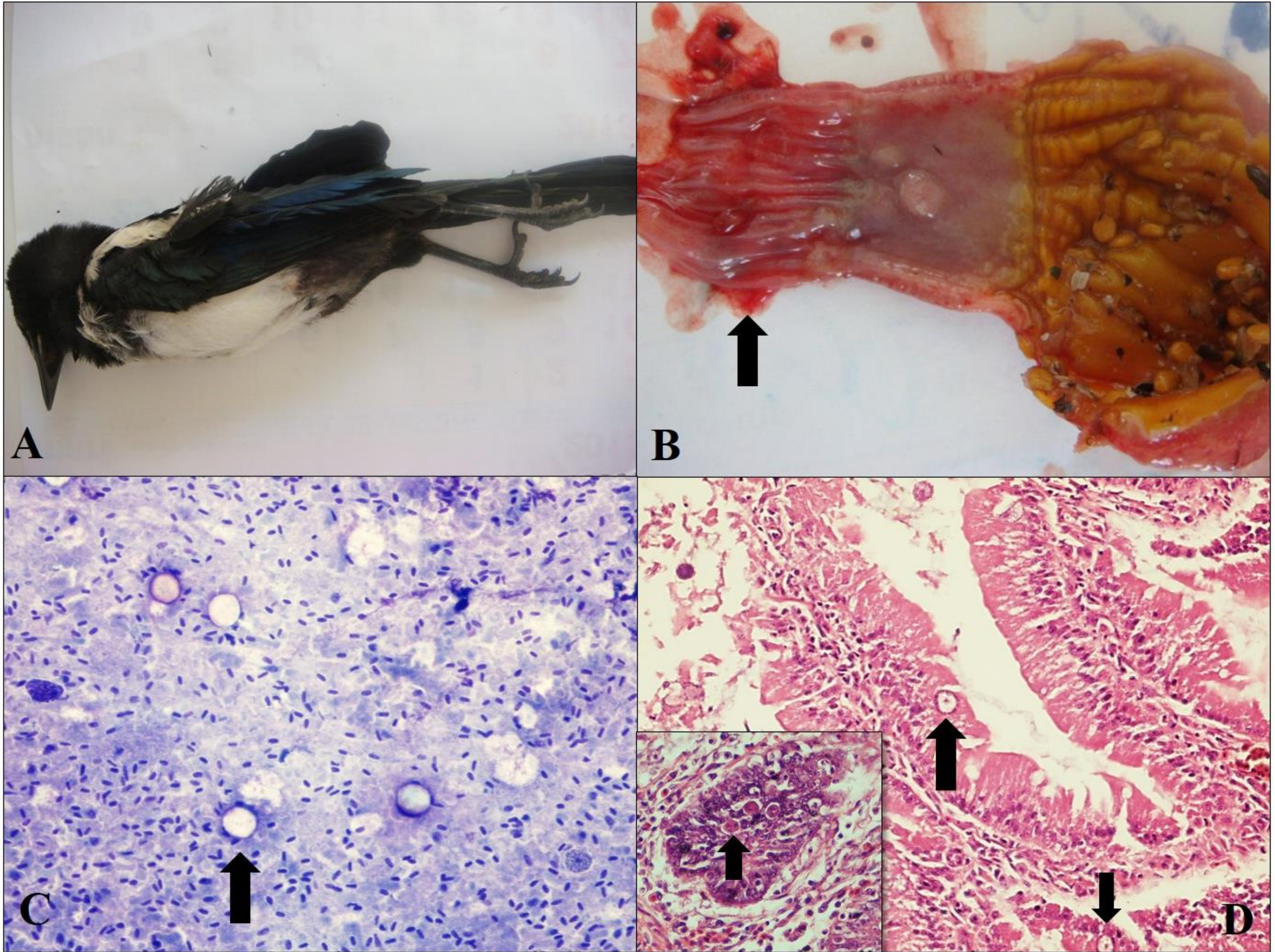

Figure 3. A) External examination of a Magpie (*Pica pica*). B) Macroscopic photograph of the proventriculus-ventriculus and intestinal mucosa. Haemorrhagic intestinal mucosa due to intestinal coccidiosis. Arrow showed duodenum. C) Touch imprint samples prepared from intestinal mucosa, Hemacolor, 10×. Arrow showed oocysts. D) Histopathologic appearance of the small intestine, H&E, 20×. Necrotic villi and numerous oocysts (arrows) are observed in the mucosa of the small intestine of magpie 3.
Şekil 3. A) Saksağanın dıştan muayenesi. B) Proventrikülüs-ventrikülüsün ve bağırsak mukozasının makroskobik fotoğrafı. İntestinal coccidiosis nedenli hemorajik intestinal mukoza. Ok, duodenumu göstermektedir. C) Bağırsak mukozasından hazırlanan tuşe preparat, Hemacolor, 10×. Ok, ookistleri göstermektedir. D) İnce bağırsağın histopatolojik görünümü, H&E, 20×. Nekrotik villi ve çok sayıda ookist (oklar), saksağan 3'ün ince bağırsak mukozasında gözlenmektedir.

Table 1. Bacteriologic, parasitologic and histopathologic findings of sampled magpies
Tablo 1. Örneklenen saksağanlarda bakteriyolojik, parazitolojik ve histopatolojik bulgular

No. of magpies	Bacteriologic	Parasitologic	Histopathologic findings
1	*Klebsiella pneumonia*	-	Subacute focal necrotic mycotic pneumonia and subacute diffuse nonpurulent enteritis
2	*Staphylococcus xylosus, S. sciuri*	*Passerilepis* sp.	Chronic nonpurulent granulomatous gastroenteritis
3	-	*Brachylaima* sp., *Isospora rochalimai*	Subacute nonpurulent verminous enteritis
4	*Escherichia coli*	-	Nonspecific findings
5	*E. coli*	-	Nonspecific findings
6	*Salmonella spp., Staphylococcus (CONs)*	-	Perivasculer cuffings in brain, cocobacilli in proventriculus and lumen of convoluted tubules, subacute multifocal necrotic granulomatous hepatitis (paratyphoid nodules), chronic granulamatous enteritis
7	-	-	Nonspecific findings
8	-	-	Nonspecific findings

The microbiological tests conducted on eight samples showed those eight pathogenic bacteria and no fungi. Bacteriological identification results for targeted material from magpies were shown in Table 1. Coagulase-negative staphylococci (n: 2, 25%) (*Staphylococcus xylosus* and *S. sciuri*), *Escherichia coli* (n: 1, 12.5%) and *Klebsiella pneumonia* (n: 1, 12.5%) were the prominent species while *Salmonella spp.* (n: 1, 12.5%) was frequently isolated from magpies. The bacteriological cluster represented (n: 6, 75%) bacteria out of 5 different bacterial species from 8 magpies. None of the samples represented *Mycoplasma spp*., *Campylobacter spp*. and germs for fungi.

Discussion and Conclusion

Birds of prey may act as a reservoir for various contagious diseases to human such as Staphylococci, Influenza A virus, West Nile and tick-borne infections (20, 27, 34). Even though this study was not conducted to detect specifically diseases that can be transmitted to humans or animals, *Staphylococcus* species and *Isospora rochalimai* determined in this study can be considered in this group.

In the study, two helminth species and one protozoan species were recovered from common magpies, which is a new record for parasitic studies in Turkey. Parasitologic findings resulted in a diagnosis of these species; this is the first record of *Brachylaima* sp., *Passerilepis* sp. and *Isospora rochalimai* in common magpies from Turkey. Passerilepis is a member of Hymenolepididae, cestodes in this family might induce catarrhal enteritis and necrosis of mucosa (32). Authors were thought that the granulomatous reaction with giant cells of the foreign body-type in magpie 2 arose from reaction to *Passerilepis* sp. Against some bacterial agents like *Mycobacterium avium* might develop a few granulomas in the intestines of wild birds. In some reports, tuberculosis and salmonellosis have been shown to be associated with crowding and the contaminated environment in wild birds (31). *Salmonella* sp. can spread to the liver, leading to toxin-induced necrotic hepatitis known as paratyphoid nodules (16, 24, 31). In our study, isolating *Salmonella* and *Staphylococcus spp.* in magpie 6 supports these studies.

All parasite species found in our study have been previously described as parasitising Passerine birds/magpies either on different parts of the world (8, 12, 35) or also from neighbouring countries of Turkey (14, 23, 25). As the parasites of magpies, there is a unique research in Turkey (6). However, researchers have detected different helminth and protozoan species than this study.

The bacteriological composition of the samples showed variability. In this study, coagulase-negative *Staphylococcus* (CONs) was the predominant species detected from clinical specimens. Staphylococci may be transient contaminants, short-term replicating residents, or long-term colonisers of the skin of animals (29). Most that cause of infection does so when the skin or mucous membranes are compromised in some way. Infections by *Staphylococcus* often began at some breach of the epithelial barrier, whether keratinized, mucosal or conjunctival body parts. Virulence of *Staphylococcus* in domestic and wild animals conceived that multifactorial mechanism, such as producing microbial surface components recognizing adhesive matrix molecules (MSCRAMMs).

Klebsiella pneumonia is an important pathogen of companion and wild birds. The organism is a contaminant of bird seeds, fruits and vegetables. Birds are easily colonized and the microorganisms are frequently isolated from the cloaca and choana of clinically normal birds. Respiratory infections, septicemia, and diarrhea are the common disease manifestations in compromised hosts. While, mycotic infections can be accompanied by immunosuppressive birds (28, 29). Although the fungal type in the lung could not be isolated and identificated by mycotic tests, microbiologically isolated *K. pneumonia* in this study might have been caused of nonpurulent enteritis in magpie 1.

Besides *Klebsiella*, *E.coli* infections should be considered as a remarkable Gram-negative pathogen for poultry and wild birds. *E.coli* occurs naturally in the lower part of intestines of human and warm-blooded animals. Commensal *E.coli* strains typically do not cause disease. However, they can be opportunistic pathogens when certain conditions exist. Pathogenic *E.coli* species are broadly grouped into two categories; extraintestinal pathogenic *E.coli* (UPEC, MNEC), and intestinal (STEC, ETEC, EPEC, EIEC, EAEC) / diarrheagenic *E.coli* depending on whether they cause disease outside or within the intestinal tract. Extra pathogenic *E.coli* strains carry a distinct set of virulence genes that enable them to cause disease outside the intestine (16). Avian pathogenic *E. coli* (APEC) caused by extra-intestinal pathogenic *E. coli* strains; is the term used to describe aerosacculitis, polyserositis, septicemia and other mainly extraintestinal diseases in chickens, turkeys and other avian species. APEC is found in the intestinal microflora of healthy birds and most of the diseases associated with them are secondary to environmental and host predisposing factors (9).

A study was conducted in Norway between the periods from 1969 to 2000; *Salmonella spp.* was isolated from 470 wild birds belonging to 26 species. The salmonella-positive birds included 441 small passerines, 15 gulls, five waterfowl, four birds of prey, three doves, and two crows. Serovar Typhimurium O:4,5,12 was identified as the most common variant found in bird species other than small passerines, mainly gulls, and this variant has been responsible for three large outbreaks of human salmonellosis in Norway (24). It has been suggested that serovar Typhimurium has established a reservoir in avian wildlife, and epidemiological and bacteriological evidence indicate that wild birds may transmit the infection to humans (17, 24).

Wild birds have been postulated as sentinels, reservoirs, and potential spreaders of antibiotic resistance through migration can be transmitted from birds to humans and vice versa (4). A research which was conducted between the years 1999-2000; it was found that *Escherichia coli* isolates were resistant to multiple antibiotics eight out of 20 magpies trapped and the most prevalent resistance trait among these isolates was to tetracycline, but resistance to ampicillin, chloramphenicol, kanamycin, sulphonamide, tetracycline and trimethoprim were also found (19). In this study, antimicrobial resistance pattern of bacterial isolates detected from magpies was not mentioned. Molecular characterisation of bacteria was not performed, also.

Coccidiosis from the genus Isospora had been reported previously in various wild birds (3, 13) and different coccidian species had identified in magpies also (35). Necropsy, histopathological and parasitological findings in our study were consistent with coccidiosis. Due to defined findings for magpie 3 which brought to the hospital with non-traumatic symptoms, coccidiosis was thought to be the cause of its death.

As a conclusion, investigation of these magpies resulted in new infectious agents for Turkey's wildlife fauna with related histopathological findings. Further investigations should be performed to detect if the agents have a potential of bird to animal or zoonotic characters.

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The effect of olive leaf extract on digestive enzyme inhibition and insulin production in streptozotocin-induced diabetic rats

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Summary: Olive leaf has natural bioactive compounds, mainly oleuropein, that are widely considered to have potentially beneficial effects on health. This study aimed to evaluate the effects of olive leaf extract (OLE) on the inhibition of carbohydrate digestive enzymes, and immunohistochemical study of insulin in the pancreas of *in vivo* streptozotocin-induced diabetic rats. Blood glucose levels, insulin, glycated hemoglobin (HbA_{1c}), α-amylase and α-glucosidase activities, and an immunohistochemical study were performed at the end of the experiment. In the OLE treated group, blood glucose levels and HbA_{1c} significantly decreased while insulin levels increased. Besides this, OLE treated group showed remarkable inhibitory activities on α-amylase and α-glucosidase compared with the Acarbose treated group. It was observed that OLE exhibited partial positive immunoreaction for insulin in β-cells through immunohistochemical analysis. Considering that OLE is more tolerable for digestion system compared to acarbose, it may be a better fitoformulation for antidiabetic medications. OLE could offer an additional beneficial effect for the treatment of diabetes.
Keywords: α-amylase, α-glucosidase, hypoglycemic, immunohistochemistry, olive leaf.

Streptozotosin ile indüklenmiş diyabetik sıçanlarda zeytin yaprağı ekstraktının sindirim enzimi inhibisyonuna ve insülin üretimine etkisi

Özet: Zeytin yaprağı potansiyel olarak sağlık üzerine yararlı etkileri olduğu düşünülen başlıca oleuropein olmak üzere biyoaktif bileşenlere sahiptir. Bu çalışma, streptozotosin (STZ) ile indüklenmiş diyabetik sıçanlarda zetin yaprağı ekstraktının (OLE) *in vivo* karbonhidrat sindirim enzimleri inhibisyonunun ve pankreasta insülin mevcudiyetinin araştırılmasını amaçlamaktadır. Deney sonunda kan glukoz seviyeleri, insülin seviyeleri, glikozillenmiş hemoglobin (HbA_{1c}), α-amilaz ve α-glukozidaz aktiviteleri analizi ile immunohistokimyasal çalışma yapıldı. OLE tedavi grubunda kan glukoz seviyeleri ve HbA_{1c} anlamlı şekilde azalırken insülin seviyeleri arttı. Bunun yanında OLE, Akarboz grubuna göre dikkat çekici şekilde α-amilaz ve α-glukozidaz aktivitelerinde inhibitör etki gösterdi. Immunohistokimyasal analizde OLE'nin β-hücrelerinde insülin için kısmi pozitif immunoreaksiyon gösterdiği gözlendi. OLE akarboz ile karşılaştırıldığında sindirim sistemi bakımından daha tolere edilebilir olduğu düşünüldüğünde antidiyabetik ilaçlara göre daha iyi bir fitoformülasyon olabilir. OLE, diyabet tedavisi için ek bir faydalı etki sunabilir.
Anahtar sözcükler: α-amilaz, α-glukozidaz, hipoglisemi, immunohistokimya, zeytin yaprağı.

Introduction

Diabetes mellitus is a heterogeneous metabolic syndrome characterized by hyperglycemia caused by a relative or absolute deficiency of insulin (11). Insulin action deficiency, a common case of diabetes, leads to impairment of carbohydrate, lipid and protein metabolism. Furthermore, insulin resistance plays a role in the pathogenesis of hyperglycemia and diabetes in tissues and cells (10). Hyperglycemia developing with impaired fasting glucose and glucose tolerance leads to serious macrovascular and microvascular diabetic complications. In addition, increased reactive oxygen species play a substantial role in the development of diabetes complications (25). Therefore, the control of blood glucose levels in diabetes is very important.

Recently, scientific studies have proven that many phytochemicals are effective both to prevent and treat diseases. The health benefits of phytochemicals depend on the amount consumed and on their bioactivity, for this reason, nutraceutical and therapeutical usage have become popular (20). The pharmacological properties of the fruit of the olive tree and its products have been defined as important components of a healthy diet due to their bioactive phenolic content (21). Nevertheless, olive leaves contain higher amounts of polyphenols than olive oil. For example, oleuropein is the main phenolic compound and the most active phenolic compound in olive leaf (23).

Although, several studies revealed that olives and olive leaf have an antihyperglycemic effect, but there is no information with respect to the inhibition effects of α-

amylase and α-glucosidase and amelioration of β-cells *in vivo*. Therefore, this study aimed to investigate the effect of olive leaf extract (OLE) on α-amylase and α-glucosidase enzymes inhibition and insulin production of β-cells on *in vivo* experimental diabetes.

Materials and Methods

All chemicals and reagents used in the study were analytical grade and obtained from Sigma Inc. (St. Louis, MO).

Olive leaf extract: Olive (*Olea europaea* L.) leaves were collected from Antalya, Turkey, in August 2013. They were then dried and powdered to make the olive leaf extract. The extract was composed of 1 g of powder combined with 50 mL of distilled water. The extraction process was carried out on a magnetic hot plate (Wisd WiseStir MSH-20D) for a period of 12 hours at 80˚C and 750 rpm. Then, it was filtered and placed in a centrifuge (Hettich Universal 320r) for 5 minutes at 4ºC, 3500 rpm in a falcon tube. The final extract obtained was transferred to vials in order to carry out content analysis. The extraction was carried out in duplicate. OLE was evaporated under reduced pressure to administer for study. Furthermore, 1.5 g of leaf sample was infused in 100 ml of water at 80ºC for 5 minutes to create a homemade extract.

Determination of extract content by HPLC: The amounts of oleuropein, hydroxytyrosol, tyrosol and verbascoside in the sample were measured quantitatively against external standards in extracted olive leaves. A Waters 1525 binary HPLC pump and Waters 2487 dual absorbance detector were used for content analysis. The measurement was made by adjusting chromatogram to 280 nm wavelengths for the determination and assignment of polyphenolic compounds.

Animals: Experiments were performed on 40 male rats (*Wistar albino,* 250–350 g and 5–6 months of age) obtained from Experimental Application and Research Center, Yuzuncu Yil University (Turkey). The rats were housed in five groups (n=8) at 20 ± 2°C with 12:12 h reverse light/dark cycle in stainless cages and fed standard chow *ad libitum* and water for 21 days. This investigation was approved by the Yuzuncu Yıl University Animal Researches Local Ethic Committee (no. 2015/08).

Experimental protocol: Blood glucose levels were measured by using a glucometer (Accu Check Nano, Germany) in tail bloods, and monitored periodically every week. Streptozotocin (STZ) was administered at 45 mg/kg intraperitonally (i.p.) (13). Blood glucose levels were measured in tail bloods at 3 days after STZ injection. Rats with glucose levels ≥200 mg/dL were considered diabetic. *Control Group (CG)*: Rats were given a single dose of 1 mL citrate buffer. *Diabetic Group (DG)*: Rats were given a single dose of 45 mg/kg body weight (bw) i.p STZ. *Diabetic+OLE (OLE)*: Diabetic rats were treated with 25 mg/kg bw OLE daily using an intragastric tube. *Diabetic+Infusion (Inf)*: Diabetic rats were treated with 1 mL infusion solution daily using an intragastric tube. *Diabetic+Acarbose (Ac)*: Diabetic rats were treated with 150 mg/kg bw Glucobay (Bayer, Turkey) daily using an intragastric tube.

Infusion and acarbose group designed for comparison of only biochemical analysis and monitoring blood glucose level.

The rats were anesthetized with ketamine+xylazine and then blood and tissue samples were collected at the end of the experiment. The pancreas and intestinal tissues were removed and rinsed with physiological saline.

Biochemical analysis: Insulin levels were measured with electrochemiluminescence immunoassay (ECLIA) method (Architect İ4000SR, Abbott Laboratories Inc.). HbA_{1c} was determined using automatic glycohemoglobin analyzer based on HPLC (ADAMS A1c HA-8180T, Akray Inc.).

Determination of α-amylase and α-glucosidase activities: α-Amylase activity was measured with Alpha-Amylase Assay kit (Abnova) by using the spectrophotometric method (Boeco S-22 UV-Vis) as specified by the supplier. An insoluble dye-coupled substrate amylose azure was cleaved by α-amylase into soluble colored products. The color intensity was measured at 595 nm in the sample.

α-Glucosidase activity was measured with Alpha-Glucosidase Assay kit (Assay bioTech) by using the spectrophotometric method (Biochrom Anthos Zenyth 200) as specified by the supplier. α-Glucosidase reacts with 4-nitrophenyl α-D-glucopyranoside and a yellow complex is formed. This complex was measured at 405 nm.

Immunohistochemistry analysis: Pancreatic tissues were fixed and embedded in paraffin. Immunocytochemical reactions were performed by ABC (avidin biotin complex) (14). Endogenous peroxidase activity was inhibited by 3% H_2O_2 for 30 min at the deparaffinized section and washed with tap water. The section was blocked by incubation with normal goat serum (DAKO, X 0907, Denmark) with PBS, diluted 1:4 to block non-specific binding. They were incubated with monoclonal insulin (18-0066, Zymed, San Francisco, CA), diluted 1/40 overnight and washed with PBS for 30 min. The sections were incubated with biotinylated anti-mouse IgG (DAKO LSAB2 Kit) for 30×2 min and washed with PBS. They were incubated AEC (Aminoethylcarbazole Substrate Kit, Zymed Laboratories) for 10 min and washed with tap water. Counterstaining was performed with hematoxylin and the sections were mounted. The tissue preparations were examined by light microscopy (Leica ICC 50).

Statistical analysis: Data are expressed as mean ($\overline{X}$) and standard deviation (±SD). Significant differences between groups were assessed using one-way analysis of variance followed by Tukey's test and Tamhane's T2. *p* value ≤ 0.05 was accepted as statistically significant.

Results

The amounts of oleuropein, hydroxytyrosol, tyrosol and verbascoside was determined at 15335.55, 461.05, 41.6 and 357.6 µg/g respectively in OLE by HPLC analysis (Figure 1). The values of blood glucose levels (BGL), insulin, HbA_{1c}, α-amylase and α-glucosidase activities are shown in Table 1. Blood glucose levels increased in STZ administered groups. The results are shown in Figure 2A. According to the results, BGL was significantly decreased in the OLE-treated group compared to the diabetic group, but it did not decrease significantly in the infusion group. Glycated hemoglobin (HbA_{1c}) is a form of hemoglobin that was determined 9±0.4 % in the diabetic group. However, OLE and infusion administration resulted in a decrease of HbA_{1c} ($p < 0.05$) as shown in Figure 2B. In this context there was a strong positive correlation between BGL and HbA_{1c} in the groups. Although insulin levels decreased in the diabetic group, it remarkably increased in the OLE-treated group (Figure 2C). Activities of the carbohydrate digestive enzymes, α-amylase and α-glucosidase, are shown in Figure 3A-B. As concerns α-amylase and α-glucosidase activities, the results of the present study showed that both activities significantly decreased in the OLE group compared with the diabetic group. Remarkably, OLE showed a much more effective reduction in α-glucosidase and α-amylase activities compared with acarbose. Infusion administration significantly induced a decrease in α-amylase activity in comparison with the diabetic group, but there was not a significant decrease in α-glucosidase activity. However, it did not display an efficient activity on both enzymes compared with acarbose.

Figure 4 shows the presence of insulin in pancreatic β-cells. Sections were counterstained with immunoperoxidase-hematoxylin. Insulin positive cells were approved by immunostaining for insulin antibodies. Insulin showed normal expression with respect to the control group (As shown Figure 4A). However, a negative reaction was determined for insulin in Langerhans islets (arrows) in the diabetic group (Figure 4B). On the other hand, the OLE-treated group exhibited partial positive immunoreaction (arrows) for insulin in β-cells (Figure 4C).

Table 1. Various parameters of control and STZ-induced diabetic rats.
Tablo 1. Kontrol ve STZ ile indüklenmiş diabetik sıçanların çeşitli parametreleri.

Analysis	CG	DG	OLE	Inf	Ac
Glucose (mg/dL)	112±9	566±37^{a}	444±41^{a,b}	520±15^{a}	511±28^{a}
Insulin (ng/mL)	0.65±0.10	0.22±0.04^{a}	0.43±0.06^{a,b}	0.32±0.04^{a}	0.27±0.03^{a}
HbA_{1c} (%)	4.5±0.2	9±0.4^{a}	7.4±0.2^{a,b}	7.7±0.4^{a,b}	8.1±0.3^{a,b}
α-Amylase (U/L)	1268±135	1950±182^{a}	1063±87^{b}	1534±129^{a,b}	1296±184^{b}
α-Glucosidase (mU/mL)	3166±119	3475±180^{a}	2696±201^{a,b}	3364±104	3132±122^{b}

^{a}: Significantly different from control (*p*<0.05). ^{b}: Significantly different from the DG (*p*<0.05).

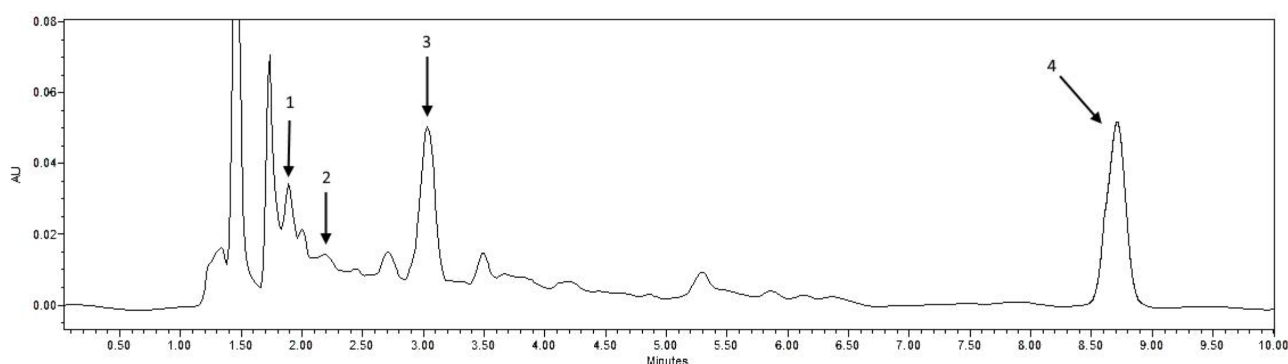

Figure 1. HPLC phenolic profile of OLE at 280 nm. (1) Hydroxytyrosol; (2) Tyrosol; (3) Verbascoside; (4) Oleuropein.
Şekil 1. OLE'nin 280 nm'de HPLC fenolik profili. (1) Hidroksitirozol; (2) Tirozol; (3) Verbaskozit; (4) Oleuropein.

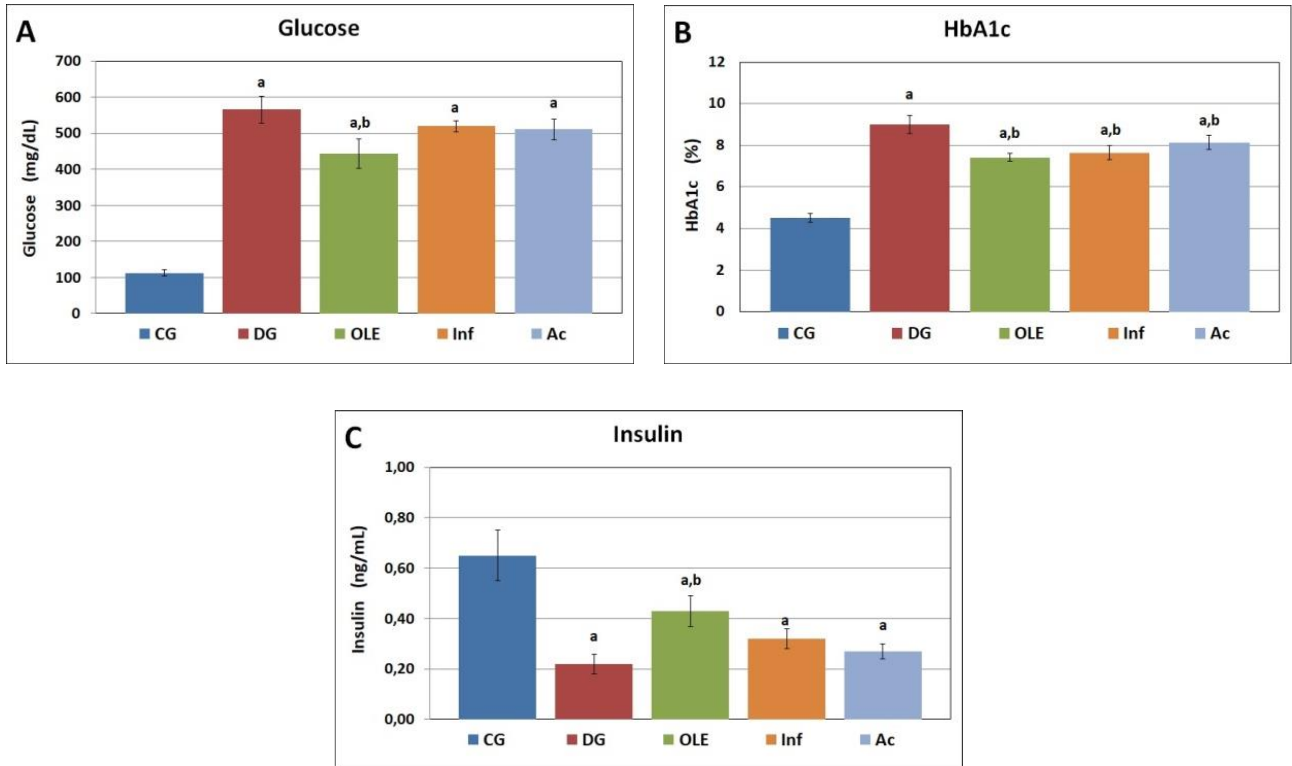

Figure 2. Blood glucose levels (A), HbA_{1c} percentages (B), and Insulin levels (C) of control and STZ-induced diabetic rats.
Şekil 2. Kontrol ve STZ ile indüklenmiş diabetik sıçanların kan glukoz seviyeleri (A), HbA_{1c} yüzdeleri (B) ve İnsulin seviyeleri (C).
[a]: Significantly different from control ($p<0.05$). [b]: Significantly different from the DG ($p<0.05$).

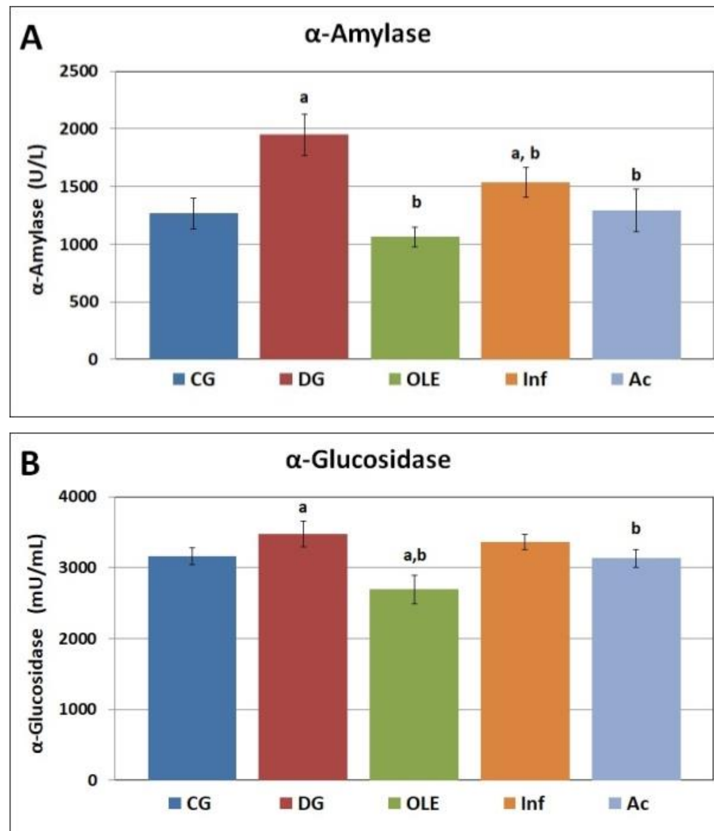

Figure 3. α-Amylase (A) and α-Glucosidase activities (B) of control and STZ-induced diabetic rats.
Şekil 3. Kontrol ve STZ ile indüklenmiş diabetik sıçanların α-Amilaz (A) ve α-Glukozidaz aktiviteleri (B).
[a]: Significantly different from control ($p<0.05$). [b]: Significantly different from the DG ($p<0.05$).

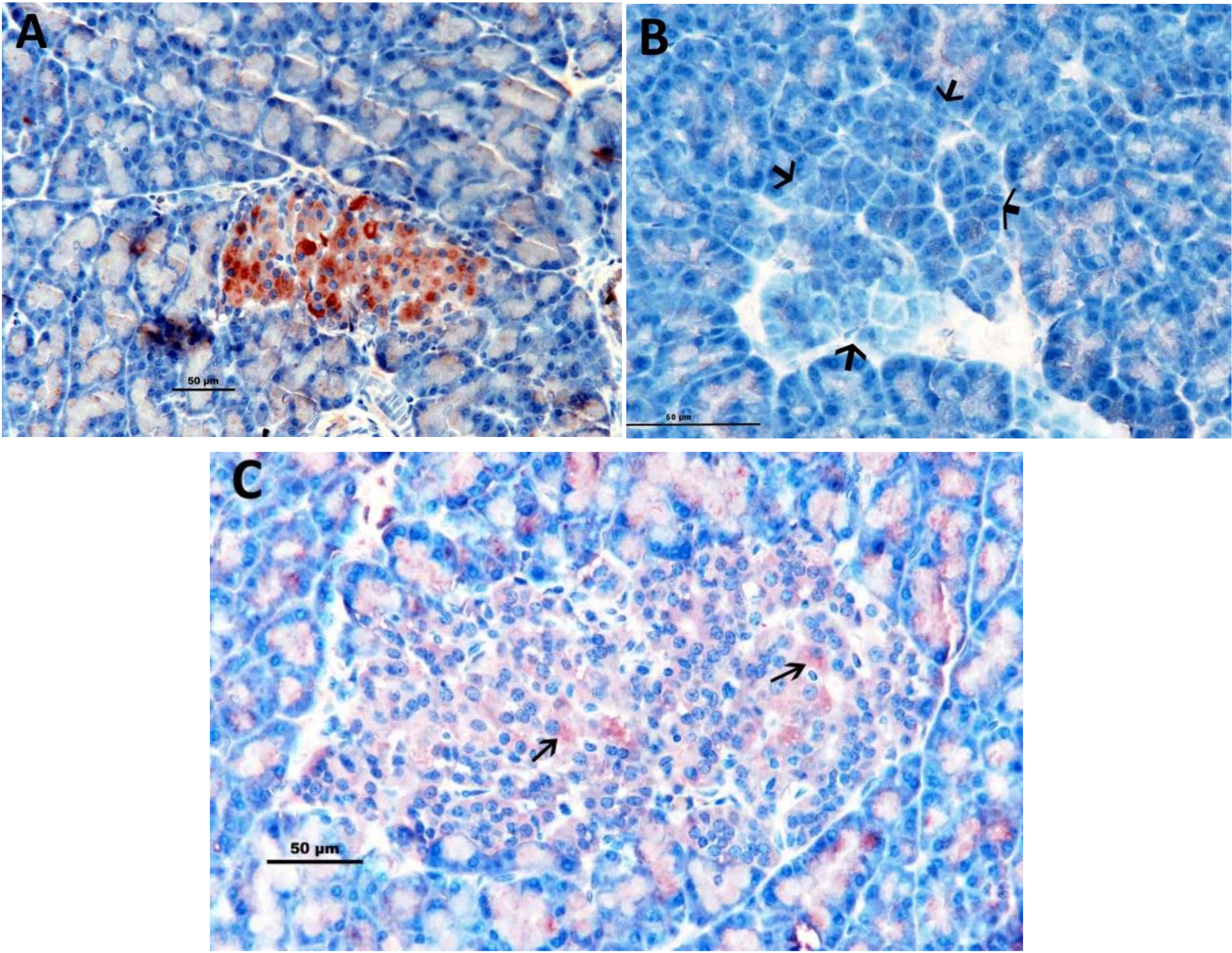

Figure 4. The Control group exhibited positive immunohistochemical reaction of pancreatic β-cells (A); negative immunohistochemical reaction of pancreatic β-cells (arrows) in the Diabetic group (B); partial immunohistochemical reaction of pancreatic β-cells (arrows) in the OLE group (C). Immunoperoxidase-Hematoxylin, Bar=50µm.

Şekil 4. Kontrol grubu pankreatik β-hücrelerinin pozitif immunohistokimyasal reaksiyonu (A); Diabetik grubta pankreatik β-hücrelerinin (oklar) negaitf immunohistokimyasal reaksiyonu (B); OLE grubunda pankreatik β-hücrelerinde (oklar) kısmi immunohistokimyasal reaksiyon (C). İmmunoperoksidaz-Hematoksilen, Bar=50µm.

Discussion and Conclusion

Recently, plant-based treatments have been considered effective for the prevention and control of diabetes because of their specific biological activities and low toxic effects. Due to these characteristic effects, they may be preferred over various antidiabetic medications (5, 22). In the present study, the α-amylase and α-glucosidase inhibitory effects and β-cell ameliorative activity of OLE were investigated on STZ-induced diabetic rats *in vivo* for the first time.

The results showed that a dose of 25 mg/kg OLE was effective in controlling blood glucose level, which decreased by about 20%. Olive leaf was reported to have a hypoglycemic effect on diabetic rabbits, rats, and humans (3, 12, 26) which is in agreement with both current findings and previous studies. In the previous study, pancreatic islet cells isolated from allaxon-induced rats were incubated with crude oleuropeoside (0.2 mg/mL) which purified from olive leaves. As a result, the presence of oleuropeoside in the islet incubation medium together with 2.7 mmol/L glucose (basal) raised insulin levels (9). Bock et al. (4) reported that OLE improves both insulin sensitivity and pancreatic β-cell secretory capacity after oral glucose challenge on the overweight males. The findings of current study are consistent with the aforementioned studies in that OLE may be effective in insulin production. Besides, partial positive immunohistochemical findings in the present study corroborate OLE’s effect on insulin production. On the other hand, treatment of diabetic rats with OLE significantly decreased HbA_{1c}. In a previous investigation, Wainstein et al. reported that HbA_{1c} significantly decreased from 10% to 8.0±1.5% in OLE-treated subjects at the end of the 14 weeks in the randomized clinical trial (26). However, Wainstein et al. (26) did not measure the physical activities and diet type of the participants in their

study, so the independent effect of OLE alone could not be determined. These data confirmed the hypoglycemic and antidiabetic effects of OLE which might be mediated through its α-glucosidase and α-amylase inhibitory activity.

Previous *in vitro* studies have indicated that olive oil, olive mill wastewater, and olive leaf extract were effective at the inhibitory concentration 50% (IC_{50}) against α-glucosidase and α-amylase (6, 16, 18). Furthermore, olive oil exhibited efficient inhibitory activity compared to acarbose because of a richer phenolic content (18). On the basis of these literatures, the results obtained in the present study provided positive support *in vivo*. A positive correlation was observed in the OLE group when compared to α-glucosidase and α-amylase enzyme activities with blood glucose level. It was indicated that the blood glucose levels markedly attenuated while the enzyme activities decreased in OLE group. OLE is considered to be effective to decrease blood glucose level by (i) inhibiting activity of carbohydrate digestive enzymes, α-glycosidase and α-amylase, or (ii) down-regulating gene expressions of these enzymes. Antidiabetic medications cause undesirable symptoms due to undigested starch in the colon (7). Thus, tending toward alternative α-glucosidase inhibitors that are derived from natural sources and nutrients may be more effective, safe, tolerable and cheaper. Although acarbose administration is known to cause bloating and diarrhea, these symptoms were not observed in the OLE treatment in the present study.

Impaired β cells function or structure causes alteration in blood glucose and insulin levels. Based on various immunohistochemical studies conducted on diabetes demonstrated that the density of insulin positive reaction area (24), amount (17) and percentage of β-cells (15) of diabetic subjects were lower compared to non-diabetic subjects. The antidiabetic activities of medicinal plants depend on the degree of β cell destruction and the presence of bioactive contents which show attenuation in the blood glucose level (19). The results obtained in this study revealed that monitored partial positive immunoreaction for insulin in β cells at the OLE group may play a role in the reduction of blood glucose due to oleuropein, which is the most bioactive phenolic. In previous studies, phytochemicals have been reported to be effective with different mechanisms in the regeneration of β-cells (1, 2, 8). For this reason, the results suggested that OLE may regenerate β cells and/or up-regulate insulin expression in intact β cells. In this way OLE may exhibit a hypoglycemic effect.

OLE administration significantly improved glycemic status and showed efficient activity for inhibitions of α-amylase and α-glucosidase. The biochemical and immunohistochemical results revealed that OLE might have a potential agonist and/or antagonist effect for the development of new antidiabetic medications. Considering these effects of OLE, it may be a better alternative phytoformulation in comparison with antidiabetic medications.

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Protective effects of resveratrol on testicular oxidative stress induced of MK-801 in mice

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Summary: Recently, it has been discovered that the doses of the MK-801 causing schizophrenia also initiate the oxidative stress in the testis. The current study investigated the protective role of the resveratrol against the MK-801 induced oxidative stress in the testis in mice. The testis weight, the total oxidant-antioxidant status, seminiferous tubules diameter, epithelial height, testicular pathology, and epididymal sperm motility were evaluated. A total of 24 male mice were equally divided into 4 groups so that each group included 6 mice. In the study, Group I (control group) was intraperitoneally received with 0.9% saline (10ml/kg). Group II was i.p. administered with MK-801 (1mg/kg), Group III was treated with i.p. MK-801 (1mg/kg) and resveratrol (40mg/kg), and the Group IV was treated i.p. with resveratrol (40mg/kg). All injections were performed for 14 days. According to the results, in the testis of mice in Group II the oxidative stress was observed. The oxidative stress affected the seminiferous tubules pathologically and decreased the weight of the testis and sperm motility. However, resveratrol protected the MK-801 induced oxidative stress in the testis. Moreover, this dose of the resveratrol increased the sperm motility compared with the controls. In conclusion, MK-801 caused oxidative stress in the testis and resveratrol had a protective effect against this damage.

Keywords: MK-801, oxidative stress, resveratrol, schizophrenia, testis.

Fare testislerinde Mk-801'le indüklenen oksidatif strese karşı resveratrolün koruyucu etkisi

Özet: Günümüzde MK-801'in şizofreniye sebep olan dozlarının testiste oksidatif strese de neden olduğu ortaya çıkmıştır. Bu çalışma fare testislerinde MK-801 kullanılarak ortaya çıkartılan oksidatif strese karşı resveratrolün koruyucu etkisini araştırmak amacıyla yapılmıştır. Testisler ağırlık, toplam oksidan-antioksidan seviyeleri, seminifer tübüllerin çapları ve epitel dokularının yükseklikleri, patolojik ve epididimal sperm motilitesi yönünden değerlendirilmiştir. Bu amaçla 24 fare alınarak grup başına 6'şar fareden oluşan 4 grup oluşturulmuştur. Grup I, kontrolden oluşmuştur ve bu gruba intraperitoneal yolla %0,9 FTS (10ml/kg) verilmiştir. Grup II'ye i.p. yolla MK-801 (1mg/kg), Grup III'e i.p. yolla MK-801 (1mg/kg) ve resveratrol (40mg/kg), Grup IV'e de i.p. olarak resveratrol (40mg/kg) verilmiştir. Uygulamalar on dört gün boyunca sürmüştür. Bu sürenin sonunda testisler çıkartılmış ve yapılan incelemeler neticesinde testiste ikinci grupta MK-801'in oksidatif strese sebep olduğu saptanmış, testis ağırlığında azalma, sperm motilitesinde düşme ve tübüllerde patolojik değişiklikler olduğu görülmüştür. Resveratrolün ise oksidatif strese karşı testisi koruduğu ortaya konulmuştur. Ayrıca resveratrol grubunda kontrole göre sperm motilitesinin arttığı gözlenmiştir. Sonuç olarak MK-801'in testiste oksidatif strese sebep olduğu ve resveratrolün de koruyucu etkisinin bulunduğu ortaya çıkarılmıştır.

Anahtar sözcükler: MK-801, oksidatif stres, resveratrol, şizofreni, testis.

Introduction

The MK-801 is an N-methyl-D-aspartate (NMDA) receptor antagonist in the glutamatergic category and acts by disrupting the Wnt signaling pathway (9, 26). The hypofunction of the NMDA receptor impairs the glutamatergic system and leads to schizophrenia illness (10, 11, 15, 26). Experimentally, the NMDA receptor could be blocked with certain doses of MK-801 (24, 25, 26). Recently, it has been discovered that the 5 days 0.5 mg/kg intraperitoneal (i.p.) dose of the MK-801 has emerged the oxidative stress in the testis because the glutamatergic system units like transporters and receptors are not only placed in the nervous system (14, 16). They are also placed in the several peripheral body parts like adrenal, pituitary, pineal glands, pancreatic islets, retina, liver, kidney, intestine, heart, lung, skeletal muscle and bone marrow (7, 14, 16). The metabotropic glutamate receptors were also found in the human and rat testis (21, 22). In addition to the human and rat, the mice testis has both glutamate transporters and receptors (7).

The MK-801 impairs the glutamatergic system in the testis and induces the oxidative stress. The oxidative stress is the abundant production of the reactive oxygen species (ROS) in the tissue. Accumulation of the ROS impairs the antioxidant system and may damage and cellular injury occur in the tissue (14, 16). Actually, the body already produces reactive oxygen metabolites. In the testis, during the spermiogenesis, the dead and abnormal spermatozoon production causes reactive oxygen metabolites normally. However, if ROS production exceeds certain limits or the antioxidant system is inadequate, tissue damage may occur due to lipid peroxidation. Because of the highly rich polyunsaturated fatty acid content of testicular membranes, the ROS impairs the membrane lipids with initiating the lipid peroxidation (1). Lipid peroxidation in the sperm cell membrane impairs the midpiece of the sperm cell and thus canceled the capacitation and the acrosome reaction (1,6,13).

Resveratrol is a phytoalexin with an antioxidant feature and especially found abundantly in the peanuts and grapes (2, 5). It could be used as anti-oxidant, anti-inflammatory, anti-cancer, anti-viral, anti-aging, anti-diabetic, and cardio-protectant (2, 5, 12, 18). Recent studies have shown that resveratrol could be used successfully as a testicular protectant against oxidative stress (5, 12, 13, 18). It protects the spermatocytes against the lipid peroxidation which appears in the sperm cell membrane and DNA damage by increasing antioxidant enzyme release (5, 18). The resveratrol may be beneficial in testicular dysfunctions and much effective than melatonin, vitamin E and α-phenyl-N-tert-butyl nitrone (17). Meanwhile, the resveratrol improves sperm quality and motility (13).

In the present study, the possible protective role of the resveratrol was aimed to evaluate against the testicular oxidative stress conducted by the chronic dose of MK-801. The previous researches were detected that the 1mg/kg i.p. injections for 14 days is the chronic dose of the MK-801 in mice (24, 25). In this study, the effects of the oxidative stress in the mice testis aimed to understand by measuring the testicular total oxidant-antioxidant parameters, pathology, seminiferous tubule diameter, epithelial height, and epididymal sperm motility.

Material and Methods

Animals: The current study was carried out on 24 male Balb/C mice housed at the Experimental Animal Research Centre of Afyon Kocatepe University following ethical committee approval (Ethical Committee for Experimental Animals, Afyon Kocatepe University, AKUHADYEK-132-16). All mice were placed in the plexiglass cages with a 12/12h light/dark cycle and fed ad libitum with the commercial food pellets and tap water. The room temperature was adjusted to 20-22 ^{0}C.

Groups and dosages: This study was performed on 24 male mice testis. The mice were divided into 4 groups and each group included 6 mice. The Group I was the control and intraperitoneally (i.p.) received with 0.9% saline (10ml/kg) (due to MK-801 (Sigma, St. Louis, MO, USA) dissolved in the saline). Group II was i.p. administered with MK-801 (1mg/kg). The Group III was treated i.p. with MK-801 (1mg/kg) + resveratrol (purchased from Terraternal Pharmaceutical, London, England) (40mg/kg) and the Group IV were treated i.p. with resveratrol (40mg/kg - resveratrol was dissolved in 0.9% saline). The saline, MK-801 and resveratrol doses in this study were selected based on the previous studies (8, 25). All injections were performed for 14 days. In Group III, resveratrol was injected in the morning and MK-801 was injected in the afternoon. After the injections, all mice were sacrificed by decapitation and all testes were collected. All the right testes were separated for the determination of the oxidative stress parameters and the other testis was separated for the histo-morphometric and pathological evaluations.

Morphometric and pathological evaluation: After the sacrification of the mice, all the left testes were weighted and fixed with the 10% buffered neutral formaldehyde solution, totally embedded in paraffin, sliced and stained by Hematoxylin-eosin for the histo-morphometric and pathological evaluations. The diameter and epithelial height of seminiferous tubules were measured using Mshot software loaded to a computer connected to the Olympus BH2 microscope attached Mshot 14 mp camera (23). The measurements were performed under X20 objective.

Biochemical analyses

Preparation of homogenate: All the right testes were trimmed and the remaining portions (each of 0.1g in weight) were washed with ice-cold 0.9% NaCl and immediately homogenized for oxidative stress analyses. Initially, the tissue was homogenized as follows. Each tissue was separately placed into a homogenizer (IKA T18, Germany), adding 1 mL of the solution containing 10% (w/v) in 0.1M phosphate buffer, pH 7.4, 1mM EDTA and the mixture was homogenized in ice for five minutes with the homogenizer. Then the homogenates were centrifuged (Nüve NF 1000R, Ankara, Turkey) at 3500 rpm/min for 10 min at 4°C. The testis samples prepared for use in the analyses were stored at -80°C, until laboratory analyses.

Measurement of oxidative stress parameters in tissue homogenate: TAS (total antioxidant status) and TOS (total oxidant status) levels which are among the oxidative stress parameters were measured using the kit (Rel Assay, Gaziantep, Turkey) working with the spectrophotometric methods (3, 4). The TAS method is calibrated with a stable antioxidant standard solution,

which is a vitamin E analog and traditionally named as Trolox Equivalent. On the other hand, TOS assay is based on the oxidation of ferrous ion to ferric ion in the presence of various oxidants in acidic medium. TAS and TOS levels were reported as mmol Trolox Equivalent/L and µmol H_2O_2 Equivalent/L, respectively.

Epididymal sperm evaluation: The forward progressive sperm motility percentage was assessed using a phase contrast microscope with a heated stage as described by Sonmez et al. (20). A glass slide was placed on a phase contrast microscope and the stage was warmed up to 37 °C. Then, several droplets of Tris buffer solution [0.3 M Tris (hydroxymethyl) aminomethane, 0.027 M glucose, 0.1 M citric acid] were dropped on the glass slide, and a very small droplet of fluid obtained from left caudal epididymis with a pipette was added to the Tris buffer solution and mixed by a coverslip. The forward progressive sperm motility percentage was evaluated visually under the microscope at 200x and 400x magnifications. The estimation of the sperm motility was performed from three different areas in each sample. The mean of the three successive estimations was accepted as the final motility score.

Statistical analysis: Data obtained from experimental animals were expressed as means and standard deviations and analyzed using one-way analysis of variance (ANOVA) followed by Duncan posthoc test on the SPSS software computer program. A difference in the mean values of $P<0.05$ was considered to be significant.

Results

The mean testis weight, spermatozoon motilities, testicular structures, and total oxidant-antioxidant levels were measured and reported with P values in Table 1 and Table 2. In the present study, in Group II, it was detected that the mean testis weight was significantly decreased compared with the others ($P<0.05$). The resveratrol administration protected the mean testicular weight treated with MK-801 in Group III.

Table 1. Effects of MK-801 (1 mg/kg), resveratrol (RES-40 mg/kg) and RES (40 mg/kg) + MK-801 (1 mg/kg) on mean testis weight, epididymal spermatozoon motility percentage, the diameter of seminiferous tubules and epithelial height of seminiferous tubules.
Tablo 1. MK-801(1 mg/kg), resveratrol (RES-40 mg/kg) ve RES (40 mg/kg) + MK-801 (1 mg/kg) kombinasyonunun ortalama testis ağırlığı, epididimal spermatozon hareketliliği yüzdesi, seminifer tubül çapı ve epitel yüksekliği üzerine etkisi.

Groups	The mean testis weight (g)	Epididymal spermatozoon motility percentage	The diameter of seminiferous tubules (µm)	The epithelial height of seminiferous tubules (µm)
Control	$0.227±0.026^a$	$71.66±4.08^b$	228±11	63±3
MK-801	$0.175±0.43^b$	$61.66±9.83^c$	224±7	61±5
MK-801+Resveratrol	$0.230±0.029^a$	$70.00±6.32^b$	228±10	62±3
Resveratrol	$0.236±0.21^a$	$81.66±4.08^a$	225±8	59±3
P	<0.011	<0.001	0.856	0.340

Values are mean ± S.D, n=6.
^{a,b,c}: In the same column values with different letters show statistically significant differences in mean testis weight and epididymal spermatozoon motility percentage ($P<0.05$)
^{a,b,c}: Aynı sütunda farklı harfleri taşıyan ortalama testis ağırlığı ve epididimal spermatozon hareketliliği yüzdesi ($P<0.05$) istatistiksel açıdan önemlidir

Table 2. Effects of MK-801 (1 mg/kg), resveratrol (RES-40 mg/kg) and RES (40 mg/kg) + MK-801 (1 mg/kg) on total antioxidant status (TAS) and total oxidant status (TOS) of mice. TAS: Total Antioxidant Status, TOS: Total Oxidant Status, g: gram, µm: micrometre
Tablo 2. MK-801(1 mg/kg), resveratrol (RES-40 mg/kg) ve RES (40 mg/kg) + MK-801 (1 mg/kg) kombinasyonunun total antioksidan durum (TAS) ve total oxidan durum (TOS) üzerine etkisi. TAS: Toplam Antioksidan Düzeyi, TOS: Toplam Oksidan Düzeyi, g: gram, µm: micrometre

Groups	Testis TAS (mmol torolox equiv./L) measurement	Testis TOS (µmol H2O2 equiv./L) measurement
Control	$0.46±0.08^b$	$2.66±0.65^b$
MK-801	$0.24±0.06^c$	$5.46±1.44^a$
MK-801+Resveratrol	$0.61±0.11^a$	$2.85±0.46^b$
Resveratrol	$0.74±0.15^a$	$1.81±0.17^b$
P	<0.001	<0.001

Values are mean ± S.D, n=6.
^{a,b,c}: In the same column values with different letters show statistically significant differences in TAS and TOS ($P<0.05$)
^{a,b,c}: Aynı sütunda farklı harfleri taşıyan TAS ve TOS değerleri ($P<0.05$) istatistiksel açıdan önemlidir

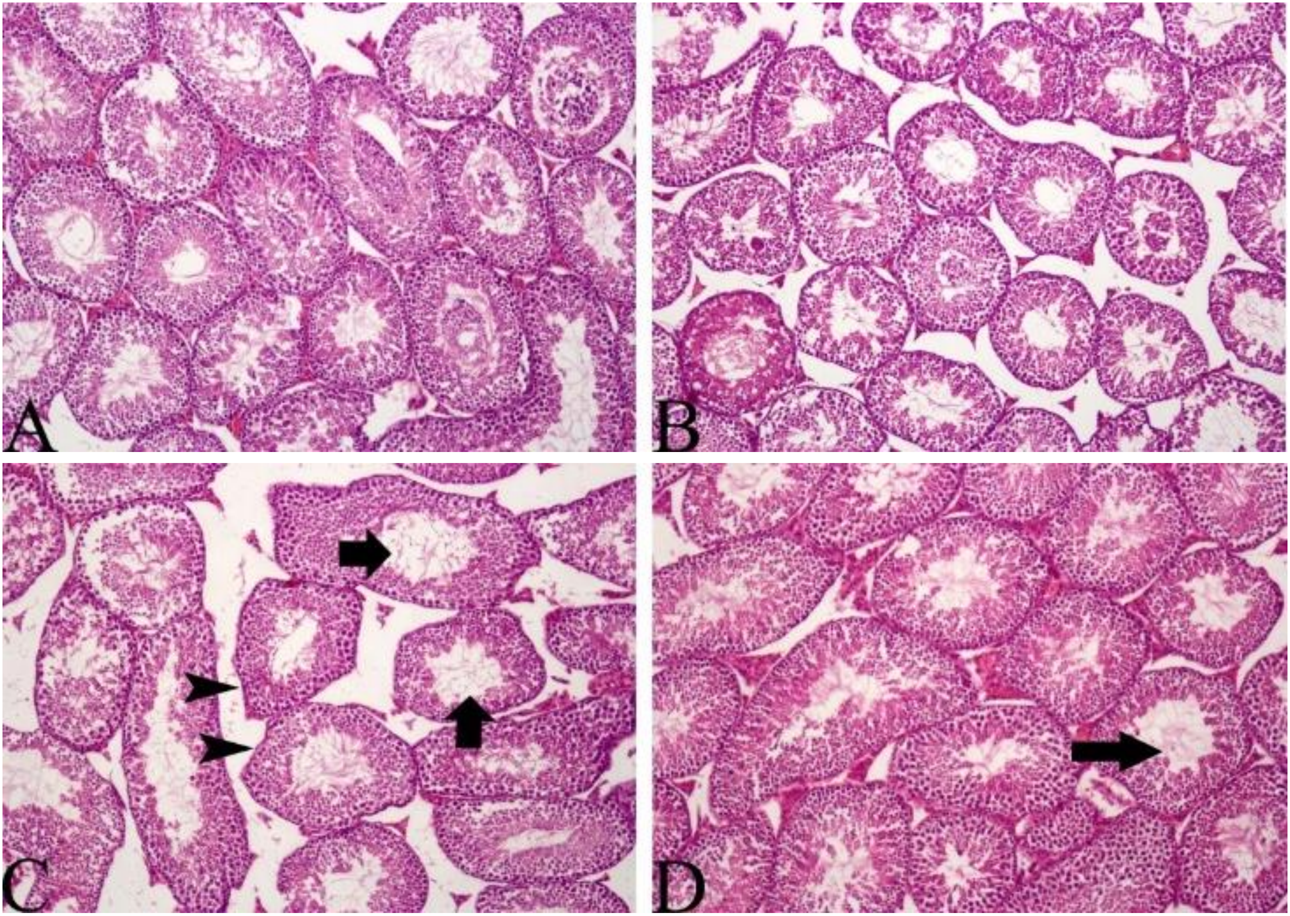

Figure 1. Effects of MK-801 (1 mg/kg), resveratrol (RES-40 mg/kg) and RES (40 mg/kg) + MK-801 (1 mg/kg) on histopathological changes in the testis of mice. A. Control group B. Resveratrol treated group. C. MK-801 treated group, Arrow: Necrobiotic and degenerative changes in the epithelial cells of the seminiferous tubules and decrease in the number of the spermatozoid, Arrowhead: Disorganisation in the basement membrane of the seminiferous tubules D. MK-801+Resveratrol treated group, Arrow: Decrease in the number of the spermatozoid Hematoxylene and eosine (10x)

Şekil 1. Fare testislerindeki histopatolojik değişiklikler üzerine MK-801(1 mg/kg), resveratrol (RES-40 mg/kg) ve RES (40 mg/kg) + MK-801 (1 mg/kg) kombinasyonunun etkisi. A. Kontrol grubu B. Resveratrol grubu C. MK-801 grubu, Ok: Seminifer tübül epitel hücrelerinde dejeneratif ve nekrobiyotik değişiklikler ve spermatozoid sayısında azalma, Ok başı: Seminifer tübül membranında disorganizasyon D. MK-801+Resveratrol grubu, Ok: Spermatozoid sayısında azalma Hematoksilen-eozin (10x)

The oxidant and antioxidant parameters showed statistical differences in Group II compared with the controls. The testicular TOS levels in other groups except Group II did not differ from each other statistically. However, TOS level in Group II was 5.46±1.44 and it was significantly increased when compared with the other groups and the TAS level in the Group II was lower than the others. So, this is clear that the oxidative stress was increased in Group II, compared with the controls and the resveratrol was protected the testicular tissue against the oxidative stress by raised the antioxidant activity in the testis ($P<0.05$). Meanwhile, it was realized that the antioxidant capacity in Group III and IV were also higher than the Group I.

When the diameter and epithelial height of seminiferous tubules were measured no statistical significance found between the groups. Moreover, pathologically, the tubules and the spermatozoids were in their normal posture in the Group I. However, the tubules and the spermatozoids were seen to be affected by the MK-801 administration. The necrobiotic and degenerative changes in the tubular epithelial cells and a decrease in the number of the spermatozoid were detected in Group II and only a decrease in the number of the spermatozoid was detected in group III. Therefore, the administration of resveratrol reduced the degenerative and harmful effects of the MK-801 in the testis (Figure 1).

The percentage of sperm motility in the groups were observed and it was revealed that the sperm motility was significantly reduced in Group II and was significantly increased in Group IV when compared to Group I ($P<0.05$).

Discussion and Conclusion

In the present study, it was aimed to evaluate the protective role of the resveratrol against the testicular

oxidative stress conducted by the chronic dose of MK-801. The excessive amount of reactive oxygen metabolites in the tissue causes oxidative stress. Actually, the reactive oxygen metabolites are already produced by the internal organs of the body while they are running and for example, in the testis small amount of oxidation is necessary for normal sperm production. Meanwhile, the balance between the oxidant and antioxidant system is protected by the antioxidants produced by the body. The chemical agent MK-801 impairs the oxidant-antioxidant system balance and thus oxidative stress appears in the tissue. One of the main points of this study is to catch the ratio of this impairment. In fact, there are various measurement tools that measure the tissue oxidative stress in the tissue. However, the TAS-TOS kit is a useful method to make a decision for the determination of the oxidation in the tissue. There are only a few studies investigated the testicular injury and protective roles of some antioxidants against the oxidative stress induced by MK-801 on the laboratory animals (14, 16). According to literature, this may be the first study researched the antioxidant protection of the resveratrol against the MK-801 induced testicular oxidative stress in the mice.

The oxidative stress in the testis depended on MK-801 firstly reported by the Ozyurt et al. (2007). Ozyurt et al. (2007) and then Parlaktas et al. (2008) were showed the increased oxidative stress parameters in the testicular tissue. In the present study, the testicular measurement of the oxidative stress value in Group II was increase parallel to the findings of these authors. Moreover, in Group III, the addition of the resveratrol against the MK-801 as a protective antioxidant agent seems to be beneficial and protectant. Because resveratrol administered mice in Group III, the TOS value stay close to Group I's level. While the TOS level was decreased, the TAS level was increased in the resveratrol injected groups. The resveratrol threw down the oxidative stress by enhanced the antioxidant activity in the tissue. Additionally, it seems that the powerful antioxidant activity of the resveratrol, tracking the antioxidant activity of the Group III and IV also better than the Group I.

Ozyurt et al. (2007) and Parlaktas et al. (2008) were reported that the MK-801 injected rats' spermatogenesis and normal tubular epithelium was affected. The seminiferous tubules and cells are degenerated and disorganized. According to the findings of these two researchers, administration of the melatonin hormone to the rats protected the testicular tissue and injections of the CAPE prevented the degeneration of the germinal cells and atrophy of the tubular epithelium in the testicular tissue. In another study on the rabbit bucks, MK-801 injection was caused a significant decrease in sperm number, antioxidant parameters and pathological changes in testis. Dietary supplementation of vitamin E, vitamin C, and olive pomace ameliorated the related parameters in the testis (19). In the present study, the emerged oxidative stress sourced by the MK-801 decreased the testicular weight same as previous studies (14, 16). However, the resveratrol was protected the organs loss of weight and kept it near in the Group I's limit. The necrobiotic and degenerative changes in the tubular epithelial cells and a decrease in the number of spermatozoids were detected in Group II. In Group III, only a decrease in the number of spermatozoids was detected. So, the resveratrol protected the testicular tissue against the necrosis and degeneration in the Group III. Meanwhile, the chronic dose of MK-801 injections didn't cause the tubular shrinkage and epithelial loss in the testis.

There are several studies investigating the healing effects of the resveratrol on the testicular tissue. In a study conducted by Reddy et al. (17) the cisplatin-induced testicular toxicity was investigated. The authors reported that the resveratrol improved the injury in the testicular tissue and also spermatogenesis. In another study (12), the protective effect of the resveratrol was observed in the varicocele testis. The varicocele had many negative effects on the testis parameters like volume, abnormal sperm amount and motility. The resveratrol as a protectant was given in a daily dose (300mg/kg by gavage) to the animals from 42 to 100 days of age. At the end of the applications, a reduction of the harmful effects of the varicocele was observed in the resveratrol group. Another research (5) reported that the 5 mg/kg i.p. a dose of resveratrol during 3 weeks alleviated the testicular oxidative stress in diabetic mice. Moreover, the resveratrol (20 mg/kg by gavage daily for 4 weeks) improved the oxidative stress and testicular dysfunctions induced by depression (18).

In the present study, it was revealed that the 40 mg/kg dose of resveratrol increased the progressive sperm motility against the Group I. In a study (13), the 10 mg/kg dose of resveratrol protected the testis from the oxidative stress; however, this dose did not affect the sperm motility. But in another recent research, the asthenospermia sperm samples were collected from the obese peoples and the 2.5, 6, 15, 30, 50 and 100 µmol/l resveratrol was added into the spermatozoa's medium. The 2.5, 6, 15, 50 and 100 µmol/l doses of resveratrol did not alter the progressive sperm motility while 30 µmol/l dose of resveratrol notably improved the progressive motility (2).

In conclusion, the results of the present study demonstrate that resveratrol was effective for the inhibition of the oxidative stress induced with MK-801 in mice testes. The resveratrol protected the testicular tissue against the oxidative stress, pathological injuries, and also sperm motility. Furthermore, in Group IV the resveratrol increased the motility of the spermatozoon than the control group.

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The anatomy of cervical sympathetic ganglia in Saanen goats*

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Summary: Sympathetic ganglions located in the cervical region are important organs that make the final synapse of the sympathetic nerve fibers reached to the head, neck, and forelimbs. As far as we know, there are not any anatomical data about cervical sympathetic ganglia in Saanen goat. In this study, we determined the nerve branches separated from the ganglia and the location of the ganglia. We also determined the expression of some enzymes and proteins such as tyrosine hydroxylase (TH), dopamine β-hydroxylase (DβH), neuropeptide Y (NPY) and substance P (SP) in ganglia. Ganglion cervicale craniale (GCC) was on the medial side of bulla tympanica. Mainly branches named as nn. carotici interni, n. jugularis and nn. carotici externi was found to be separated from this ganglion and thin branches joined to the nearby nerve. It was found that n. vertebralis, the two branches that constitute the ansa subclavian, and the thin nerve branches involved in the surrounding tissues and organs separated from ganglion cervicothoracicum (GCT) that located in the first intercostal space. A total of five ganglion cervicale medium (GCM) found at the junction of the two branches forming the ansa subclavia. Another ganglion was not found on where cervical part of truncus sympathicus in all dissections and histological examinations. DβH, TH, NPY and SP were revealed to be express in all ganglia. DβH and NPY in CCG, TH in MCG, DβH, NPY and TH in GCT were found to be more intense staining.

Keywords: Anatomy, immunohistochemistry, Saanen goat, sympathetic ganglia.

Saanen keçilerinde servikal sempatik ganglion'ların anatomisi

Özet: Cervical bölgede yer alan sempatik ganglion'lar baş, boyun ve ön ektremite'lere giden sempatik sinir liflerinin son sinaps yaptığı önemli organlardır. Saanen keçisinde servikal sempatik ganglion'lara ait anatomik bilgiye rastlanılmadı. Bu nedenle, bu çalışma ile bahsi geçen ganglion'ların yeri, ganglion'dan ayrılan sinir kolları ve ganglion'lardaki dopamin β-hidroksilaz (DβH), tirozin hidroksilaz (TH), Neuropeptid Y (NYP) ve Substans P (SP) gibi bazı enzim ve protein expresyonu belirlendi. Bulla tympanica'nın medial'inde yer alan ganglion cervicale craniale (GCC)'den başlıca nn. carotici interni, n. jugularis, n. caroticus externus ve çevre sinirlere katılan ince kollar ayrıldığı görüldü. Birinci interkostal aralıkta yer alan ganglion cervicothoracicum (GCT)'dan başlıca n. vertebralis, ansa subclavia'yı oluşturan iki kol ve çevre doku ve organlara katılan ince sinir kollarının ayrıldığı belirlendi. Ansa subclavia'yı oluşturan iki kolun birleşim yerinde toplam 5 adet ganglion cervicale medium (GCM)'a rastlandı. Yapılan tüm diseksiyon ve histolojik incelemelerde truncus symphaticus'un cervical bölümünde başka bir gangliona rastlanmadı. DβH, TH, NPY ve SP'nin tüm ganglion'larda exprese olduğu, GCC'de DβH ve NPY'nin, GCM'da TH'ın, GCT'da DβH, NPY ve TH'ın daha yoğun boyanma gösterdiği belirlendi.

Anahtar sözcükler: Anatomi, immunohistokimya, Saanen keçisi, sempatik ganglion.

Introduction

In the sympathetic nervous system, two neurons function between the center and the effector organ. Synapses between these neurons occur in the ganglion (35). GCC and GCT are always present in domestic mammals, whereas the presence of GCM varies with species or even individuals (14, 16, 35). The presence of ganglion, also named to as ganglia intermedia, has been reported in some species (27).

The immunoreactivity of enzymes such as TH and DβH and proteins such as NYP and SP known to be expressed by sympathetic neurons in the ganglia trunci sympathici have been reported (2, 3, 5, 8, 13, 24). NPY that originated from the sympatho-adrenomedullary nervous system has a vasoconstrictive and mitogenic effect on blood vessels. It functions in blood pressure regulation and angiogenesis (23). SP which performs functions such as pain perception, emotional behavior, stress, smooth muscle contraction and saliva production

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has a wide spread in the body (9, 10, 11, 36). In addition, SP in the neuropeptide structure is also found in preganglionic neurons of sympathetic ganglia (12). Catecholamine neurons, such as dopamine, norepinephrine and epinephrine contain the TH enzyme. After this enzyme converts tyrosine to a composition called as dopa, dopa is also turned into dopamine neurotransmitter. In addition to TH, neurons that use norepinephrine as a neurotransmitter also have the DβH which converts dopamine to norepinephrine (1).

It is stated that cause dysfunctions in the head, neck and forelimb of pathological conditions that may occur in cervical sympathetic ganglia due to some metabolic diseases (21) or arterial insufficiency (28). One of them is Horner's syndrome (29), the other is the suppression of melatonin release expressed in the pineal gland (26). Damages occurred in GCT for different reasons can lead to arrhythmias in the heart (22).

The literature on the anatomy of the cervical sympathetic ganglia that have important functions is not encountered in Saanen goats. The purpose of this study was to determine the location and size of cervical sympathetic ganglia, nerve branches separated from them, and also demonstration of the presence of sympathetic neurons via DβH, NYP, TH and SP antibodies known to be expressed by sympathetic neurons in the ganglia

Materials and Methods

In this study, a total of 14 samples were evaluated by examining each half of 7 adult female Saanen goats separately. Our study was approved by our local Ondokuz Mayıs University Animal Experiment Local Ethical Committee (Ethics committee number: 2012/28). After perfusion, all the materials were fixed with 10% formaldehyde solution. The dissections were performed under a stereomicroscope (Olympus SZ61 TRC). Photos were taken with a digital camera (Olympus C-5060). Measurements were measured with digital caliper (Mitutoyo, Japan). These measurements were analyzed via ordinary least squares (OLS) technique. Nomina Anatomica Veterinaria (30) was used for anatomic denomination.

Four blocks from each of GCC, GCM and GCT were prepared for histological and immunohistochemical examinations. Tissue sections were in 5 µm thick. The prepared sections were stained with haematoxylin-eosin (H&E) and were processed for immunohistochemical investigation with primary antibodies Tyrosine Hydroxylase Polyclonal antibody (PA1-4679, Thermo Fisher Scientific, USA), anti-Neuropeptide Y antibody (PA5- 19568, Thermo Fisher Scientific, USA), Substance P Polyclonal antibody (Bs-0065R, Bioss, USA) and Anti-Dopamine β Hydroxylase antibody (Millipore AB 1585, USA) by using standard streptavidin-biotin peroxidase complex method (SBPC) with a commercial kit (Zymed, USA). The reaction product was visualized by aminoethylcarbazole (AEC) chromogen (Zymed, USA) and counterstained with Mayer haematoxylin. Immunohistochemistry results were interpreted using a light microscope (Nikon Eclipse E600). For the quantification of the immunological staining in the tissue the analysis was initiated on the basis of the high intensity reaction fields. All of the sections were examined at a magnification of 400 X. The staining densities of cells that are positive in each area [(0) no reaction; (1) poor; (2) medium; (3) intense staining] was determined.

In addition, the blocks prepared for determination of whether or not any ganglions in the cervical part of the truncus sympathicus between GCC and GCM or GCT were examined by staining with H&E (25).

Results

GCC (Figure 1-a) and GCT (Figure 2-a) were found to be present in all examined Saanen goats. While GCM (Figure 2-b) existed in some materials, there was no another ganglion in the cervical part of the truncus sympathicus in all examined materials.

Ganglion cervicale craniale: GCC located in the ventral of art. atlantooccipitalis, at the medial and ventral of bulla tympanica and at the caudal of lnn. retropharyngei mediales. The average length, width, and thickness of this ganglion, which was generally oval (10/14) and sometimes spindle (4/14) shaped, were determined as 9.35 ± 0.99 mm, 4.03 ± 0.44 mm and 3.21 ± 0.32 mm, respectively.

It was observed that two nerves separated from the cranial half of the GCC. One of these was nn. carotici interni (Figure 1-b), and the other was n. jugularis (Figure 1-c). It was determined that the n. jugularis joined to the n. glossopharyngeus (Figure 1-d) and n. vagus (Figure 1-e). The nn. carotici interni usually established of two (11/14), very rarely one (2/14) or three (1/14) thin nerve branches. This nerve which accompanied the a. caroticus internus after leaving the ganglion constituted the plexus caroticus internus around this artery. The nn. carotici interni ended in sinus cavernosus.

It was determined that generally (10/14) 3, sometimes (4/14) 4 nerve branches separated from the ventral half of the ganglion. One of these joined to ramus pharyngeus (Figure 1-e ') which separated from n. vagus, the other one participated directly n. vagus. Nervus caroticus externus originated as a single nerve from the ventral side of the ganglion (Figure 1-f) was observed to separeted two branches (4 samples) immediately after the distinction. In three samples, these branches formed plexus caroticus externus around the a. caroticus externus the same named artery. In one sample, while one of the branches shaped the plexus, the other participated in the n. laryngeus cranialis (Fig. 1-f '). The nerve branches directly participating in cervical nerves from GCC could not observed in this study.

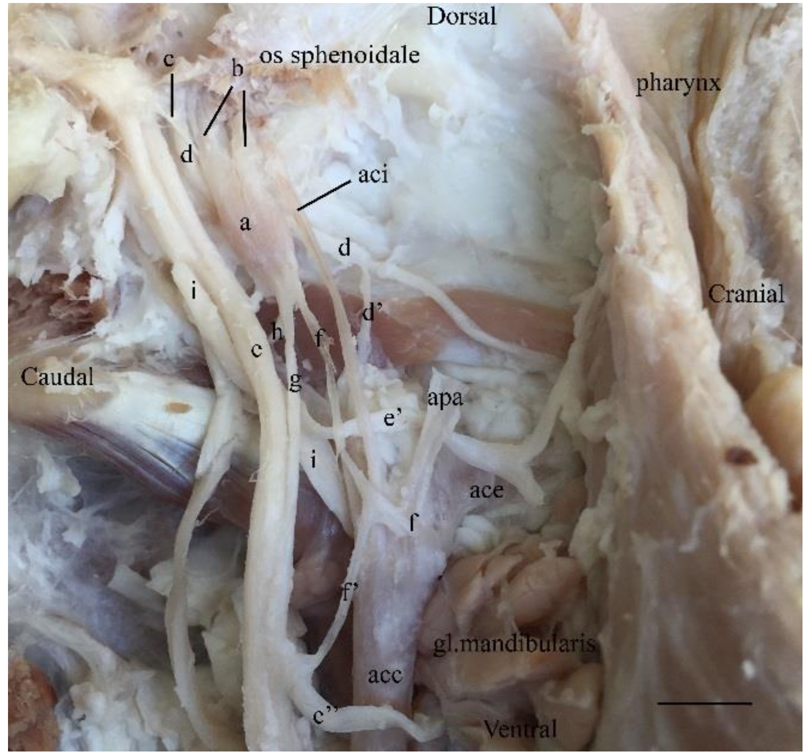

Figure 1. Medial view of GCC in Saanen goat.
a) GCC, b) nn. carotici internii, c) n. jugularis, d) n. glossopharyngeus, d') the branch that separates from n. glossopharyngeus and extented to glomus caroticus, e) n. vagus, e') ramus pharyngeus of n. vagus, e'') n. laryngeus cranialis, f) n. caroticus externus, f') the branch that separates from the n. caroticus externus and joins to the n. laryngeus cranialis, g) cervical part of tr. sympatheticus, h) branch that separates from CCG and joined to both n. vagus and the ramus pharyngeus of n. vagus, i) n. hypoglossus, apa) a. pharygea ascendes, acc) a. carotis communis, ace: a. carotis externa, aci) a. carotis interna, bar) 1 cm.

Şekil 1. Saanen keçisinde GCC'nin medial görünümü
a) CCG, b) nn. carotici internii, c) n. jugularis, d) n. glossopharyngeus, d') n. glossopharyngeus'tan ayrılan ve glomus caroticus'a uzanan kol, e) n. vagus, e') n. vagus'un ramus pharyngeus'u, e'') n. laryngeus cranialis, f) n. caroticus externus, f') n. caroticus externus'tan ayrılan ve n. laryngeus cranialis'e katılan kol, g) tr. sympatheticus'un servikal bölümü, h) CCG'den ayrılan ve hem n. vagus'a hem de onun ramus pharygeus'una katılan kol, i) n. hypoglossus, apa) a. pharygea ascendes, acc) a. carotis communis, ace) a. carotis externa, aci) a. carotis interna, bar) 1 cm.

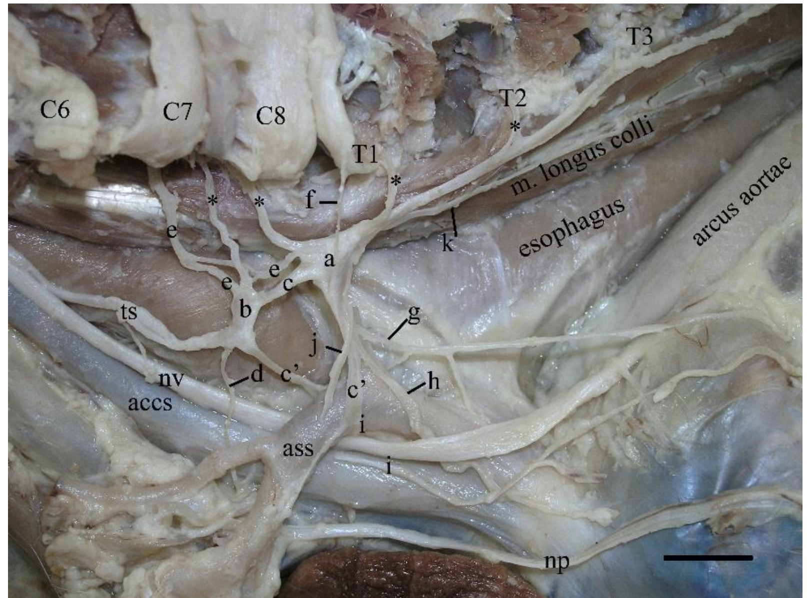

Figure 2. The view from left side of GCT and GCM in Saanen goat.
a) GCT, b) GCM, c) cranial branch of ansa sublavia, c') caudal branch of ansa sublavia, d) branch that separated from GCM and accompanied to a. subclavia sinistra, e) n. vertebralis, f) the branch joined from GCT to T1 n.spinalis, g) the branch that separates from caudovenral of GCT and joined to plexus aorticus, h) the branch that separates from ventral of GCT and dispersing to heart at the level of the left auricula, i) the branch that separated from ventral of GCT and dispersed to heart, j) the branch that separates from GCT and extented a long with a. sublavia sinistra, k) branch that dispersed on m. longus colli, accs) a. carotis communis sinistra, ass) a. subclavia sinistra, np) n. phrenicus, nv) n. vagus, ts) cervical part of the truncus sympathicus, C6, C7, C8) ventral branches of the 6th, 7th, and 8th nn. cervicalis spinalis, T1, T2, T3) ventral branches of the 1st, 2nd and 3rd nn. thoracalis spinalis, *: rami communicantes

Şekil 2. Saanen keçisinde CTG ve MCG'nin sol taraftan görünümü.
a) CTG, b) MCG, c): ansa sublavia'nın cranial kolu, c') ansa subclavia'nın caudal kolu, d) CTG'dan ayrılan ve a. subclavia sinistra'nın kolları ile seyreden kol, e) n. vertebralis, f) CTG'dan T1 spinal sinire katılan kol, g) CTG'un caudoventral'inden ayrılan ve plexus aorticus'a katılan kol, h) CTG'un ventral'inden ayrılan ve auricula sinistra düzeyinde kalbe dağılan kol, i) CTG'un ventral'inden ayrılan ve kalbe dağılan kol, j) CTG'dan ayrılan ve a. sublavia sinistra'ya boyunca uzanan kol, k) m. longus colli üzerinde seyreden sinir kolu, accs) a. carotis communis sinistra, ass) a. subclavia sinistra, np) n. phrenicus, nv) n. vagus, ts) tr. sympathicus'un boyun bölümü C6, C7, C8: 6. 7. ve 8. cervical spinal sinirlerin ventral kolları T1, T2, T3: 1. 2. ve 3. thoracal spinal sinirlerin ventral kolları *: rami communicantes.

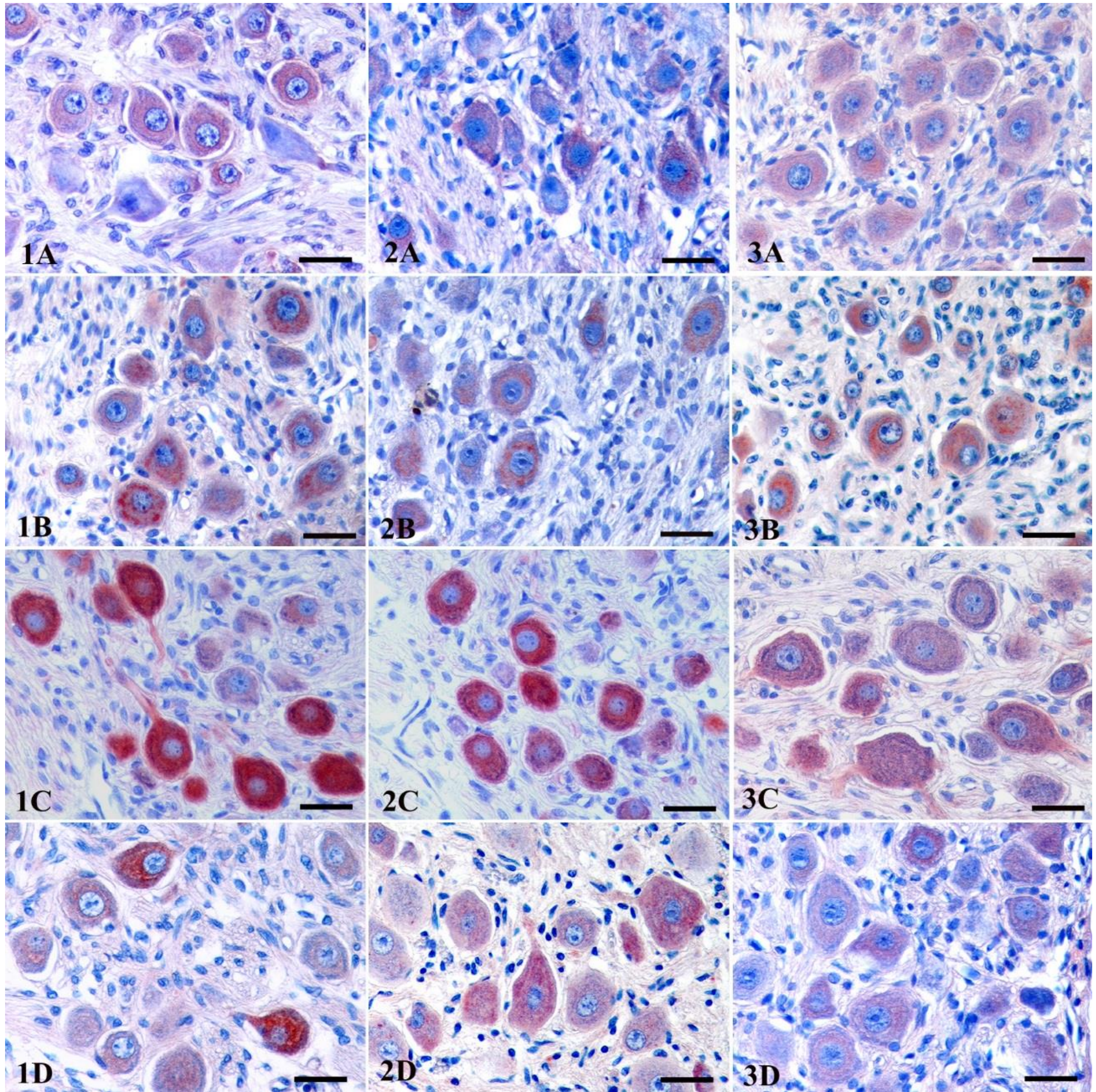

Figure 3. The expression of dopamine-β-hydrohsiase (A), Neuropeptide Y (B), Tyrosine hydroxylase (C) and Substance P (D) in GCC (1), GCM (2), GCT (3), bar), 25µm.
Şekil 3. CCG (1), MCG (2), CTG (3)'da Dopamin - β -hidroksilaz (A), Neuropeptid Y (B), Tirozin hidroksilaz (C) ve Substans P (D)'nin ekspresyonu, bar), 25µm.

Ganglion cervicale medium: The oval-shaped GCM (Figure 2-b) was at the entrance of the apertura thoracis cranialis and where join of the ansa cranialis (Figure 2-c) and ansa caudalis (Figure 2-c') of CTG. The length, width and thickness average measurements of GCM that observed as total 5 numbers (three left, two right) were in 4.78 ± 0.46 mm, 4.25 ± 0.22 mm and 2.72 ± 0.46 mm, respectively. Whereas some branches that separated from GCM added to a. subclavia dextra and a. subclavia sinistra (Figure 2-d), it was determined that some branches extended to the heart. In one sample, one branch that came from the GCM joined to n. vertebralis (Figure 2-e). In same sample, one branch which separated from ventral branch of 7th nervus cervicalis participated to GCM (Figure 2- *).

Ganglion cervicothoracicum: The GCT located in first intercostal space (Figure 2-a) and between the m longus colli and esophagus on the left, m longus colli and trachea on the right. GCT was triangular, round or spindle shape. It observed to be formed by the combination of the last cervical ganglion sympathica and first thoracal ganglion sympathetica (in a sample, the second thoracal

ganglion sympathetica also joined). Thin nerve branches involved from GCT to ventral branches of 8th cervical n. spinalis and first thoracal n. spinalis (Figure 2-f). The mean length, width and thickness measurements of GCT were in 12.84 ± 1.07 mm, 5.56 ± 0.93 mm and 3.26 ± 0.66 mm, respectively.

It was observed that thin nerve branches added from GCC to the vertebral nerve (Figure 2-e), to ansa cranialis (Figure 2-c) and ansa caudalis (Figure 2-c') forming ansa subclavia and in addition, to plexus aorticus (Figure 2-g) and heart (Figure 2-h-i). Apart from these branches, there were one (Figure 2-j) or two more branches separating from the ventral of the ganglion. Some branches (Figure 2-d) originating from GCM also added to these branches that accompany with a. subclavia sinistra and dextra and forming perivascular plexus on them. It was seen to participation of one branch (Figure 2-e) from GCM to n. vertebralis in only one sample.

Neurons in the cervical sympathetic ganglion stained immunopositive with DβH, NPY, SP and TH antibodies (Figure 3). In all three ganglia, the cytoplasm of some neurons with DβH antibody stained medium intensity and homogeneous character. It was observed that the staining with NPY antibody in the perinuclear region of some neurons determined to be granular. Although the cytoplasm of neurons with SP antibody was homogeneous staining, it was noted that the intensity of staining in all three ganglia was different. It was determined with TH antibody that almost all neurons in each of the three ganglia reacted immunopositive and the intensity of staining of most neurons was intense.

Discussion and Conclusion

It is important to know the location of the sympathetic ganglia and the distribution of the nerves separated from them, as the conduction disorders in postganglionic nerve fibers in the sympathetic nervous system may cause important clinical symptoms (18, 19).

The GCC was oval and spindle shaped in our study. This stiuation was similar to literature (6, 14, 15) The dimensions of the GCC have been reported in goat (27), Tibetan cattle (34) and roe deer (15). In this study, the width and thickness measurements of the GCC were similar to those reported as 3.67 mm and 3.07 mm in the roe deer (15). The length of the ganglion in the Saanen goat was lower than the value reported as 13.85 mm in the roe deer (15), as and higher than the value reported as 8 mm in the goat (27).

The separating of three main nerves as the nn. carotici interni, n. caroticus externus and n. jugularis from the GCC and the formations a plexus around the same named arteries of branches separated from nn. carotici interni and n. caroticus externus was compatible with the literature (6, 14, 31, 34). It has been reported in the literature (6, 7, 15, 27, 31) that there are differences in the numbers of the nn. carotici interni and n. caroticus externus. In this study, while the separating as usually to two branches of the nn. carotici interni was similar to literature (7, 27), as very rarely, the separating of the single branch was created a difference. In this study, the separation as a single branch of the n. caroticus externus from the ventral of GCC was consonant to literature. (6, 7, 27, 31). In four materials, the nerve which separated as a single branch from the ganglion immediately divided to two. This situation was similar to state of Kabak and Onuk (15).

In the literature (14, 15), it has been reported that the nerve branch separating from GCC is involved to the n. laryngeus cranialis. In this study, although one nerve that separating from the ganglion and joined directly to n. laryngeus cranialis was not presence, the participation to the mentioned nerve of the one branch separating from the n. caroticus externus and cervical part of the truncus sympathicus constituted an important difference.

The presence of connecting branches one (6, 15, 27, 31, 35) two or three (14) between the GCC and the nn. cervicales is mentioned. The absence of any linkage in this study was suggest that this was due to dissection errors.

Differences are reported about the presence of GCM at the junction of ansa cranialis and ansa caudalis in the literature (27). A total of 5 numbers GCM, 3 on the left and 2 on the right were observed in this study. In cervical part of truncus sympathicus of goat, the GCM and ganglia intermedia have reported by Getty (27). While it was only present GCM in our study, the ganglia intermedia were not found. This situation was consistent with the reported in the roe deer (16).

The localization of GCT within the first intercostal space in the Saanen goat was consistent with the literature (16, 27, 32, 33). Although the shaped of GCT is reported as oval, star, half moon, pear and inverted L letter (17, 27, 32, 33), the appearance like as triangular and spindle in this study was similar to roe deer (16). The length, width and thickness measurements of the GCTs reported in different species (16, 17, 27, 32, 33) determined to be 12.84 x 5.56 x 3.26 mm in the Saanen goat, respectively, and these values were similar to the goat (27).

It has been reported that GCT is caused by the combination of the last ganglion cervicale with the first (33), second (16, 27) or third (32) ganglia thoracica. In Saanen goats, the same ganglion usually formed by the coalescence of the last ganglion cervicale and first ganglia thoracica. In one sample, the second ganglia thoracica also participated in the formation of GCT. Kabak et al. (16) has reported that one branch of each extended to the ventral branches of the last n. cervicalis and second n.thoracalis from GCT, and two branches extended to the ventral branch of the first thoracal n. spinalis. In this study,

similarly to the literature (17, 27, 32, 33), it was observed that one branch participated in the last cervical n. spinalis and first thoracal n. spinalis. In one sample and on the left side, the extending of one branch to the ventral branch of the 7th cervical n. spinalis from GCM considered as a significant difference. The presence and distribution of the other branches leaving the ganglion were consistent with the literature (16, 17, 27, 32, 33).

In this study, immunohistochemical examination of GCC, GCM and GCT revealed that DβH, NYP, TH and SP antibodies were immunopositive. The immunopositivity of SP varies between species and ganglions. It has been reported that SP does not show immunoreactivity in pig GCT (13), in sheep (2) and dog GCM (8) and in rat GCC (4). The immunopositivity observed in all ganglia was an important difference for SP antibody in our study. While DβH, NPY, and TH antibodies have showed intense immunopositivity in GCM of sheep (2), it has been expressed that only NPY antibody has showed intense immunopositivity in pig (13) and cat (24) GCT, in dog ganglia trunci sympathici (8) in rat GCC (20). In the Saanen goats, three antibodies were observed to be positive and intensely stained in all the ganglia. This situation can be interpreted as having intensively synapses of sympathetic nerves in all ganglia.

In conclusion, the shape, size and location of cervical sympathetic ganglia (GCC, GCM and GCT), the nerve branches separating from ganglion, and the relation between these branches extremite and peripheral organs and vessels determined in detail in Saanen goat. Although there were some minor differences in shape, size, location of GCC, GCM and GCT and major nerves and interconnected branches separated from ganglion in Saanen goats, our results were observed to be generally consistent with the literature. Besides these similarities, it was remarkable some different findings. One of these was that the nerve branches separated from caudodorsal of GCT did not see. The other one was a branch extending from GCM to both the ventral branch of the 7th n. cervicalis and the n. vertebralis in one sample. Also, DβH, NPY, TH and SP showed immunopositive reaction in all examined ganglia. We think that the findings obtained as a result of this study will contribute to the literature of the anatomy and will be a source for the studies to be made about the subject.

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Effects of *Allium tuncelianum* on hyperglycemia and oxidative stress in the kidney and liver tissues in rats with diabetes mellitus induced by streptozotocin

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Summary: In this study, it was aimed to investigate the effects of *Allium tuncelianum* extract on hyperglycemia and oxidative stress in the kidney and liver tissues in rats with diabetes mellitus induced by streptozotocin. The rats were randomly divided into 4 groups with 10 animals in each group: Control group (C) was intraperitoneally (i.p.) treated with physiological saline solution, diabetic control (DC) group i.p. with a single dose of 50 mg/kg streptozotocin (STZ), diabetic + insulin (D+I) group i.p. with a single dose of 50 mg/kg STZ and subcutaneously (s.c.) with 2 IU insulin for 28 days (Levemir Flexpen), diabetic + *Allium tuncelianum* extract (D+AT) group i.p. with a single dose of 50 mg/kg STZ and orally with 250 mg/kg *Allium tuncelianum* extract for 28 days. The serum glycated hemoglobin (HbA1c), insulin levels and the kidney and liver thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), and catalase (CAT) activities were determined by using ELISA kits. Increased blood glucose levels ($p<0.001$), increased TBARS levels ($p<0.001$, $p<0.01$ respectively), and decreased SOD and CAT activities ($p<0.001$) in the kidney and liver tissue homogenates were determined in diabetic control group compared to control group. *Allium tuncelianum* extract had potent antioxidant activities in the diabetic rats, and demonstrated improvement effects by increasing insulin levels, decreasing glycated hemoglobin levels, and attenuating oxidative stress in the diabetic rats. This study suggests that *Allium tuncelianum* extract may have therapeutic potential for patients with diabetes.

Keywords: *Allium tuncelianum*, antioxidant, diabetes, oxidative stress, rat.

Streptozotosin ile diyabetes mellitus oluşturulan sıçanlarda *Allium tuncelianum*'un hiperglisemi ve böbrek ve karaciğer dokularında oksidatif stres üzerine etkileri

Özet: Bu çalışmada streptozotosin ile diyabetes mellitus oluşturulan sıçanlarda *Allium tuncelianum* ekstraktının hiperglisemi ve böbrek ve karaciğer dokularında oksidatif stres üzerine etkilerini araştırmak amaçlandı. Sıçanlar her bir grupta 10 hayvan olacak şekilde rastgele 4 gruba ayrıldı: Kontrol grubuna (C) periton içi (i.p.) fizyolojik tuzlu su, diyabetli kontrol (DC) grubuna i.p. tek doz 50 mg/kg streptozotosin (STZ), diyabet + insülin (D+I) grubuna i.p. tek doz 50 mg/kg STZ ve 28 gün süre ile deri altı (s.c.) 2 IU insülin (Levemir Flexpen), diyabet + *Allium tuncelianum* ekstrakt (D+AT) grubuna i.p. tek doz 50 mg/kg STZ ve 28 gün süre ile ağızdan 250 mg/kg *Allium tuncelianum* ekstraktı ugulandı. Serum glikozile hemoglobin (HbA1c), insülin düzeyleri ve böbrek ve karaciğer tiyobarbitürik asit reaktif maddeler (TBARS), süperoksit dismutaz (SOD) ve katalaz (CAT) aktiviteleri ELISA kitleri ile belirlendi. Kontrol grubuna göre diyabetli kontrol grubunda yüksek kan glikoz düzeyleri ($p<0.001$), böbrek ve karaciğer homojenatlarında TBARS düzeylerinde artma ($p<0.001$, $p<0.01$, sırasıyla) ve SOD ve CAT aktivitelerinde azalma ($p<0.001$) belirlendi. *Allium tuncelianum* ekstraktı diyabetli sıçanlarda güçlü antioksidan aktivitelere sahipti ve diyabetli sıçanlarda insülin düzeylerini artırarak, glikozile hemoglobin düzeylerini azaltarak ve oksidatif stresi hafifleterek iyileştirici etkiler gösterdi. Bu çalışma *Allium tuncelianum* ekstraktının diyabetli hastalar için terapötik potansiyele sahip olabileceğini göstermektedir.

Anahtar sözcükler: *Allium tuncelianum*, antioksidan, diyabet, oksidatif stres, sıçan.

Introduction

Diabetes mellitus is a common metabolic endocrine disorder (11). Insulin-dependent diabetes mellitus is characterized by autoimmune destruction of pancreatic β-cells, and severe insulin deficiency due to loss of insulin-producing β-islet cells (39), resulting in hyperglycemia (18). It causes vascular complications such as nephropathy, neuropathy, retinopathy and peripheral vascular disease. These complications are related to the advanced glycation end products, oxidative stress, and

inflammation (11). Pancreatic islet β-cell destruction and apoptosis, and decreased insulin secretion could be associated with oxidative stress (32). Oxidative stress in type-1 diabetes mellitus develops due to hyperglycemia, the accumulation of advanced glycosylation end products, glucose oxidation (41), and the oxidation of protein, DNA and lipids (12, 26). In diabetic conditions, increase of reactive oxygen species (ROS) produced by NADPH oxidase (13,31) for example superoxide anion (30), hydrogen peroxide, and nitric oxide (10) and decrease of antioxidant defenses for example superoxide dismutase and catalase activities (30) are reported. Moreover, it is shown that oxidative stress in diabetic rats causes increased mitogen-activated protein kinases, increase of inflammation (increased nuclear factor-kappa B activation, tumor necrosis factor-α and interleukin-6 expression), and fibrosis (21).

Medicinal herbs with hypoglycemic activity have been recently investigated as adjunctive treatments for diabetes mellitus. One of the most effective plants for lowering blood glucose levels is *Allium sativum* (7, 28).

Garlic is a member of the *Liliaceae* family and has bioactive compounds (8). It has been used for centuries as traditional medicine to treat several diseases (35) and consumed as food. Garlic has been suggested to have antidiabetic and antioxidant (38), anti-inflammatory (14), antifungal and antibacterial (23), and anti-atherosclerotic activities (42).

Allium tuncelianum (*A. tuncelianum*) is an endemic garlic species that naturally grow in a limited area between the regions of Sivas and Erzurum, especially in the Ovacık district within the plots of the Munzur Mountains. *A. tuncelianum* comprises bioactive compounds such as organosulfur compounds allyl methyl sulfide, diallyl disulfide, and diallyl trisulfide, phenolic substances, various fatty acids such as oleic and linoleic acids and vitamins and minerals (37). According to the author's knowledge, there have been no studies on the hypoglycemic and anti-oxidant activities of *A. tuncelianum* in diabetic rats. Therefore, the aim of this study was to investigate the effects of *A. tuncelianum* extract on hyperglycemia and oxidative stress in streptozotocin-induced diabetic rats.

Materials and Methods

***Chemicals*:** Streptozotocin was purchased from the Sigma Chemical Company in China. The other reagents used in this study were obtained from various companies (Sigma, Riedel-de Haen, Fluka Companies, all in Germany).

***A. tuncelianum extraction process*:** *A. tuncelianum* bulbs were obtained from Ovacık district of Tunceli, Turkey. The *A. tuncelianum* extraction process was performed according to the method of Ozkan et al. (27). The bulbs were dried in a dark room. *A. tuncelianum* bulbs of 1000 g were finely minced, kept in 5000 ml absolute ethyl alcohol at room temperature in a dark condition for 20 h, and mingled with a period of five hours. Then, this mixture was concentrated by evaporating ethyl alcohol. The final product as *A. tuncelianum extract* was stored at -20 ºC.

***Animals*:** In this study, 40 female Sprague-Dawley rats, 2 months old, weighing 200 ± 20 g, were supplied by the Laboratory Animal Research Center at Fırat University, Elazığ, Turkey. The rats were housed in standard conditions (23±1 ºC, 12 h light/12 h darkness). This study was approved by the Local Animal Care Ethics Committee at Kafkas University, Kars, Turkey, and was in compliance with the International Guidelines for Care and Use of Laboratory Animals (2006/063, 21.04.2016). The rats were randomly divided into 4 groups with 10 animals in each group: Control group (C) was intraperitoneally (i.p.) treated with physiological saline solution; Diabetic control (DC) group was i.p. treated with a single dose of 50 mg/kg Streptozotocin (STZ; dissolved in citrate buffer, pH 4.5) (17); Diabetic + insulin (D+I) group was i.p. treated with a single dose of 50 mg/kg STZ and subcutaneously (s.c.) with 2 IU insulin for 28 days (Levemir Flexpen) (40); Diabetic + *A. tuncelianum* extract (D+AT) group was i.p. treated with a single dose of 50 mg/kg STZ and orally with 250 mg/kg *A. tuncelianum* extract (dissolved in physiological saline solution, 1:1) for 28 days (38).

72 hours after STZ administration, the fasting blood glucose level of each rat was determined by glucometer (Bayer Contour TS, Germany). Rats with fasting blood glucose of 200 mg/dl were considered diabetic and included in the study (40).

The serum glycated hemoglobin (HbA1c), insulin levels (Catalog No: E-EL-R2466) and the kidney and liver thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD) (Catalog No: E-EL-R1424), and catalase (CAT) (Catalog No: E-EL-R2456) activities were determined by using ELISA kits (Elabscience, USA).

Upon completion of the study, all rats were sacrificed by sodium pentobarbital anesthesia (30 mg/kg). Blood samples were collected by intra-cardiac route from animals under sodium pentobarbital anesthesia and then centrifuged at 4000 g for 5 min to obtain serum samples. The kidney and liver tissues and serum samples were stored at – 20 ºC until biochemical analysis. Before the biochemical analysis both tissue and serum samples were allowed to dissolve at room temperature. After the liver and kidney tissues were separated into pieces, one part of the tissue samples was homogenized (Wiggen Hauser, Germany) in cold phosphate-buffered saline (1:9) and centrifuged at +4 ^{0}C, 10.000 g for 5 min to obtain a liver and kidney homogenate.

***Statistical analysis*:** A confidence level of 95% ($p < .05$) and power of 75% were used. The calculation gave a sample size of 10. All statistical evaluations were made using the SPSS 18 package program. One way analysis of variance and Tukey's test were used to determine differences between the groups. All parameters were given as mean ± SD. Significance was considered at $p<0.05$.

Results

The blood glucose, serum HbA1c, and serum insulin levels of the rats in this study are shown in Table 1. The kidney and liver TBARS levels, SOD and CAT activities of the rats in this study appear in Figure 1.

Our data showed that the blood glucose levels were significantly higher in the DC group compared to the control group ($p<0.001$). From the 7th to the 28th day in the D+AT group, the blood glucose levels were non-significantly lower at all measurements compared to the DC group. However, from the 7th to the 28th day in the D+I group, the blood glucose levels were significantly lower at all measurements compared to the DC group ($p<0.05$, $p<0.05$, $p<0.01$, respectively).

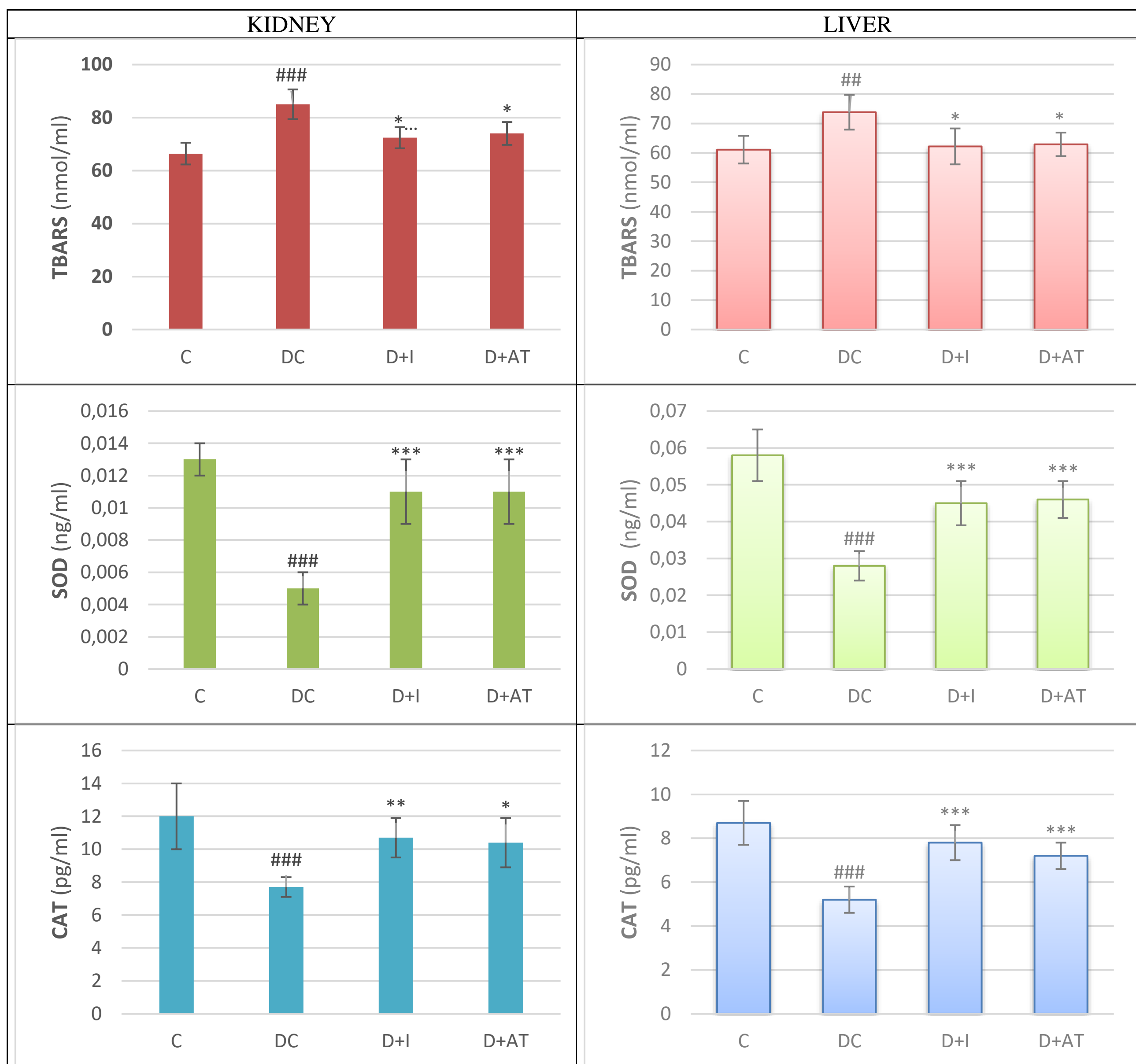

Figure 1. Effects of *Allium tuncelianum* extract on the kidney and liver TBARS, SOD and CAT levels in diabetic rats.
Şekil 1. Diyabetli ratlarda böbrek ve karaciğer TBARS, SOD ve CAT düzeyleri üzerine *Allium tuncelianum* ekstraktının etkileri.
C: Control group; DC: Diabetic control group; D+I: Diabetic+insulin group; D+AT: Diabetic+*Allium tuncelianum* group. The kidney and liver TBARS, SOD and CAT levels are provided as mean ± SD.
#P<0.05 versus control group, ##P<0.01 versus control group, ###P<0.001 versus control group, *p<0.05 versus diabetic control group, **p<0.01 versus diabetic control group, ***p<0.001 versus diabetic control group.

Table 1. Effects of *Allium tuncelianum* extract on blood glucose, serum HbA1c and serum insulin levels in diabetic rats.
Tablo 1. Diyabetli ratlarda kan glikoz, serum HbA1c ve serum insülin düzeyleri üzerine *Allium tuncelianum* ektraktının etkileri.

Parameters	C	DC	D+I	D+AT
Initial Glucose (mg/dl)	84.0±3.0	84.4±5.0	87.5±5.2	87.2±4.5
Glucose on diabetes development (mg/dl)	86.5±4.6	399.0±9.8###	393.9±14.1	403.3±36.7
Glucose on day 7 (mg/dl)	81.7±3.4	389.7±12.1###	359.3±29.4*	379.8±12.2
Glucose on day 14 (mg/dl)	86.3±6.2	392.7±15.7###	358.3±27.9*	382.2±25.9
Glucose on day 21 (mg/dl)	83. 7±4.5	394.0±14.7###	362.0±8.9***	383.8±14.9
Glucose on day 28 (mg/dl)	84.3±4.4	401.7±10.7###	347.6±43.0**	389.2±10.6
HbA1c (ng/ml)	185.8±17.9	355.1±22.6###	285.0±15.4***	319.2±28.9*
Insulin (ng/ml)	133.6±17.9	90.8±13.3###	150.7±15.2***	105.5±10.6

C: Control group; DC: Diabetic control group; D+I: Diabetic+insulin group; D+AT: Diabetic+*Allium tuncelianum* group. Blood glucose, serum HbA1c and serum insulin levels were given as mean ± SD.
#P<0.05 versus control group, ##P<0.01 versus control group, ###P<0.001 versus control group, *p<0.05 versus diabetic control group, **p<0.01 versus diabetic control group, ***p<0.001 versus diabetic control group.

In the DC group, the serum HbA1c level significantly increased and the serum insulin level significantly decreased compared to the control group (p<0.001). In the D+AT and D+ I groups, the serum HbA1c levels significantly decreased compared to the DC group (p<0.05, p<0.001 respectively). The serum insulin levels only significantly increased in the D+I group compared to the DC group (p<0.001). However, in the D+AT group, the serum insulin level non-significantly increased compared to the DC group.

The kidney and liver TBARS levels significantly increased in the DC group compared to the control group (p<0.001, p<0.01, respectively). The kidney and liver TBARS levels were significantly reduced in the D+I and D+AT groups compared to the DC group (p<0.05). The kidney and liver SOD and CAT activities in the DC group were significantly reduced compared to the control group (p<0.001). In the D+I and D+AT groups, the kidney and liver SOD activities were significantly enhanced compared to the DC group (p<0.001). In the D+I and D+AT groups, the kidney CAT activities were significantly enhanced compared to the DC group (p<0.01, p<0.05, respectively). In the D+I and D+AT groups, the liver CAT activities were significantly enhanced compared to the DC group (p<0.001).

Discussion and Conclusion

The development of diabetes mellitus in rats in this study was induced by a single dose of 50 mg/kg i.p. streptozotocin injection. In the D+AT group, from day 7 through 28 the blood glucose levels non-significantly decreased compared to the DC group. In addition, the serum insulin levels in the D+AT group non-significantly increased compared to the DC group. The serum glycated hemoglobin levels significantly decreased in the D+AT group compared to the DC group. In this study, the decreased glycated hemoglobin levels revealed that the improvement effects in diabetic rats were developed by the treatment of *A. tuncelianum* extract at a dose of 250 mg/kg. However, the treatment dosage of 250 mg/kg of *A. tuncelianum* extract to reduce higher blood glucose levels in the D+AT group was likely low. For example, Thomson et al. (38) found a significant decrease in blood glucose levels and a significant increase in serum insulin levels in the treatment of diabetic rats with either 300 or 600 mg/kg dose of aged garlic.

Some studies revealed hypoglycemic effects of garlic extracts, but other studies have not. Shiju et al. (34) found a significant decrease in the glycated hemoglobin levels with the treatment of 500 mg/kg aged garlic (*Allium sativum*), but did not found a hypoglycemic effect in the diabetic rats. In contrast, Saravanan et al. (33) have suggested that S-allylcysteine derived from garlic at a dose of 150 mg/kg normalizes blood glucose levels in the STZ diabetic rats. Similarly Rajani Kanth et al. (29) have revealed that garlic treatment at a dose of 250 and 500 mg/kg significantly normalizes the blood glucose levels in the diabetic rats. In addition, Hassan et al. (15) have stated that garlic aqueous extract (150 mg/kg) in alloxan-induced diabetic rats significantly decrease the blood glucose levels (56%). Nasiri et al. (25) have suggested that the blood glucose levels in diabetic rats were significantly decreased via garlic extract treatment (2 g/kg/day for 30 days). This can be attributed to the treatment doses of garlic in diabetic rats or the species of garlic.

The pathogenesis of diabetes mellitus has been implicated in oxidative stress via increased free radicals which are formed by glucose oxidation, glycosylated proteins, and then oxidative degradation of glycosylated proteins (2, 16, 22). In addition, hyperglycemia has been revealed to promote oxidative stress by mitochondrial dysfunction and endoplasmic reticulum stress (32). Enhanced expressions of advanced glycation end products in the kidney and liver tissues were demonstrated in STZ

diabetic rats (3), and enhanced advanced glycation end products have been indicated to have a role in the intracellular reactive oxygen species production, proinflammatory cytokines secretion, and collagen synthesis (20).

Several studies have indicated the development of oxidative stress in diabetic rats via increase in TBARS or lipid peroxide levels (1, 4, 5) and decrease in antioxidant enzymes such as CAT, GPx activities (29) and SOD activity (9). In this study, it was found that the kidney and liver TBARS levels were significantly enhanced, while the kidney and liver SOD and CAT activities were significantly reduced in the DC rats compared to the control rats. This suggested that oxidative stress in the kidney and liver tissues occurred in rats with the STZ-induced diabetes.

Oxidative stress causes impairment of membrane functions and disruption of the membranes (1). Decreased pancreatic islet cells, fibrosis of the pancreas, and liver degenerations due to oxidative stress have been indicated in diabetic rats (19). In this study, a decrease in the serum insulin levels of the diabetic rats may be associated with the occurrence of oxidative stress, increase in glycated hemoglobin levels and possible pancreatic β-cell disruption and pancreatic damage.

Garlic has been indicated to have antioxidant (37) and hypoglycemic activities and to attenuate β-cell destruction (19, 24). Similarly, *Allium hookeri* contributed to the regeneration of some β-cells and insulin secretion in the pancreas derangement caused by STZ (32). S-allyl cysteine sulfoxide as a garlic antioxidant compound (*Allium sativum* Linn) has been showed to ameliorate the diabetic rats induced by alloxan and to control lipid peroxidation (6). Similarly it has been shown that garlic administration significantly reduces ROS levels and significantly increases SOD, and CAT activities in hearts of diabetic rats (36). However, according to the author's knowledge, there have not been any studies on the hypoglycemic activity of *A. tuncelianum* extract in diabetic rats. In this study, *A. tuncelianum* extract had nonsignificant hypoglycemic effects. In addition, *A. tuncelianum* extract significantly increased the kidney and liver SOD and CAT activities in the DC rats, suggesting that *A. tuncelianum* extract has potent antioxidant activities.

In conclusion, the results of this study confirmed that *A. tuncelianum* extract had potent antioxidant activities in diabetic rats, and improvement effects by increasing insulin levels, decreasing glycated hemoglobin levels, and attenuating oxidative stress in the diabetes treatment in rats. Further studies are required for the assessment of higher dosages of *A. tuncelianum* extract on hypoglycemic activities in diabetic rats.

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Identification of bacteria isolated from dairy goats with subclinical mastitis and investigation of methicillin and vancomycin resistant *Staphylococcus aureus* strains

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Summary: The objective of this study was to determine methicillin and vancomycin resistance phenotypically by agar disc diffusion method (ADDM) and minimal inhibitory concentration (MIC) test and genotypically by polymerase chain reaction (PCR) in *Staphylococcus aureus* strains isolated from goat milk. A total of 466 milk samples were collected from 233 goats in herds with mastitis problems in Burdur province of Turkey. The microorganisms were isolated from 122 (26.18%) of goat milk samples and among these microorganisms 53 (42.06%) were coagulase negative staphylococci (CNS), 43 (34.23%) were *Staphylococcus aureus*, 16 (12.08%) were *Escherichia coli*, 10 (7.94%) were *Candida* spp. and 4 (3.17%) were *Brucella melitensis*. Seven of *S. aureus* isolates were determined resistant to methicillin by ADDM and five of these isolates were found resistant to methicillin by MIC. *mec*A and *van*A genes can not be determined in *S. aureus* isolates by PCR. *Staphylococcus aureus* isolates were found to be susceptible to vancomycin by ADDM and MIC test. In conclusion, *S. aureus* and CNS are found to be the most isolated species from goat milk in Burdur province. In addition to that, the absence of *mec*A and *van*A genes in the *S. aureus* isolated from goat milk showed that goat milk does not play a significant role in the spreading of MRSA.

Keywords: Goat, methicillin, *S. aureus*, vancomycin.

Subklinik mastitisli keçilerden izole edilen bakterilerin identifikasyonu ve metisilin ve vankomisin dirençli *Staphylococcus aureus* suşlarının araştırılması

Özet: Bu çalışmanın amacı, keçi sütlerinden izole edilen *Staphylococcus aureus* izolatlarında fenotipik ve genotipik metisilin ve vankomisin direncinin agar disk difüzyon (ADDM), minimal inhibitory konsantrasyon (MİK) ve polimeraz zincir reaksiyonu (PZR) metodları ile belirlenmesidir. Bu amaçla, Burdur ilinde mastitis problemi yaşanan sürülerde bulunan 233 adet keçiden 466 adet süt örneği toplandı. Keçi sütlerinin 122 (%26.18)'sinden mikroorganizma izole edildi. İzole edilen mikroorganizmaların 53 (%42.06)'ü koagülaz negatif stafilokok (KNS), 43 (%34.23)'ü *Staphylococcus aureus*, 16 (%12.08)'sı *Escherichia coli*, 10 (%7.94)'u *Candida* spp. ve 4 (%3.17)'ü ise *Brucella melitensis* olarak belirlendi. *Staphylococcus aureus* izolatlarının 7'si ADDM ile metisiline dirençli bulunurken, bu izolatların 5'i MİK ile fenotipik olarak metisiline dirençli bulundu. *Staphylococcus aureus* izolatlarında *mec*A ve *van*A genleri PZR ile belirlenemedi. *Staphylococcus aureus* izolatları ADDM ve MİK ile vankomisine duyarlı bulundu. Sonuç olarak *S. aureus* ve KNS'nin Burdur ilinde keçi sütlerinden en sık izole edilen bakteriler olduğu belirlendi. Bununla birlikte, keçi sütlerinden izole edilen *S. aureus* suşlarında *mec*A ve *van*A genlerinin saptanamaması, MRSA'nın yayılmasında keçi sütlerinin önemli bir rol oynamadığını göstermiştir.

Anahtar sözcükler: Keçi, metisilin, *S. aureus*, vankomisin.

Introduction

Staphylococcus aureus (S. aureus) causes clinical and subclinical mastitis in farm animals (4,15) and food borne infection in human due to the contamination of goat, sheep milk for traditional caprine and ovine milk products are not subjected to pasteurization (23). Although several infectious agents have been isolated from goat mastitis (4, 19, 21, 36, 45), *S. aureus* is the most important mastitis pathogen, due to economical loses and decrease in milk production of dairy goat in worldwide (21). β-lactam antibiotics were generally preferred for the treatment of *S. aureus* infections in humans and animals (15, 29, 36). But, methicillin resistance *S. aureus* strains with use of β-lactamase resistant penicillins have started to attract attention all over the world and are responsible for hospital infections (9).

Methicillin resistance in staphylococci is mediated by *mec*A gene, which encodes a penicillin binding protein 2a (PBP-2a). This gene leads to reduce affinities to β-lactam antibiotics and present in all of methicillin resistant

staphylococci (17). Methicillin resistance *S. aureus* (MRSA) is the most important pathogen isolated from human nosocomial infections and MRSA infections of humans are increasing substantially in worldwide that is not only related to the resistance to β-lactam antibiotics, but also related with resistance to other antibiotics (aminoglycosides, macrolides and quinolones etc.) (3, 22, 25, 43). Penisillinase resistant penicillins are not used in veterinary medicine except cloxacilline performed intramammary in cattle. In recent years, MRSA has been isolated from various animals and animal products such as milk and cheese (2, 17, 39, 43). But, the studies about the presence of MRSA in goat milk and milk products has limited. MRSA has been detected from individual milk sample, bulk tank milk of goats and nasal swab of farm personnel (39). Similarly, Cartimiglia et al., (16) reported that MRSA was determined in bulk tank milk from dairy goat farms in Northern Italy. Chu et al., (12) announced that eleven MRSA isolates were identified from four goat farms. Altough MRSA was detected in cattle milk and milk products, it was not reported in goat milk and milk products in studies except for one study in Turkey. Aras et al., (2)'s study is the first report of MRSA isolation from goat mastitis in Turkey. In this study, two MRSA strains were isolated from mastitis and identified as MRSA by PCR (2). In methicillin resistant strains, chromosomal genes that are different from the *mec*A gene and which are necessary for expression of resistance are identified and these genes are named as fem factors (9). It is reported that *fem*A gene is only a feature of *S. aureus* strains and not found in other staphylococci (9).

Vancomycin has been a final choice antibiotic used for the treatment of MRSA infections in humans. Vancomycin resistance in enterococci is encoded by five *van* genes. Noble et al. (30) showed that the *van* genes have been found in *S. aureus* strains and methicillin resistant strains can acquire these genes. Hiramatsu et al., (20) reported the first MRSA with reduced susceptibility to vancomycin in Japan in 1996. Subsequently, vancomycin intermediate *S. aureus* (VISA) isolates have been reported in worldwide (31, 35, 37, 41). After a short time, in 2002, vancomycin resistant *S. aureus* (VRSA) was identified in Michigan (35). In all of these cases, patients had been treated with vancomycin or teicoplanin within six months (32). In studies (10, 35, 42), it was reported that VRSA isolates recovered from humans can carry *mec*A and *van*A genes. *In vivo* and *in vitro* transmission of *van*A genes from *Enterococcus faecalis* to *S. aureus*, VRSA can also be seen in animals, if MRSA and VRE are in the same animal (30). Although vancomycin resistance was reported by phenotypical tests in farm animals (1, 6), the presence of *van* genes have not yet been showed in livestock (1, 6, 8, 31, 41).

In this study, we aimed to identify microorganisms causing goat mastitis and to determine methicillin and vancomycin resistance in *S. aureus* isolates recovered from goat milk samples.

Materials and Methods

***Sampling*:** Four hundred sixty six milk samples were collected from 233 goats in herds with subclinical mastitis in five villages (Kurna, Düver, Güneyyayla, Kayaaltı and Kökez) of Burdur province of Turkey. Sampling was done in enterprises that had mastitis problem and samples were collected from the animals suspected of subclinical mastitis. The teats were cleaned by using 70% alcohol. After, the first few streams of foremilk were discarded, the milk samples were aseptically collected into sterile tubes.

***Bacterial isolates*:** Each milk samples were inoculated onto blood agar base (Oxoid, CM0055, UK) added 5% sheep blood and then the plates were incubated at 37°C for 18-24 h, aerobically. The microorganisms were identified by conventional microbiological procedures such as colony morphology, Gram staining, haemolysis, catalase, coagulase, DNAse, Voges-Proskauer, acetoin test, carbonhydrate fermentation tests etc. (47). The presence of *fem*A genes in the isolates identified as *S. aureus* using phenotypic tests were investigated by PCR and these isolates were confirmed as *S. aureus. Staphylococcus aureus* isolates were stored at -20°C in brain hearth infusion broth (Oxoid, CM1135, UK) containing 15% (v/v) glycerol.

***Antimicrobial susceptibility tests*:** The phenotypic resistance of the *S. aureus* strains to oxacillin was determined by ADDM and MIC test. *S. aureus* strains were cultured in blood agar with 5% sheep blood for 18-24 h at 37°C and strains were suspended in tryptic soy broth (TSB) (Oxoid, CM0129, UK) for McFarland Standard No. 0.5. The broth was cultured onto Mueller Hinton agar (Oxoid, CM0337, UK) with 2% NaCl for oxacillin resistance and onto Mueller Hinton agar for vancomycin resistance. The oxacillin (1 µg, Oxoid, UK) and vancomycin disc (30 µg, Oxoid, UK) were added and incubated for 24 h at 37°C. Inhibition zone diameters were evaluated according to Clinical and Laboratory Standards Institute (CLSI) (14).

The MIC for oxacillin (Sigma Chemical Co., St. Louis, MO, USA) were determined using a broth macrodilution method according to CLSI (9). *Staphylococcus aureus* isolates were tested into Mueller-Hinton broth (Oxoid, CM0405, UK) supplemented with 2% NaCl containing oxacillin in concentrations ranging from 0.5 to 256 µg/ml. MIC test for vancomycin was performed with Mueller Hinton broth containing vancomycin concentrations ranging from 0.5 to 256 µg/ml. The tubes containing oxacillin and vancomycin

were incubated at 35°C for 24 h and the MIC was defined as the lowest concentration of antibiotics that prevented the visible growth. The isolates were evaluated as susceptible (S), intermediate (I) and resistant (R) according to the MIC breakpoints of CLSI (14). In addition to the test organisms, MICs of the following control strains were also tested: *S. aureus* 27R (methicillin resistant), *S. aureus* 25923 (methicillin susceptible) and *Enterococcus faecalis* (*E. faecalis*) poultry isolate (vancomycin resistant).

β-lactamase hyperproduction cause borderline resistance in *mec*A negative isolates. For determination of overproduction of staphylococcal β-lactamase, ADDT was performed with amoxycillin clavulanic-acid (30µg, Oxoid, UK) disc.

***DNA* extraction**: *S. aureus* isolates and the control strains were inoculated into brain hearth infusion broth and incubated at 37°C for 24 h. Then the culture was centrifuged in 12000 rpm for 10 min. Bacterial pellet was resuspended in 200 µl of phosphate buffer solution (PBS) and centrifuged in 12000 rpm for 10 min again. The supernatant was transferred mini centrifuge tube. DNA samples were kept at -20°C until use.

***Detection of mec*A, *fem*A *and van*A genes:** Primers, target genes, PCR product sizes and references used for PCR protocols are presented in Table 1. PCR amplifications for each of *mec*A, *fem*A and *van*A genes were performed in a volume of 25 µl by using primers in Table 1. The reaction mixture of *mec*A, *fem*A and *van*A contained 5 µl template DNA, 12.5 µl 2×PCR Mastermix (Applied Biosystem, Roche, USA) and 1 µl each primers (100 pmol). PCR assay for *fem*A was preformed as follows: initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C 45 s, annealing at 54°C for 45 s and extention at 72°C for 45 s. Finally, a 5 min extention period at 72°C was carried out (28). The amplification of *mec*A gene was performed as previously described by Ardic et al., (3). PCR assay for *van*A amplification; reaction was performed with the following program: an initial denaturation at 95°C for 10 min, 30 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s and polymerisation at 72°C for 30 s, and a final extension at 72°C for 10 min (13). *Staphylococcus aureus* 27R strain and an *E. faecalis* poultry isolate were used as positive control for *mec*A and *van*A genes, respectively. *S. aureus* 25923 was used as positive control strain for *fem*A gene and negative control strain for *mec*A gene.

PCR products were electrophoresed (Scie-Plas, HU10, UK) in 1.5% agarose gel stained with 0.5 µg/ml ethidium bromid at 100 V for 45 min and bands were imaged (EDAS 290, Eastman Kodak Company, Rochester, NY, USA) under UV light (UV-transilluminator, CLP, USA).

Results

In this study, all of 466 milk samples were collected from 233 goats with subclinical mastitis. Bacterial cultures were positive in 122 (26.18%) of 466 milk samples and 126 microorganisms were isolated from the samples. The isolated microorganisms were 53 (42.06%) CNS, 43 (34.23%) *S. aureus*, 16 (12.08%) *E. coli*, 10 (7.94%) *Candida* spp. and 4 (3.17%) *B. melitensis*.

Seven *S. aureus* isolates were found to be oxacillin resistant by ADDM. However, five of these isolates were found to be resistant to oxacillin by MIC test. MIC values for oxacillin were >256 µg/ml in 1 isolate, >128 µg/ml in 2 isolates and >32 µg/ml in 2 isolates. β-lactamase hyperproducers may cause to borderline resistance in *mec*A negative isolates. Thus, the overproduction of staphylococcal β-lactamase was determined with the susceptibility tests to amoxycillin-clavulanic acid (30 µg, Oxoid) in phenotypically MRSA isolates and five isolates were found to be susceptible to this antibiotic.

All of the *S. aureus* isolates (100%) were found susceptible to vancomycin by ADDM and MIC values. Forty three isolates were investigated for and vancomycin resistance genes (*mec*A and *van*A). According to the PCR results, all of the isolates were positive for *fem*A genes. However, *mec*A and *van*A genes were not detected in *S. aureus* isolates.

Table 1. Primers, target gene, PCR product size and references used in the study.
Tablo 1. Çalışmada kullanılan primerler, hedef gen, PZR ürün büyüklüğü ve referanslar.

Target gene	Sequences (5'-3')	Amplicon size (bp)	Reference
*mec*A	5'-CCT AGT AAA GCT CCG GAA-3' 5'-CTA GTC CAT TCG GTC C-3'	314	Choi et al. (11); Ardic et al. (3)
*fem*A	5'-AAAAAAGCACATAACAAGCG-3' 5'-GATAAAGAAGAAACCAGCAG-3'	132	Mehrotra et al. (28)
*van*A	5'-CAT GAA TAG AAT AAA AGTTGCAATA-3' 5'-CCCCTTTAACGCTAATACGATCAA-3'	1030	Clark et al. (13)

Discussion and Conclusion

Mastitis is an important problem in dairy goat herds, due to difficulties in treatment and control. Several researchers reported that CNS was isolated from dairy goats with subclinical and clinical mastitis and the isolation rate was changed between 40%-88.5% (4, 15, 18, 19, 21, 36, 45). In this study, the milk samples were collected from dairy goat herds with mastitis problem. CNS was determined to be the most frequently isolated bacterium (42.06%) from the goat milk. These results confirmed that CNS is the most commonly reported agent in subclinical mastitis of goats (4, 15, 19, 36, 45). However, Bergonier et al., (5) stated that CNS was isolated from subclinical mastitis and *S. aureus* was isolated from clinical mastitis. But, *S. aureus* was the second bacterial agent (34.23%) isolated from goats with subclinical mastitis in this study. This result was found to be higher than the results reported by other studies (4, 15, 18, 19, 21, 36, 45). In this study, *E. coli*, *Candida* spp. and *B. melitensis* were isolated from goat milks, too. The researchers were reported that different microorganisms may cause goat mastitis (18, 19, 21, 27). Mastitis agents may vary depending on the conditions of care in goat farms and the area in which the study is performed.

Treatment of mastitis has been generally made by using antibiotics (7, 15, 36). The use of antibiotics randomly without antibiotic susceptibility test leads to resistance to antibiotics. MRSA isolates with multidrug resistance are generally isolated from human, but these agents have been isolated from several animal species in recent years, too (2, 17, 24, 33). In this study, phenotypic and genotypic methicillin and vancomycin resistance were investigated in *S. aureus* isolates by ADDM, broth macrodilution method and PCR. Phenotypic methicillin resistance was detected in only seven isolates by ADDM. Five of these isolates were determined as methicillin resistant by broth dilution method. MIC values were changed between 32 µg/ml and 256 µg/ml in the isolates. But, *mec*A gene was not detected in these isolates. We thought that these isolates may be shown borderline resistance due to overproduction of β-lactamase. Borderline isolates that do not contain *mec*A gene that have been reported in several studies (9, 22, 25). The hyperproduction of β-lactamase in staphylococci can be determined using β-lactamase inhibitors, such as clavulanate and sulbactam. Thus, we tested the oxacillin resistant isolates by ADDM and macro dilution test with amoxycillin clavulanic-acid disc to determine overproduction of β-lactamase. The isolates were found susceptible to amoxycillin clavulanic-acid. These results support the researchers who reported that the over production of staphylococcal β-lactamase can leads to phenotypic methicillin resistance (9, 22, 25).

*mec*A is a gene encoding resistance to methicillin and PCR is a gold standard method in molecular diagnosis of methicillin resistance (9). Some researchers (38, 45) did not detect *mec*A gene in *S. aureus* isolates from goat milk. However, Aras et al. (2) reported that 2 out of 42 *S. aureus* isolated from goat with clinical mastitis were identified as MRSA by disc diffusion test in Turkey. Also, they were determined the presence of *mec*A gene in *S. aureus* isolates by PCR. According to the study results, the researchers suggested that MRSA might be causative agent for goat mastitis represent a major concern for public health. But, our results support the researchers (2, 38, 45) who reported that goat milk does not play a significant role in the spreading of MRSA and this situation does not represent a great public health concern.

*fem*A gene is a marker used for genotypic identification at the species level of *S. aureus* isolates (3, 9). In this study, the *fem*A gene was investigated in the isolates and the control strains, and all of them were found positive for *fem*A. MRSA is the most important pathogen isolated from human nosocomial infections in recent years, and MRSA has been isolated from various animals (2, 17, 39, 43), too. The treatment of MRSA infections in humans has been made by vancomycin being final choice (9, 20, 31). Presence of VRSA has been investigated by phenotypic methods in goat and cattle milk and phenotypic vancomycin resistance were not determined in *S. aureus* isolates (26, 45, 46). On the other hand, Umaru et al., (44) reported that phenotypic VRSA was isolated from fresh and fermented milk in Nigeria. But, in this study, the presence of *van*A gene which is regarded as the gold standard was not investigated by PCR. Similarly, Bhattacharyya et al. (6) reported VRSA and VISA strains in goat milk. But, the presence of *van*A could not be determined in this study. Although several genes encoding vancomycin resistance have been determined in enterococci, *van*A gene is generally detected in VRSA isolates (13). Until today, VRSA isolates including *van* genes encoding vancomycin resistance were not determined in farm animals (6, 13, 26, 34, 40, 45, 46). Although MRSA was generally reported in mastitis cases of farm animals (2, 12, 16, 17, 22, 43), VRSA has not been determined in animals, yet. In this study, MRSA and VRSA were not detected in goat herds with mastitis problems, too.

In summary, this study shows that *S. aureus* and CNS are found to be the most isolated species from goat milk in Burdur province. However, the absence of *mec*A and *van*A genes in the *S. aureus* isolated from goat milk showed that goat milk does not play a significant role in the spreading of MRSA.

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Relationship between seropositivity of *Encephalitozoon cuniculi* and renal biochemical markers in clinically healthy rabbits

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Summary: *Encephalitozoon cuniculi* can cause latent disease, especially in lagomorphs and many wild and domestic animals in various countries. This infection is important for veterinary and public health because it is caused by a potentially zoonotic and opportunistic pathogen. The aim of this study was to investigate the relationship between seropositivity of *E. cuniculi* and renal function markers, which may be useful in predicting the disease in clinically healthy rabbits. In this study, the seropositivity of *E. cuniculi* infection in clinically healthy rabbits was determined, and necropsy findings were evaluated along with the results of renal function tests. In a laboratory rabbit breeding facility, enzyme-linked immunosorbent assay (ELISA) tests showed that 48 (49.5%) of 97 rabbits were seropositive against *E. cuniculi*. Blood urea nitrogen (BUN) and creatinine levels were significantly elevated in seropositive animals. Two seropositive rabbits were necropsied to confirm the infection. According to histopathological findings in the kidney, degenerative changes and *E. cuniculi* spores were identified in the tubule epithelia. Serum creatinine ($p<0.001$) and BUN ($p<0.01$) levels were found to have a statistically significant relationship with the serological status of rabbits. Serological and histopathological methods are not routinely used in rabbits to diagnose *E. cuniculi* infection. The kidneys were one of the most affected organs in encephalitozoonosis in rabbits. As revealed in this study, the testing blood urea nitrogen and creatinine levels would be useful for the evaluation of general health status and renal function of the seropositive rabbits, and clinical interpretation as well.

Keywords: Biochemical markers, *Encephalitozoon cuniculi,* rabbit, renal function.

Sağlıklı tavşanlarda *Encephalitozoon cuniculi* seropozitifliği ile böbrek biyokimyasal belirteçleri arasındaki ilişki

Özet: *Encephalitozoon cuniculi,* özellikle tavşangillerde ve çeşitli ülkelerdeki birçok yaban ve evcil hayvanlarda latent hastalığa neden olabilmektedir. Bu enfeksiyon potansiyel olarak zoonotik ve fırsatçı bir patojenden kaynaklandığından veteriner ve halk sağlığı için önemlidir. Bu çalışmanın amacı, klinik olarak sağlıklı tavşanlarda *E. cuniculi* seropozitifliği ve böbrek fonksiyon belirteçleri arasındaki ilişkinin, hastalığın tanısında yararlı olabileceğini araştırmaktır. Bu çalışmada, sağlıklı tavşanlarda *E. cuniculi* enfeksiyonunun seropozitifliği enzim bağlantılı immünosorbent testi (ELISA) ile belirlenmiş ve böbrek fonksiyon test sonuçları ile otopsi bulguları birlikte değerlendirilmiştir. Bir laboratuvar tavşan yetiştirme tesisinde, ELISA testi, 97 tavşanın 48'inin (%49.5) *E. cuniculi*'ye karşı seropozitif olduğunu göstermiştir. Seropozitif hayvanlarda kan üre nitrojen (BUN) ve kreatinin düzeyleri anlamlı olarak yüksek bulunmuştur. Serum kreatinin ($p<0.001$) ve BUN ($p<0.01$) düzeylerinin tavşanların serolojik durumu ile istatistik olarak anlamlı bir ilişkisi olduğu anlaşılmıştır. Enfeksiyonun doğrulanması için iki seropozitif tavşan otopsi edildi. Böbrekteki histopatolojik bulgulara göre tübül epitelinde dejeneratif değişiklikler ve *E. cuniculi* sporları tespit edilmiştir. *Encephalitozoon cuniculi* enfeksiyonunu teşhis etmek için tavşanlarda serolojik ve histopatolojik yöntemler rutin olarak kullanılmamaktadır. Böbrekler tavşanlarda ensefalitozoonosisde en çok etkilenen organlardan biridir. Çalışmanın da gösterdiği üzere, kan üre nitrojen ve kreatinin düzeylerinin belirlenmesi, seropozitif tavşanların böbrek fonksiyonlarının değerlendirilmes, klinik ve genel sağlık durumunu yorumlanması adına yararlı olabileceği anlaşılmaktadır.

Anahtar sözcükler: Biyokimyasal belirteçler, böbrek fonksiyonu, *Encephalitozoon cuniculi,* tavşan.

Introduction

Encephalitozoon cuniculi is a Gram-positive, obligate intracellular parasite distributed worldwide. This parasite can cause a potential latent infection in many wild and domestic animal species, including humans, and is best documented in the lagomorphs (12, 15, 16, 20). Encephalitozoonosis is one of the most common health problems in rabbits, and it is found at industrial and family farms and in pet, zoo and laboratory rabbits in many countries (16, 21, 37). The determination of antibody

serostatus is useful to confirm exposure to the parasite, but antibody serostatus is also reported not to be correlated with clinical signs of a true infection (6, 7, 33), which ranges from a total lack of symptoms to sudden death in infected rabbits. Serologic assays such as enzyme-linked immunosorbent assay (ELISA) is considered to be the most useful tools for antemortem diagnosis of the infection in different populations (6, 8).

The rabbits' main organs primarily affected by the parasite are kidneys and brain. The antibodies develop three to four weeks after infection, parallel to the increased antibody titers over time, normally the first alterations occur in the kidney and later in the brain. The pathogen primarily localized in renal cortical tubular epithelium and sometimes in the glomerular epithelium. Consequently, histopathological changes occur and it may result in kidney failure. Later, renal disease leads to clinical polydipsia and polyuria (10, 16, 17, 33). In addition, postmortem findings such as histopathological signs and anatomic localization of the lesions are not proportionally correlated with antemortem symptoms (19, 33). Postmortem diagnosis of *E. cuniculi* is required for definitive diagnosis. Using this method, the spores can be identified in many chronically infected tissues (1, 33, 36). Postmortem morphological lesions in the kidneys are characteristic pitted appearance of the shrunken fibrotic kidneys. The lesion often considered to be diagnostic for confirmation of the infection in rabbits. On the other side, renal damage in living animals, biochemical parameters mainly creatinine and blood urea nitrogen are used as important kidney function markers in the accurate diagnosis of the renal impairment. In rabbit breeding, changes in biochemical parameters can also be used as indicators of welfare status and provide important information for clinicians for monitoring many diseases (9, 25, 29).

The main objective of this study is to determine the relationship between *E. cuniculi* seropositivity and biochemical renal function markers that may be useful in predicting the disease in clinically healthy rabbits. For this purpose, the serology of *E. cuniculi* infection and the results of renal function tests were evaluated in the context of autopsy findings in a laboratory rabbit breeding facility.

Materials and Methods

All animal practices have been assessed and approved by the National Animal Experiment Central Ethics Board in accordance with the national ethical regulation (2018/111334-2b).

Animals and blood samples: In this study, New Zealand white rabbits were breed as *in vivo* test material in a laboratory rabbit breeding facility. The animals were housed individually in Noryl cages in a controlled room under a temperature of 18 to 20°C, relative humidity of 50±5% and a 12:12-hour light:dark cycle. The rabbits were fed a standard commercial pellet diet and provided with water ad libitum. The rabbits (62♂, 35♀/3-41 months of age) were monitored for the health status. Each rabbit was checked daily for water and feed intake, and their urine and feces output was also tracked. The animals were examined monthly for weight gain. Blood samples were taken for serological and biochemical analysis under aseptic conditions from the marginal ear veins of each animal immobilized in a restraint box. Then the sera were separated and stored at -20°C until the serological and biochemical analyses.

Serology: To determine the *E. cuniculi* specific antibody responses in the rabbits, an ELISA kit (Express Biotech International-USA) containing positive and negative controls (rabbit serum) was used according to the manufacturer's instructions. The difference between sample optical density (OD) and negative-control OD (Δ) was greater than or equal to 0.300, the sample was evaluated as positive.

Biochemical analyses: In the serum samples, activity aspartate amino transferase (AST); alanine aminotransferase (ALT); gamma-glutamyl transferase (GGT); lactate dehydrogenase (LDH); alkaline phosphatase (ALP); concentrations of glucose, triglycerides, total cholesterol, albumin, total protein, creatinine and blood urea nitrogen (BUN); uric acid; creatin kinase (CK); total bilirubin (TB); calcium (Ca); phosphorus (P) and magnesium (Mg) were determined using a fully automated BT 3000 plus biochemistry analyzer (Biotecnica Instruments S.p.A., Italy) according to the manufacturers' instructions. Commercial diagnostic kits (Quimica Clinica Aplicada) were used for the determination of biochemical parameters. Analyzer compositions were completely adjusted to our needs for clinical chemistry. The biochemical analyzer was calibrated for the reference point, which facilitates accurate results for each biochemical marker. Reference standard controls were also run before each determination, and the values obtained for the different biochemical parameters were always within the expected ranges.

Histopathology: First, necropsy was performed to confirm the infection and to show how the infection had damaged the tissues affected. For this reason, two seropositive rabbits were selected randomly and examined postmortem after species specific-euthanasia protocol (xylazine 60 mg/kg and ketamine hydrochloride 100 mg/kg via intramuscular injection), and all organs and tissues were examined. The pathological changes were recorded according to general macroscopic criteria. In particular, the changes in kidneys, brain, lung and liver were examined thoroughly. Samples from the tissues were collected for histopathological examination during the necropsy, and fixed in buffered 10% formalin for 24 hours. After the fixation, tissues were embedded in paraffin wax and processed according to routine methods.

Sections of five microns in thickness were cut from paraffin blocks and stained with hematoxylin and eosin (H&E). The findings, mainly in kidney and brain, were evaluated under a digital light microscope (Euromex).

Statistical analyses: The Kolmogorov-Smirnov and Shapiro Wilk normality tests were performed, with the number of subjects in infected and non-infected groups, respectively, to be more or less than fifty. After the normality tests, the logarithmic transformation was first applied to the variables with no normal distribution, then the variables were tested again in the normality test. The analysis determined that all the variables in the dataset showed the normal distribution feature. The difference between the mean values of the variables examined in infected and non-infected animals was analyzed via an independent sample t-test. The effects of gender and age on infected animals were analyzed via two-way analysis of variance, and the difference between the mean values of the variables according to gender were compared using a Bonferroni correction. Mean values of antibody titer were compared via an independent sample t-test. The descriptive statistics for the variables are given in terms of mean and standard deviation. In all statistical analyses, $p<0.05$ was taken as significant. Statistical analyses were performed using the SPSS V.22 statistical package program.

Results

Animals: The appetite of the animals was normal, and lethargy, weight loss, polydipsia and polyuria were not observed in the animals throughout the study. No clinical signs and no mortalities due to infection were seen. All the animals (n: 97) were evaluated as clinically healthy.

Serology: The examination of 97 rabbits using ELISA showed that 48 (49.5%) were seropositive against the parasite. The gender distribution was 73% males and 27% females in seronegative animals and 54% males and 46% females in the seropositive group. In the seropositives, sex and age groups alone and the combined effects of these two factors were not statistically significant ($p>0.05$).

Biochemistry: The biochemical parameters detected in the rabbits are given in Table 1. In seropositive rabbits, the levels of protein, ALP, BUN, creatinine, cholesterol, P and glucose were significantly higher than in seronegative animals. No significant differences were observed in terms of other biochemical markers between *E. cuniculi* seronegative and seropositive serum samples. Levels of albumin ($p<0.001$) and urea ($p<0.01$) in seropositive females were lower than in seropositive males. Furthermore, creatinine ($p<0.001$) and urea ($p<0.001$) levels of seropositive males were higher than those of seronegative males. On the other hand, P ($p<0.001$), cholesterol ($p<0.001$) and glucose ($p<0.05$) levels of seropositive males were lower than the seronegative males. In seropositive female rabbits, levels of creatinine ($p<0.001$) and protein ($p<0.001$) were higher than in seronegative females, but cholesterol ($p<0.05$) and ALP ($p<0.001$) levels of seronegative females were found to be higher than those of seropositive females. According to the two-way ANOVA results, gender-based differences between mean values in albumin, protein and urea variables were significant ($p<0.05$) in seropositive rabbits.

Table 1. Changes in biochemical parameters of rabbits with *Encephalitozoon cuniculi* infection.
Tablo 1. *Encephalitozoon cuniculi* enfeksiyonu olan tavşanların biyokimyasal parametrelerinde değişiklikler.

PARAMETERS	CLINICALLY HEALTH ANIMALS								*P*
	INFECTED				NON-INFECTED				
	N	MEAN	S.E.M	%95 C.I	N	MEAN	S.E.M	%95 C.I	
Albumin (g/L)	48	46.87	0.57	45.20-48.15	49	47.76	0.91	45.59-48.88	*p*>0.05
AST (U/L)	48	33.90	3.51	27.89-39.84	48	33.98	2.29	27.11-40.51	*p*>0.05
Protein (g/L)	48	72.83	0.99	70.95-74.37	47	68.00	0.76	65.40-69.26	***p<0.001***
ALP (U/L)	48	48.98	5.81	29.72-98.18	47	156.72	13.22	151.0-194.3	***p<0.001***
ALT (U/L)	48	27.29	4.16	20.30-33.39	47	28.17	2.18	23.49-38.15	*p*>0.05
GGT (U/L)	48	10.09	0.83	8.80-11.44	47	9.75	0.40	8.25-11.22	*p*>0.05
BUN (mg/dL)	48	43.44	1.66	39.80-46.30	47	36.62	1.73	33.16-40.50	***p<0.01***
Creatinine (mg/dL)	48	1.16	0.04	1.06-1.26	47	0.82	0.05	0.69-0.91	***p<0.001***
Ca (mg/dL)	48	13.16	0.17	12.89-13.44	47	13.29	0.07	12.98-13.60	*p*>0.05
CK (U/L)	48	479.29	45.61	382.4-527.4	47	565.11	51.20	410.7-624.5	*p*>0.05
LDH (U/L)	48	357.19	27.18	310.1-404.9	47	368.70	21.64	282.0-388.8	*p*>0.05
TB (mg/dL)	48	0.27	0.032	0.22-0.33	47	0.26	0.023	0.18-0.31	*p*>0.05
Mg (mg/dL)	48	2.75	0.11	2.50-3.02	47	2.42	0.14	2.25-2.84	*p*>0.05
P (mg/dL)	47	4.06	0.18	3.73-4.40	47	5.23	0.15	4.93-5.68	***p<0.001***
Cholesterol (mmol/L)	46	38.65	3.22	33.70-45.14	47	53.21	3.56	54.80-67.40	***p<0.01***
Uric Acid (mg/dL)	45	0.47	0.05	0.37-0.58	47	0.50	0.05	0.37-0.60	*p*>0.05
Triglyceride (mg/dL)	45	92.98	9.41	77.66-110.4	47	102.26	6.62	86.64-122.2	*p*>0.05
Glucose (mg/dL)	45	116.16	3.82	106.6-126.4	47	130.32	5.75	122.5-127.9	***p<0.05***

C.I: Confidence interval.
C.I: Güven aralığı.

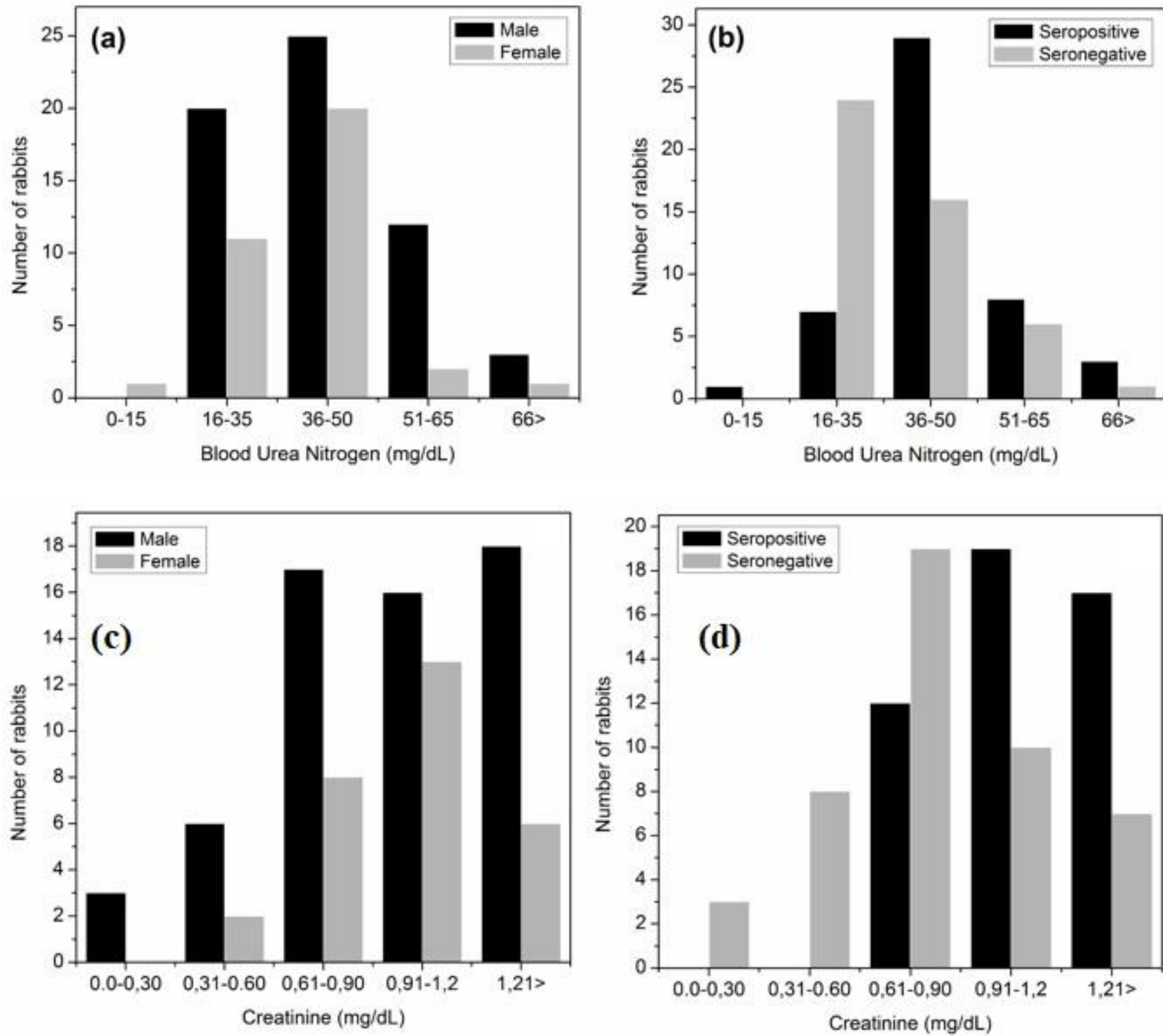

Figure 1. The blood urea nitrogen (a and b) and the creatinine (c and d) level of serum according to serostatus (b and d) of animals and gender (a and c) of rabbits.
Şekil 1. Hayvanların serolojik durumuna (b ve d) ve cinsiyete (a ve c) göre kan üre nitrojen (a ve b) ve serum kreatinin düzeyi (c ve d).

Serum, BUN and creatinine levels were significantly elevated in seropositive animals (Fig 1). However, the serum urea levels in these animals exhibited non-significant changes when compared to seronegative rabbits. Although the seropositive rabbits had azotemia and increased creatinine concentration, no clinical symptoms indicating the renal failure were inspected. In terms of these criteria, possible differences between genders have been evaluated, but no significant variation was detected.

Histopathology: Two seropositive rabbits were necropsied to confirm the infection, and specimens from their kidneys, brain, lung and liver were stained with H&E and examined under a light microscope at high magnifications for histopathological analysis. At necropsy, kidney tissues were observed to be normal in appearance, and there was no macroscopic damage (Fig 2A). The both cortical and medullar tubules were degenerated in kidneys of both animals according to histopathological findings. The degenerative changes were related to acute cell swellings and vacuolar degeneration. Most of the tubule epithelia had lost their typical pink cytoplasm. Their nuclei had lost their chromatin in many corticomedullar areas. *E. cuniculi* spores were identified in degenerated tubule epithelia. In the interstitial tissue of some corticomedullary junctions of the kidneys, mononuclear cell infiltrations composed of lymphocytes and macrophages were inspected multifocally. In the renal medulla, only a milder inflammatory response was seen, and such changes were observed only in a few interstitial areas (Fig 2B and C). In the brain, the meninges of both the cerebrum and

cerebellum were hyperemic. The brain tissues were edematouse in appearance (Fig 2D). Histopathology of all brain tissue revealed that there were karyopyknosis in Purkinje cells and some demyelination areas in the substantia alba of the cerebellum. *E. cuniculi* spores were seen freely in some demyelinated areas, as well as degenerated neurons (Fig 2E and F). Additionally, there were some lesions in the liver. In the livers of both animals, mottled appearance and discolorisation were observed, with hyperemic and possibly degenerative areas (Fig 2G). Histopathology confirmed that there were hyperemia in the central veins and sinusoids and acute cell swelling in the hepatocytes, as well as Kupffer cell activations (Fig 2H). Another affected organ was the lungs. The lungs were hyperemic (Fig 2I); however, there were no mononuclear cell infiltrations and/or cell degenerations like those found in the kidney and the liver, except hyperemic capillaries.

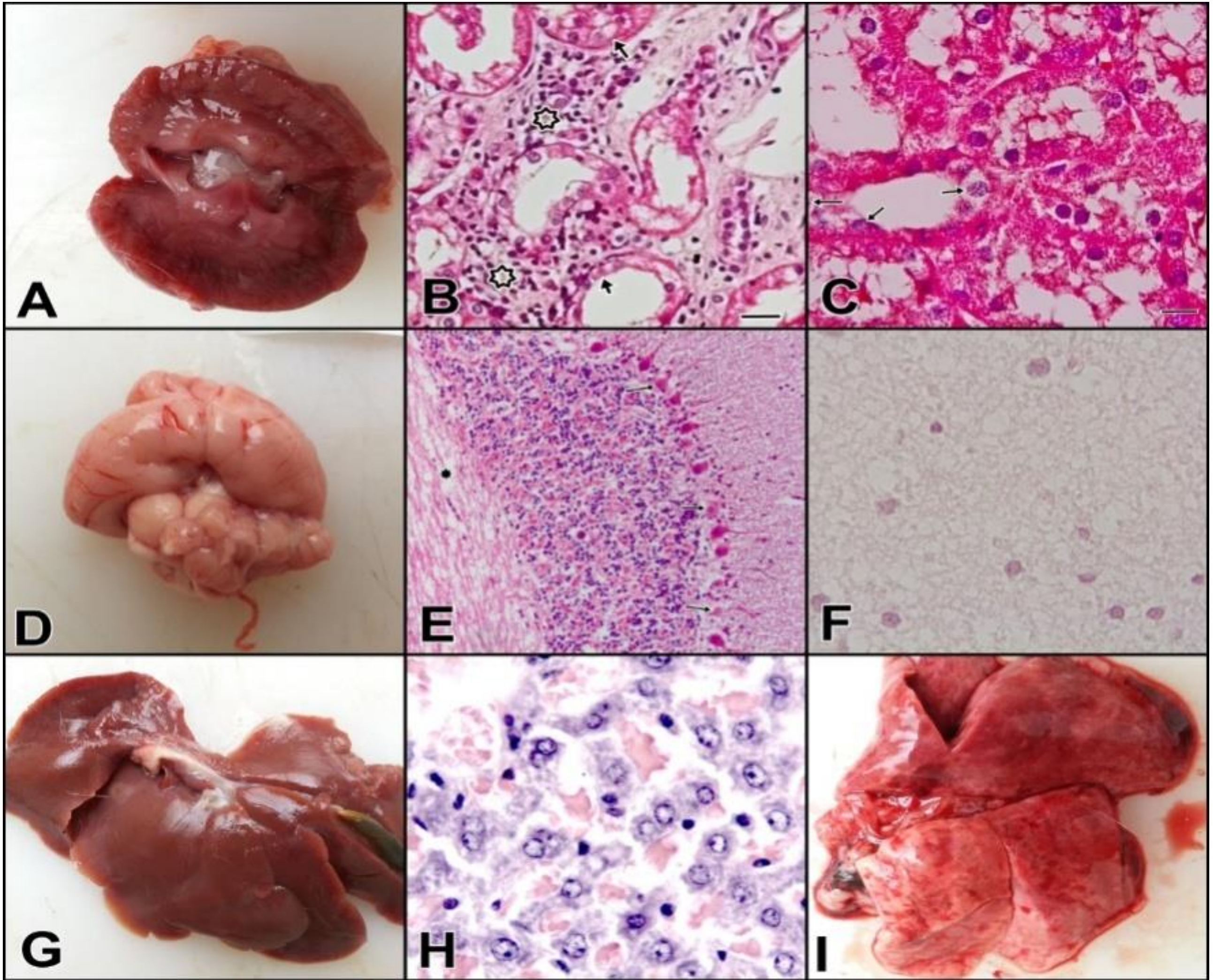

Figure 2. Macroscopical and histopathological findings in organs. **A:** Pale appearance in some cortex areas and hyperemia in the kidney. **B:** Mononuclear cell infiltration at interstitium (arrows) and degeneration in tubul epitheliums (arrows), x100, H&E. **C:** Vacuolar degeneration in tubul epitheliums (arrows), kidney, x400, H&E. **D:** Hyperemia in the cerebrum and cerebellum. **E:** Karyopyknosis in Purkinje cells (arrows) and demyelination areas (asterix) in cerebellum, x40, H&E. **F:** *Encephalitozoon cuniculi* parasites in demyelinated areas of substantia alba in cerebellum, x250, H&E. **G:** Vascular congestion and mottled discoloration due to pale areas in liver. **H:** Increase in Kupffer cell activations (arrows) and parenchymatous degeneration in hepatocytes (arrowheads), x400, HXE. **I**: Vascular congestion in lung.

Şekil 2. Organlarda makroskopik ve histopatolojik bulgular. **A**: Bazı korteks bölgelerinde solgun görünüm ve böbrekte hiperemi. **B**: İnterstisyumda mononükleer hücre infiltrasyonu (oklar) ve tubul epitelyumlarında dejenerasyon (oklar), x100, H&E. **C**: Tubul epitelyumlarında vakuolar dejenerasyon (oklar), böbrek, x400, H&E **D**: Serebrum ve serebellumda hiperemi. **E**: Serebellumda Purkinje hücrelerinde (oklar) ve demiyelinasyon bölgelerinde (asteriks) karyopyknosis x40, H&E **F**: Serebellumda substantia albanın demiyelinize bölgelerinde *Ensephalitozoon cuniculi* parazitleri x250, **G**: Karaciğerdeki damar tıkanıklığı nedeniyle soluk alanlar ve benekli renk değişimi. **H**: Kupffer hücre aktivasyonlarında artış (oklar) ve hepatositlerde (ok başı) parankim dejenerasyonu. x400, H&E **I**: Akciğerde konjesyon.

Discussion and Conclusion

Rabbits are widely used as a model for *in vivo* studies, including in biomedical studies, surgery, atherosclerosis research, antibody production (5, 39, 43). *E. cuniculi* was first recognized in laboratory rabbits by Wright and Craighead. It has been reported since 1922 in various studies in veterinary and human medicine employing different serologic diagnostic tools in numerous sample sizes and hosts (23, 30, 31, 38, 40). Most latent *E. cuniculi* infections do not cause clinical symptoms, so the ability to diagnose these infections is very limited. In addition, these infections can have a significant effect on the outcome of *in vivo* studies. Therefore, screening for this infection has been recommended by the Federation for Laboratory Animal Science Associations for the health monitoring programme of breeding and experimental colonies, particularly in small laboratory animals (22). In the present study, we investigated the role of renal function biochemical markers, which may be useful in predicting the infection in clinically healthy rabbits.

The infection is routinely diagnosed with antemortem clinical findings, but the clinical diagnosis is not easy in living rabbits because *E. cuniculi* infection has a subclinical course. Specific antibodies develop within 21 days post-infection (11, 33). In clinically healthy rabbits, the present study showed that the seropositivity rate for *E. cuniculi* infection was 49.5%, which is similar to rates (50–53%) of infection in previous studies (6, 14, 20), although it is higher than the rates of infection reported in some (4, 13, 17, 26, 28, 30-32). In contrast, in the current study, the seropositivity rate of the disease was lower than those previously reported (71–93%) in various countries (3, 10, 21, 37). Serostatus was also investigated by sex, and no difference was found between sexes in terms of seropositivity (Fig 1). There is strong variability between reports, which may be due to varying breeding practices, husbandry conditions and hygiene rules across facilities and immune status of animals. It is difficult to reduce the prevalence of the infection because the disease is spread by spores excreted in the urine of infected animals.

In infected rabbits, clinical symptoms of renal insufficiency such as weight loss, numbness and loss of appetite may be seen, and some rabbits may develop urinary incontinence and become polydipsic and polyuric (36). In our work, no clinical signs were seen throughout the study. Nevertheless, laboratory analysis can be helpful in identifying the cause and degree of renal damage in living animals, and for this reason, biochemical markers of kidney function such as creatinine, urea, uric acid and electrolytes play an important role in the accurate diagnosis of renal impairment (17, 25, 33). In rabbits, from another angle, the results of biochemical tests can be difficult to interpret in the diagnosis of renal disease, but levels of serum urea nitrogen and creatinine are still assessed as indicators of impaired renal function in animals. Harcourt-Brown (10) also reported that elevated urea and creatinine concentrations in blood are usually associated with renal disease and antibody response to *E. cuniculi.* In the current study, no direct clinical evidence was determined for renal disease in apparently healthy animals, but BUN and creatinine concentrations were found to be higher in seropositive animals than in seronegative ones (Fig 1). High levels of urea and, especially, high creatinine levels could be associated with *E. cuniculi* infection in rabbits. Significantly increased serum creatinine levels in seropositive animals could be attributed to the renal lesions induced by *E. cuniculi* spores. In our study, serum creatinine ($p<0.001$) and BUN ($p<0.01$) levels were found to have a statistically significant relationship with the serological status of rabbits (Table 1). Our results are consistent with those of previous studies (1, 3, 10, 33). On the other hand, numerous studies have reported that blood biochemistry parameters can be used to measure differences between animals. There are some factors related to the animals (age, species, strain and sex), environmental conditions, bleeding, sampling procedures and analysis method (24, 27). In this respect, apart from these factors, the autopsy was performed to demonstrate and confirm that the damage to the rabbit kidney was pathogen-dependent after serological and biochemical analysis. Twenty-eight days after infection, pathological lesions appear in the kidneys, and at this time, spores can be found in the urine. In rabbits, the first and most significant alterations are in the kidneys. The infection causes severe inflammation, interstitial nephritis and finally chronic renal impairment, but the most common clinical findings are neurological signs (1, 2, 18, 25, 33, 41, 42). In the early stages of encephalitozoonosis, the spores are found in renal tubules, epithelial cells or macrophages and within the collecting ducts as free, with minimal involvement of the glomeruli. In our study, after evaluating all tissues mainly kidney, brain, as well as tissues of other parenchymatous organs, such as the liver and the lungs tissue was selected for post-mortem diagnosis because parasite spores were detected more frequently in kidney tissue than in any other tissue. According to previous data pertaining to histological studies, an area containing dark red and grey, depressed, irregular pits and subcapsular foci on the surface of the renal cortex is considered indicative of encephalitozoonosis during necropsy (1, 34-36). In our study, there were no conspicuous findings macroscopically. However, histopathologically, epithelia of the cortical and medullary tubules were degenerated due to acute cell swellings and vacuolar degeneration in the early stages of encephalitozoonosis. The organism can be found within epithelial cells, macrophages,

inflammatory foci or free within collecting tubules. Tubule epithelia had lost their pink cytoplasm, and *E. cuniculi* spores were detected in degenerated tubule epithelia. Mononuclear cell infiltrations were seen in the interstitium of renal tissues. The spores caused a milder inflammatory response in the renal medulla. These changes were observed only in a few interstitial areas. Rodríguez-Tovar et al. (36) reported that the inflammatory reaction could have occurred in the cortical regions of the kidney. In our study, inflammatory foci were also observed in the interstitium of kidneys. On the other hand, the same authors again have documented that granulomatous foci in other organs might be seen, and these foci might mask the *E. cuniculi* spores. In our study, we did not encounter any granulomatous foci or *E. cuniculi* spores in the liver or the lungs. Other internal and parenchymatous organs did not already show either these inflammatory reactions or degeneration caused by the parasite. The lesions were located only in the liver and the lungs. Particularly, the hepatocytes degenerated as well as the Kupffer cell activations. This situation demonstrates that the parasite can select a particular organ, such as the kidneys and the central nervous system.

In conclusion, recent studies on healthy rabbits, in particular, have shown that more than 50% of the rabbits were positive for *E. cuniculi* antibodies, but the clinical interpretation of the results was difficult. Serological and histopathological methods are not routinely used in rabbits to diagnose the infection. In *E. cuniculi* infection in rabbits, there are several clinical evidences including kidney failure, reduced appetite, weight loss, excessive water consumption and increased urine output. Because the pathogen was primarily localized in the kidney was affected organ, testing blood urea and creatinine levels would be useful for the evaluation of general health status and renal function of the rabbits, and for clinical interpretation as well. For this reason, possible organ damage caused by the infection in rabbits was interpreted according to the blood analysis results. We believe that serological tests, increased BUN and creatinine levels, in particular, might be useful indicators for diagnoses of renal damage in *E. cuniculi* infected in laboratory animals.

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Kuzu enteritislerinde Cryptosporidiosis hastalığının patolojik yöntemlerle araştırılması*

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Özet: Çalışmanın amacı kuzu ishallerinde önemli bir patojen olan *Cryptosporidium parvum (C. parvum)* etkenlerinin bağırsak smearı ve parafin kesitlerden, Modifiye Ziehl-Neelsen (MZN), histopatoloji, immunohistokimya (İHK) ve immunfloresan (İF) boyama yöntemleri ile belirlemek ve Cryptosporidiosis hastalığının 30 günlüğe kadar olan kuzulardaki yaygınlığını ortaya koymaktır. Bu amaçla, ishal belirtisi gösteren ve agoni halde iken nekropsi yapılmış 60 adet ve yine ishal belirtisiyle ölmüş 85 adet (toplam 145 adet) kuzudan alınan bağırsak örnekleri kullanılmıştır. MZN boyama sadece, agoni halde iken nekropsileri yapılan 60 kuzudan alınan taze incebağırsak örneklerinden hazırlanan smear örneklerine yapılmıştır. Bu 60 örnekle birlikte ölü olarak getirilen 85 kuzudan (toplam 145) elde edilen incebağırsak örneklerinden hazırlanan parafin kesitlerden ise histopatoloji, İF ve İHC boyamaları prosedürüne uygun olarak yapıldı. MZN boyamalarda (60 örnek) %13.3 oranında *Cryptosporidium spp.*, parafin blokları hazırlanan toplam 145 örnekten alınan kesitlerin IF ve IHC boyamalarda ise %13.8 oranında *C. parvum* pozitif reaksiyon gözlenmiştir. Etken tespit edilen bağırsak kesitlerinin histopatolojik incelemelerinde; değişen derecelerde olmak üzere; epitel hücrelerinde nekroz, deskuamasyon ve villuslarda kütleşme, epitel yüzeyi boyunca *Cryptosporidium spp.* etkenleri, propria mukozada hiperemi ve mononükleer hücre infiltrasyonu, kript epitelinde mitoz artışı, lenfatik kanalda genişleme, intraepitelyal lenfositler ve bazı örneklerde *Eimeria spp* gözlendi. Sonuç olarak çalışmada İF, İHC ve MZN boyamalarının kuzularda Cryptosporidiosis hastalığını ortaya koymada etkili olduğu görülmüştür.

Anahtar sözcükler: *C. parvum*, Cryptosporidiosis, enteritis, histopatoloji, immunfloresan, immunohistokimya.

Examination of Cryptosporidiosis in lamb enteritis by pathological methods

Summary: The aim of this study is to determine *C. parvum* in small intestinal samples of lambs (up to 30 days old) with enteritis. For this aim, fresh intestinal smears prepared from 60 lambs (euthanized due to diarrhea) and stained with Modified Ziehl-Neelsen (MZN). In addition to these 60 fresh samples, 85 different small intestinal samples obtained from dead lambs (total 145 small intestinal samples) were embedded in paraffin for histopathology, immunofluorescence (IF) and immunohistochemistry (IHC). Slides were stained according to protocols (MZN, hematoxylin-eosin, IHC and IF). In MZN staining, 13.3% of smear samples were found positive for *Cryptosporidium spp*. Besides, *C. parvum* positivity was detected in the rate of 13.8% in IF and IHC stains. In the histopathologic examination, desquamation and necrosis of intestinal epithelium, villus atrophy, *Cryptosporidium spp.* along the surface of villus epithelium, hyperemia, mononuclear cellular infiltration, mitosis in crypt epithelium, intraepithelial lymphocytes, dilatation in the lymphatic canal and *Eimeria spp.* in some cases were observed in varying degree. In conclusion, IF, IHC and MZN stains were found effective in revealing cryptosporidiosis in lambs.

Keywords: *C. parvum*, Cryptosporidiosis, enteritis, histopathology, immunofluorescence, immunohistochemistry.

Giriş

Cryptosporidiosis, birçok hayvan türü ve insanda Apicomplexa sınıfına ait koksidian bir protozoon olan Cryptosporidium'lar tarafından oluşturulan, özellikle ruminantların neonatal dönemlerinde etkisini gösteren ve aynı zamanda zoonoz karakterli bir enfeksiyondur (7, 8, 14, 31). Merogoni, gametogoni ve sporogoni dönemleri enfekte epitel hücrelerinin fırçamsı kenarlarında gelişen etkenler, konakçının hücre membranı ile çevrili vakuoller içinde bulunurlar (5). Sporozoit membranında bulunan özel bir protein olan CP47, epitel hücreleri boyunca fırçamsı kenarda yer alan hedef hücrelerdeki bir glikoprotein olan P57 aracılığı ile hedef hücrelere bağlanır (26). *C. parvum* kuzularda alt jejenum ve ileumda yerleşirken, şiddetli olgularda sekum, kolon ve rektuma kadar yayılabilir (7).

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Cryptosporidiosis genç ruminantlarda çoğunlukla enterotoksijenik *E. coli*, rotavirus ve coronavirus ile birlikte bulunacağı gibi primer patojen olarak da etkili olabilir (5, 10). İntestinal Cryptosporidiosis tüm türlerde değişen derecede villus atrofisi ile kendisini gösterir. Mukoza epiteli çoğu zaman kübik, yuvarlak ya da kısa prizmatik değişikliğe uğrar. Villus epitelinin mikrovillus yüzeyi boyunca çok sayıda *Cryptosporidium spp.* görülebilir. Böylece absorptif hücre yüzeyleri büyük oranda cryptosporidium etkenleri tarafından tutulmuştur. Mukoza yüzeyinde olgunlaşmamış epitel hücre populasyonunda artış vardır. Başta prostoglandinler olmak üzere yangısal mediatörlerin salgılanması mukozal sekresyonu ve epitel hücrelerinin permeabilitesini arttırmıştır. Villus atrofisi ile birlikte gelişen absorpsiyon bozukluğuna bağlı olarak ishal gelişir (5, 25). Günümüzde genom dizi analizleri tam olarak ortaya konulan türler *C. parvum ve C. hominis* olup *C. xiaovi, C. bovis, C. ubiquitum* türler de son yıllarda yaygınlığı bakımından çalışma konusu olmaktadır (14, 15).

Sunulan çalışma, Konya ve çevresinde ishal bulgusu gösteren kuzularda (30 günlüğe kadar), MZN, histopatoloji, İHK ve İF boyama yöntemleri ile *C. parvum* teşhisi yapmak ve ishalli kuzularda Cryptosporidiosis hastalığının yaygınlığını ortaya koymak amacıyla yapılmıştır.

Materyal ve Metot

Atatürk Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu (31.05.2013/2) tarafından çalışmanın yürütülmesi uygun görülmüştür.

Çalışma materyali: Konya ve çevresinde 129 farklı sürüden, 2013-2014 yıllarında, işletme sahiplerinin verdiği anamnez bilgileri doğrultusunda, yaşları 30 günlüğe kadar olan 85'i ishal belirtisiyle ölmüş, 60'ı ise klinik olarak ishal bulgusu gösterip agoni halinde ötenazi ve nekropsi yapılan, toplam 145 adet kuzudan elde edilmiş ince bağırsak örnekleri, çalışmada materyal olarak kullanılmıştır.

MZN boyaması: MZN boyama agoni haldeyken nekropsi yapılan 60 kuzudan taze hazırlanmış ince bağırsak mukozası kazıntı örneklerine uygulanmıştır. Metil alkol ile tespit edilen kazıntı örnekleri ticari temin edilen boyama seti (ZIEHL-NEELSEN modified for Cryptosporidium, Bio-optica, 04–110803, İtalya) ile protokolüne uygun olarak boyanmıştır.

Histopatoloji İşlemleri: Agoni halde getirilmiş ishalli (60 kuzu) ve ishal belirtisiyle ölmüş (85 kuzu) toplam 145 kuzudan alınan ince bağırsak örnekleri %10'luk tamponlu formalin solusyonunda tespit edildi. Rutin histopatoloji takip işlemleri sonrasında hazırlanan parafin bloklardan 5 mikron kalınlığında kesitler alınarak hematoksilen-eozin (HE) ile boyama yapıldı.

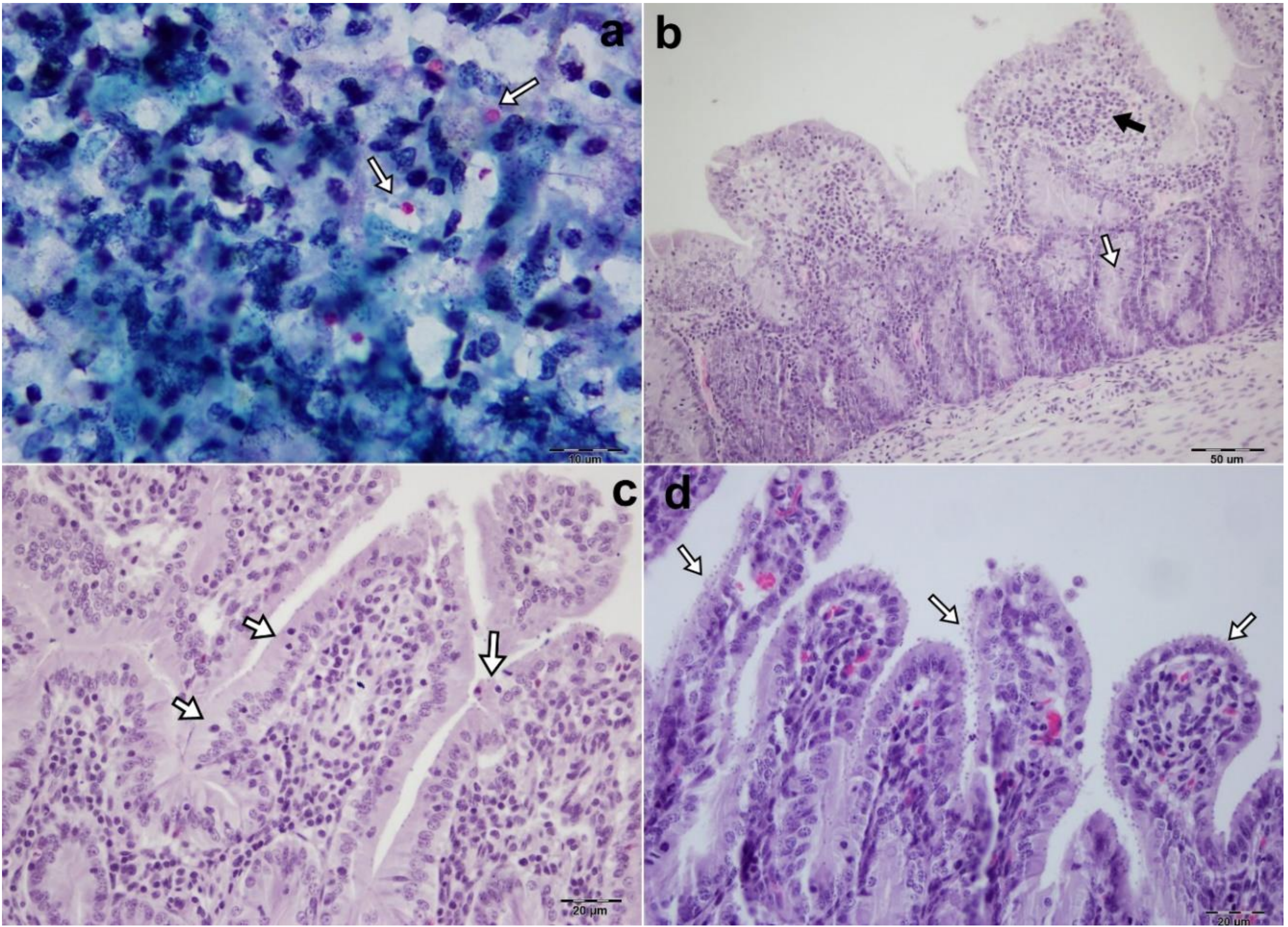
İHK boyama: parafin blokları hazırlanmış 145 örnekten mikrotomla alınan 5 mikronluk kesitler, 3x5 dk. ksilol serisinden geçirilerek deparafinize edildi. Rehidrasyon için 2×2 dk. 99˚, 1dk. 96˚ alkol kullanıldı ve distile suda yıkandı. Endojen peroksidaz aktivitesini ortadan kaldırmak için kesitlere nemli ortamda 10 dakika süre ile %3'lük H_2O_2 uygulandı. Daha sonra dokular 2 defa 5 dakika boyunca PBS ile yıkandı. Dokulardaki antijeni açığa çıkarma işlemi için 3×5 dk. 500 watt'da sitratlı tampon çözeltisi (10mM sitrik asit, %0.05 Tween 20, pH6) uygulandı. Bu aşamadan sonra kesitler sitratlı tampon çözeltisi içerisinde oda ısısına gelene kadar bekletildi. İki kez 5 dakika boyunca PBS ile yıkanana örneklerin üzerini kaplayacak şekilde protein bloklama solüsyonu eklenerek oda ısısından 5 dakika bekletildi. Protein bloklama solüsyonu uzaklaştırıldıktan sonra örneklerin üzerine antikor dilusyon buffer (Dako S0809, Carpinteria CA,ABD) ile 1/50 (1/50, 1/100 ve 1/500 ön denemeleri yapılmıştır) oranında sulandırılmış primer antikor (*Cryptosporidium parvum* antibody, BDI370, mouse monoclonal IgG1, Santa Cruz Biotechnology, Katolog no: sc-57693) konuldu ve 37˚C de 45 dakika inkubasyona bırakıldı. İnkubasyonu takiben primer antikor uzaklaştırılarak PBS ile yıkandı (2 kez 5 dakika). Biotinlenmiş sekonder antikor ile oda ısısında 10 dakika muamele edilen kesitler tekrar PBS ile yıkandı (2 kez 5 dakika). Sonrasında streptavidin-peroksidaz ile oda ısısında 10 dakika inkübe edildi. PBS ile yıkanan kesitler 3,3-diaminobenzidine (DAB) ile muamele edildi. Çeşme suyu ile yıkanan kesitler Mayer's hematoxylin ile 45 saniye boyandı. Alkol ve ksilol serileri sonrasında lamel ile kapatıldı.

İF boyama: İHK boyamada uygulanan prosedürün primer antikor ile inkubasyon ve PBS yıkama kısmına kadar olan bölümü İF boyamada da benzer şekilde uygulandı. Bu aşamadan sonra dokular 1/20 oranında sulandırılmış Goat anti Mouse Ig FITC (Biox, Katalog No. BIO156) ile inkubasyona bırakıldı (37˚C de 45 dakika). Tekrar PBS ile yıkanan (5 dakika) kesitlerin üzerine gliserin damlatılıp, lamel ile kapatıldı.

Mikroskobik incelemeler: MZN, HE ve İHK boyanmış tüm kesitler ışık mikroskobunda (DP72 kameralı Olympus BX51, Tokyo, JAPONYA), İF boyamalar ise floresan mikroskopta (Carl Zeiss axioskop A1, Colibri 2 led fluorescence) incelendi.

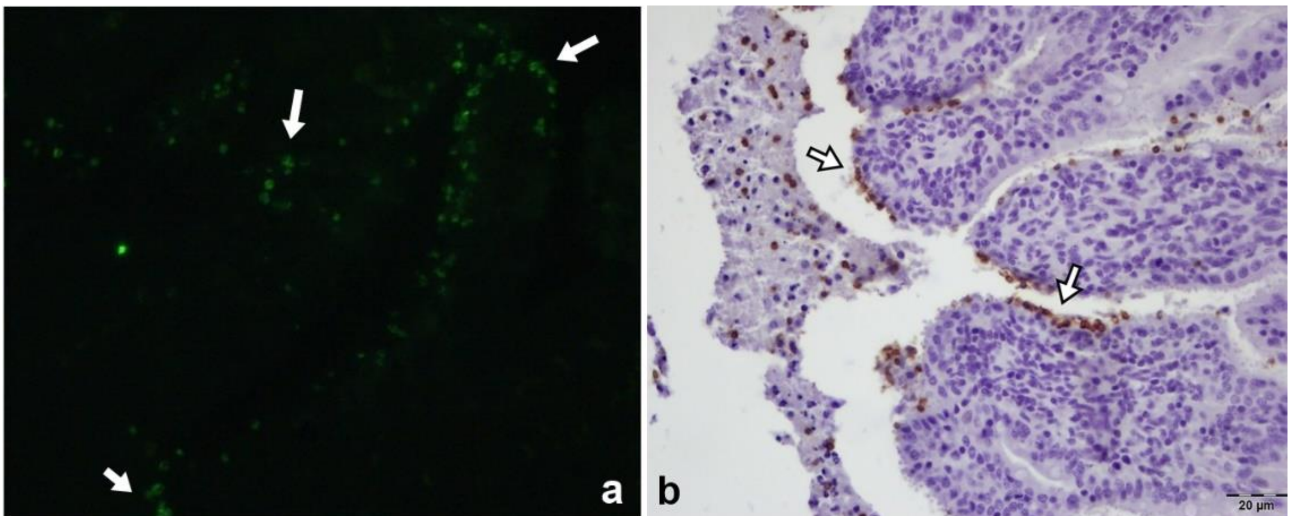
Bulgular

MZN boyama sonuçları: Taze elde edilmiş 60 adet ince bağırsak smearlarının MZN boyamalarında, 8 örnekte (%13.3) *Cryptosporidium spp.* pozitiflik tespit edildi. Koyu pembe ile açık kırmızı tonlarda boyanma özelliği gösteren etkenler yuvarlak yapılı, 3-4 mikron çapında ve kümelenme özelliği göstermeden, dağınık olarak yayıldıkları görülmüştür (Şekil 1a).



Şekil 1. a) *Cryptosporidium spp.* (oklar), İnce bağısak smearı. MZN boyama, Bar=10µ. b) villuslarda kütleşme, propriyada yangısal hücre infiltrasyonu (siyah ok) ve kript epitelinde mitoz (beyaz ok), HE, Bar=50µ. c) İntraepitelyal lenfositler (oklar), HE, Bar=20µ. d) villus epitel yüzeyi boyunca *Cryptosporidium spp.* gelişim evreleri (oklar). HE, Bar=20µ.

Figure 1. a) *Cryptosporidium spp.* distributed with intestinal epithelium and inflammatory cells (arrows), small intestine smear. MZN, Bar=10µ. b) Atrophic villi, inflammatory cell infiltration within the propria mucosae (black arrow) and mitosis in crypt epithelium (white arrow), HE, Bar=50µ. c) Intraepithelial lymphocytes (arrows), HE, Bar = 20µ. d) Developmental stages of *Cryptosporidium spp.* along the villi's epithelial surfaces (arrows), HE, Bar = 20µ.



Şekil 2. a) Parafin kesitlerde pozitif reaksiyon. *C. parvum* antibody-mouse monoclonal IgG1, Goat anti Mouse Ig FITC. b) Parafin kesitlerde villus epitel yüzeyi boyunca ve eksudatta *C. parvum* pozitif reaksiyon. *C. parvum* antibody-mouse monoclonal IgG1, streptavidin- peroksidaz, DAB kromojen ve Mayer'in hematoksileni ile zıt boyama. Bar = 20µ.

Figure 2. a) Positive reaction in paraffin sections. C. parvum antibody-mouse monoclonal IgG1, Goat anti Mouse Ig FITC. b) Positive reaction along surface of villus epithelium and exudate in paraffin sections. *C. parvum* antibody-mouse monoclonal IgG1, streptavidin-peroxidase, DAB chromogen and counterstaining with Mayer's hematoxylin. Bar = 20µ.

Tablo 1. İHK ve İF sonuçlarına göre *C. parvum* pozitif ve negatif olgularda histopatolojik bulguların oranları
Table 1. The rate of histopathological changes in *C. parvum* positive and negative cases according to IHC and IF.

Histopatolojik değişiklik	*C. parvum* (+) örneklerde (n=20)		*C. parvum* (-) örneklerde (n=125)	
	Görülme sayısı	%	Görülme sayısı	%
Propriyada hiperemi	14	70	106	84.8
Propriada nötrofil granülosit	3	15	21	16.8
Propriada eozinofil granülosit	10	50	27	21.6
Propriyada mononükleer hücre	18	90	88	70.4
Epitel hücre deskuamasyonu	15	75	84	67.2
Villus epitelinde nekroz	17	85	39	31.2
Villuslarda kütleşme	12	60	56	44.8
Lenfoid dokuda azalma	2	10	29	23.2
İntraepitelyal lenfosit	14	70	67	53.6
Kript epitelinde mitoz	13	65	71	56.8
Lenfatik kanallarda genişleme	6	30	11	8.8
Eimeria sp.	3	15	11	8.8

Histopatoloji sonuçları: Villuslarda kütleşme ve kript epitelinde mitoz artışı (Şekil 1b), villus epitelinde nekroz ve deskuamasyon, propriada hiperemi, nötrofil, eozinofil ve mononükleer hücre infiltrasyonu (Şekil 1b), lenfatik kanallarda genişleme, intraepitelyal lenfositler (Şekil 1c), peyer plakları lenfoid dokusunda azalma dikkati çekti. Villus epitel hücre yüzeyleri ve yer yer kript çevrelerinde ise *Cryptosporidium spp.* lokalizasyonu gözlendi (Şekil 1d). Histopatolojik incelemelerde bazı kesitlerde *Eimeria spp.* saptandı.

İHK ve İF boyama sonrası *C. parvum* pozitif olarak tespit edilen 20 ve diğer negatif bulunan 125 ince bağırsak örneğinde tespit edilen histopatolojik değişiklikler Tablo 1'de sunulmuştur.

İF ve İHK bulguları: Kesitlerin İF incelemelerinde 20 örnekte (%13.8) pozitif reaksiyon elde edildi (Şekil 2a). Pozitif boyanmalar tipik olarak zincir şeklinde sıralanma göstermekteydi. İHK boyamalarda da İF boyamalarda olduğu gibi 20 örnekte (%13.8) pozitif reaksiyon gözlendi (Şekil 2b). Pozitif reaksiyon veren antijenik lokalizasyonların bağırsak villuslarının epitel yüzeyi boyunca dizildiği dikkati çekti. Benzer boyanmalar eksudat içerisinde ve daha sınırlı düzeyde kript epiteli çevresinde de gözlendi.

Tartışma ve Sonuç

Türkiye'de neonatal kuzu problemleri üzerine yapılan bir araştırmada morbidite oranı % 48.6 olarak belirlenmiş, bunların içerisinde ishal olgularının ise % 15.4 ile en başta yer aldığı bildirilmiştir (12). Cryptosporidiosis erken dönem kuzu ishallerinin en önemli sebeplerinden birisi olarak görülmektedir (4, 12). Neonatal dönem olarak kabul edilen 0-28. günler arasında ishalli kuzuların dışkı örneklerinde yapılan bir çalışmada; *E. coli* F5 %10.5, *E. Coli* O157 %10.9, *C. parvum* %21.05, rotavirus %5.3, coronavirus %21.4 oranında belirlenmiştir (12). Munoz ve ark (20) tarafından yapılan başka bir çalışmada da *C. parvum* %45, *E. coli* %48, rotavirus %8.1, *Cl. perfringens* %10.8 olarak belirlenmiştir. Araştırma sonuçlarına bakıldığında Cryptosporidiosis'in erken dönem kuzu sağlığı açısından önemli bir hastalık olduğu dikkati çekmektedir.

Türkiye'de kuzularda Cryptosporidiosis üzerine yapılan araştırmalar incelendiğinde; ilk olarak Özer ve ark (23) ishalli kuzuların dışkı muayenesinde %12 oranında Cryptosporidium ookisti tespit etmişlerdir. Sonraki yıllarda yapılan çalışmalarda ise; İzmir yöresinde %23.3 (9), Aydın yöresinde %46.5 (34) oranlarında rapor edilmiştir. Konya merkez ve köylerinde yapılan diğer bir çalışmada MZN yöntemiyle %2.97, ELISA ile %9,13 oranında pozitiflik tespit edilmiştir (30). Bu verilere ilave olarak, hastalık Van yöresinde %13.63 (22), Kars yöresinde ise iki farklı çalışmada %21.05 (13) ve % 38.8 (29) oranlarında rapor edilmiştir. Dışkı örnekleri veya seroloji ağırlıklı olarak yapılan bu çalışmaların sonuçlarına göre, Türkiye'de ishalli kuzularda Cryptosporidiosis oranının, %2.97 ile %46.5 arasında oldukça değişkenlik gösterdiği dikkati çekmektedir. Araştırma sonuçlarının farklılık göstermesinde örnekleme şekilleri, alındığı dönem ve metot farklılıkları gibi faktörlerin sonuçlar üzerine etkili olduğu söylenebilir. Sunulan çalışmamızda şüpheli bağırsak kesitlerinin İF ve İHK boyamalarında %13.8 oranında *C. parvum* antijen varlığı tespit edilmiştir. Çalışmamızda örnek toplama işleminin belirli bir periyoda yayılması, farklı sürülerden toplanması ve doku düzeyinde teşhisinin yapılması sebebiyle araştırma sonuçlarımızın da hastalığın durumunu ortaya koyması bakımından değerli olduğu düşünülmüştür.

Yapılan çalışmalarda, enfeksiyon görülme sıklığının, hastalanma yaşı ile yakın ilişkili olduğu ortaya konulmuş,

hastalıktan etkilenme yaşının kuzularda en çok 1-21. günler olduğu 30. günden sonra ise azaldığı bildirilmiştir (6, 10). Sevinç ve ark (30) tarafından yaş gruplarına göre ayırdıkları 300 buzağı üzerinde yapılan bir çalışmada, hastalığın görülme oranı, 1-10 günlüklerde %50.75; 10-20 günlüklerde %35.71; 20-30 günlüklerde %25.45; 30-45 günlüklerde %14.71 ve 45 günden büyüklerde ise %13.24 oranında bildirilmiştir. Giadinis ve ark (11) da Kuzey Yunanistan'da 54 ayrı sürüden 4-15 günlük 292 oğlaktan topladıkları dışkı örneklerinde örneklerde *Cryptosporidium spp*. ookist oranını %76.4 olarak bildirmişlerdir. Arslan ve ark (2) ise 6 aydan küçük kuzu ve oğlaklarda *Cryptosporidium spp* oranını %9.6 olarak tespit etmişlerdir. Sunulan çalışmamızda 30 günlüğe kadar olan ishalli kuzularda *Cryptosporidium parvum* oranı %13.8 olarak belirlenmiştir.

Cryptosporidiumların merogoni, gametogoni ve sporogoni dönemleri enfekte epitel hücrelerinin fırçamsı kenarlarında yerleştiği, villus atrofisi ile birlikte gelişen absorpsiyon bozukluğunun ishalin temel sebebi olduğu belirtilmiştir. Bu durumda absorptif hücre yüzeylerinin büyük oranda Cryptosporidium etkenleri tarafından işgal edildiği, mukoza yüzeyinde olgunlaşmamış epitel hücre populasyonunda artış olduğu ve başta prostoglandinler olmak üzere yangısal mediatörlerin salgılanmasını, mukozal sekresyonu ve epitel hücrelerinin permeabilitesini artırdığı bildirilmiştir (5). Araştırmamızda histopatolojik bulgular değerlendirildiğinde, bağırsak değişikliklerin özellikle epitel hücrelerinde nekroz, deskuamasyon ve villuslarda kütleşme, propria hiperemi, nötrofil, eozinofil ve mononükleer hücre infiltrasyonları, intraepitelyal lenfosit, kript epitelinde mitoz artışı, lenf kanalarında genişleme ve bazı olgularda lenfoid doku kayıpları olduğu gözlenmiştir. Cryptosporidium etkenlerinin ise, tipik olarak enfekte hücrelerin mikrovillus kenarlarında yerleştiği dikkati çekmiştir. Araştırmada gözlemlenen mikroskobik bulgular önceki araştırma sonuçları ile uyumlu bulunmuştur (3, 5, 19). Histopatolojik değişiklikler daha çok epitel bölgesinde olmasının sebebi etkenin biyolojisi ile ilişkilendirilebilir. Enfeksiyonun durumunu, bireysel direnç yanında birlikte seyredebileceği *E. coli*, rotavirus ve coronavirus ile oluşabilecek miks enfeksiyonlar da etkileyebileceğinden dolayı (2, 4, 13, 20) araştırmamızda gözlemlenen histopatolojik bulguların ne düzeyde *C. parvum* enfeksiyonu ile ilişkili olduğu hakkında bir fikir yürütmek zor olacaktır. Tüm bunların yanında ana amacı ishalli kuzuların bağırsak kesitlerinde *C. parvum* antijenlerini belirlemek olan araştırmamızda, etken tespit edilen bağırsak kesitlerinin histopatolojik değerlendirmesinde, villuslarda kütleşme, epitel kaybı, eozinofil ve mononükleer hücre artışı, intraepitelyal lenfositler, kript epitelinde mitoz artışı ve lenfatiklerde genişleme öne çıkan bulgular olarak dikkati çekmiştir.

Cryptosporidiosis tanısı için en güvenilir yöntem olarak, dışkı örneğinde ookistlerin görülmesi veya antijenlerinin belirlenmesi olarak bildirilmiştir (21). Ookist tespiti için dışkı örneklerinin Ziehl-Neelsen yanında Auramine boyamalarının da oldukça etkili hatta daha pratik olduğu Kuhurana ve ark (16) tarafından rapor edilmiştir. Bununla birlikte, Sevinç ve ark (32) tarafından yapılan bir araştırmada, aynı dışkı örneklerinde MZN yöntemiyle %2.97, ELISA tekniği ile %9.13 oranında pozitiflik tespit etmişlerdir. Bu sonuç doğrultusunda dışkı örneklerinden MZN yöntemi ile hastalık teşhisinin yetersiz kaldığı düşünülebilir. Bu hipotezi destekleyici olarak, klinik gözlemlerde asemptomatik olan bazı hayvanlarda, ookist saçılımının yetersiz olduğu durumlarda ookist tespitinin de zor olduğu belirtilmektedir (21). Çalışmamızda dışkı örneğinden farklı olarak, smearlar doğrudan bağırsak mukoza kazıntılarından hazırlanmıştır. Bu yolla taze hazırlanan 60 smear örneğinin MZN boyamalarında %13.3 oranında etken tespit edilmiştir. Bu sonuç doku kesitlerinden yapılan İHK ve İF boyama sonuçlarına da (%13.8) oldukça yakın bulunmuştur. Elde ettiğimiz sonuçlar doğrultusunda, özellikle nekropsi sırasında taze hazırlanacak ince bağırsak smearları üzerine yapılacak MZN boyamalarının, hastalığın teşhisinde etkili olacağı öngörülmektedir. Bağırsak smearlarının MZN boyamalarının ışık mikroskobik incelemelerinde, dışkı boyamalarına benzer şekilde (8,17) etkenlerin pembe-kırmızı renkte, morfolojik olarak oval-yuvarlak şekilli olup, kümelenme göstermeden dağınık yayıldıkları görülmüştür.

Önceki yapılan çalışmalarda *C. parvum* antijenlerinin İF ile teşhisine yönelik çalışmaların genelde dışkı örneklerinden (1, 27, 28, 33) daha az olarak da parafin kesitlerden (3) yapıldığı görülmektedir. Çalışmamızda da parafin kesitlerinden yapılan İF boyamalarla %13.8 oranında pozitiflik tespit edilmiştir. *C. parvum* lokalizasyonlarına enfekte hücre yüzeyleri boyunca tespit edilmiş ve bu bulgular Bejan ve ark (3) ile uyumlu bulunmuştur. Araştırmamızda doku kesitlerinin İHK boyamalarında da İF boyamalarla uyumlu olarak %13.8 oranında pozitiflik tespit edilmiştir. Pozitif reaksiyon veren antijen lokalizasyonlarının villus epitel hücreleri boyunca dizildiği dikkati çekmiş ve önceki çalışmalarla (18, 24) uyumlu bulunmuştur. Çalışmada kullanılan primer antikorun İHK ve İF boyamalar için 1/50 sulandırmasında daha etkili sonuçlar alınmıştır.

Sonuç olarak, Konya ve çevresinde ishal sebebiyle ölmüş 30 günlüğe kadarki kuzularda *C. parvum* oranı %13.8 olarak belirlenmiştir. Bağırsak smearlarının MZN boyamaları ile birlikte İHK ve İF boyamaların benzer sonuçlar verdiği ve histopatolojik tanıyı kesinleştirmek için her üç testin de etkili olduğu sonucuna varılmıştır.

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Evaluation of Tularemia cases in Ankara province, Turkey

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Summary: Tularemia; caused by *Francisella tularensis* is a zoonotic disease which can be seen endemic in the northern hemisphere. The distribution of tularemia cases according to years and the characteristics of the disease in Ankara province were evaluated in this descriptive study. The number of tularemia cases according to years in Ankara were as follows: 129 cases in 2010, 349 in 2011, 87 in 2012, 2 in 2013, 0 in 2014, 23 in 2015, 86 in 2016, 16 in 2017 and 4 in 2018. 52.3% of cases seen in the years 2010-2018 were female (n = 364) and 47.7% (n = 332) were male and the mean age was 38.99 ± 18.82. In these cases, the use of waterwork was 66.7%, the use of public fountains was 40.3%, the use of well water was 7.0%, the presence of rodents around was 25.6%. To control tularemia, well water, spring water and fountains outside the grid system must be controlled; used water tanks must be maintained and cleaned at regular intervals. It is important that the water against microbiological contamination are chlorinated by automatic devices and the residual chlorine measurements are made without interruption.

Keywords: *Francisella tularensis*, rodent diseases, tularemia, waterborne diseases.

Ankara ilinde görülen Tularemi olgularının değerlendirilmesi

Özet: Tularemi, etkeni *Francisella tularensis* olan, kuzey yarı kürede endemik olarak görülebilen zoonotik bir hastalıktır. Bu tanımlayıcı tipteki çalışmada, Ankara İli'nde görülen tularemi olgularının yıllara göre dağılımı ve bazı özellikleri değerlendirilmiştir. Ankara genelinde yıllara göre tularemi olgu sayıları şöyledir: 2010'da 129, 2011'de 349, 2012'de 87, 2013'te 2, 2014'te 0, 2015'te 23, 2016'da 86, 2017'de 16 ve 2018'de 4 vaka. 2010-2018 yıllarında görülen olguların %52.3'ü kadın (n=364), %47.7'si ise (n=332) erkektir; yaş ortalaması 38.99±18.82'dir. Bu olgularda şebeke suyu kullanımı %66.7, halk çeşmelerinden su kullanımı %40.3, kuyu suyu kullanımı %7.0; çevrede kemirgen varlığı %25.6, olarak saptanmıştır. Tulareminin kontrol edilmesi amacı ile şebeke sistemi dışındaki kuyu suyu, kaynak suları ve halk çeşmelerinin kontrolü sağlanmalı; kullanılan su depolarının bakımları yapılmalı ve düzenli aralıklar ile temizlenmelidir. Mikrobiyolojik kirlenmeye karşı suların otomatik cihazlar ile klorlanması ve bakiye klor ölçümlerinin aksatılmadan yapılması önemlidir.

Anahtar sözcükler: *Francisella tularensis*, kemirgen hastalıkları, su kaynaklı hastalıklar, tularemi.

Introduction

Tularemia, caused by *Francisella tularensis* is a zoonotic disease which is endemic in the northern hemisphere. *F. tularensis* is a small gram-negative, coccobacillus bacterium (10). More than 125 animal species have been reported as the host of *F. tularensis*. Many animals, such as rabbits, various wild birds, rat, mouse, squirrel, tick, cats, dogs, sheep and bears, serve as hosts for bacteria (4).

The risk factors include hunting and eating wild rabbit meat, use of spring and well water, unhygienic food consumption, contact with rodent extracts, increased number of rodents inside and around the house and nature-related activities. While the world's most frequent transmission is contact with infected animals and ticks, consumption of spring water and non chlorinated drinking water is the most important transmission route in Turkey (3-12-13).

In recent years, parallel to climate changes, tularemia epidemiology has changed significantly in the world due to unsuitable living conditions, changes in reservoirs and vector population as well as distribution and improper living conditions due to war and migration significant increases in number of cases have been observed (3).

Ministry of Health General Directorate of Primary Health Care published a circular, numbered 2005/61 in 11/04/2005 about tularemia. In the circular in 2005, Tularemia was put on the list of Group C diseases, in the Reporting and Communication System for Communicable Diseases Standard Diagnosis, Surveillance and Laboratory Guidance. In this way, standardization has improved for tularemia; sampling and sending rules for diagnosis laboratory criteria and case definition were formed.

While tularemia was common in Marmara Region and Western Black Sea Regions before 2005, new cases were reported in the first half of 2009-2010 especially from Central Anatolia Region (16). There were 5.434 new

cases reported in Turkey between 2005 and 2012; %41.2 of them were from Central Anatolia Region (13).

Because of the increase in the tularemia cases in Ankara in 2010, "Ankara Provincial Public Health Committee (PHC) has started works such as rehabilitation of water tanks in villages, towns and districts, disinfection of the using or drinking water by automatic device or system and protection of the storages from the tularemia disease with the 2010/3 numbered PHC decisions. When tularemia cases increased again in 2016, Ankara PHC convened and took decisions with heavy sanctions especially related to water sanitation.

Ministry of Health made a change in the regulation on the Water Intended for Human Consumption on 20/10/2016. According to the modification, disinfection of the drinking water by consuming the chlorine and chlorinated compound with the automatic chlorination equipment and disinfection process starts according to flow rate and water pressure with regulatable automatic chlorination machines and measurements at the end points stabled at the point of free chlorine levels of 0.2-0.5 mg/L. "In case of failure in chlorination, back up chlorination units have to be made by local administration" law brought from the administration (2).

Surveillance and evaluation of tularemia trend and its characteristics is important in determining the epidemics in a timely manner and taking control measures. The aim of this study is to investigate the trend of tularemia cases and related characteristics in Ankara Province, Turkey.

Material and Methods

Our research was a descriptive study. In the scope of research, numbers of tularemia cases have been detected by Ankara Provincial Health Directorate Public Health Services Presidency, in behalf of "Directorate of Public Health Services Primary Health Statistics Module" between 2010-2018. The characteristics of tularemia cases in the years 2015-2018 were analyzed.

Within the scope of the study, information about the number and characteristics of the water reservoirs in the province and the protection and control measures taken regarding the tularemia were taken from the related units of the Ankara Provincial Directorate of Health.

In order to use the data in the study, permission was obtained from the Ankara Provincial Health Directorate Public Health Services Presidency Provincial Research Demands Evaluation Commission with the decision of the meeting dated 08/08/2018.

Descriptive analyses were presented using number and percentage for categorical variables; mean, standard deviation, median, minimum and maximum values for continuous variables. The statistical analysis was carried out by using OpenEpi Version 3.01. The Chi-square test was used to compare categorical variables in different groups. A p-value of less than 0.05 was considered to show statistically significant result.

Results

Table 1 demonstrates the distribution of tularemia cases by months and years in Ankara Province. Grafic 1. shows distribution of the total tularamia cases by months between 2010-2018. The distribution of cases by sex and age according to years are given in Table 2. Tularemia cases in Ankara are generally seen in rural areas, but they are caused by feeding in contaminated foods during their visits to villages and rural areas, and by bringing drinking water from drums and drinking water to the house. After the onset of tularemia cases, the first case cluster occurred in November 2010 in Bala, followed by in Pursaklar District and in Çubuk District. In December 2010, the cases of tularemia occurred in Mamak District and later in many villages and towns of Haymana, Polatlı, Kızılcahamam, Bala, Şereflikoçhisar, Beypazarı, Çamlıdere. The residence addresses of these cases are shown in Figure 1.

Table 1. Distribution of tularemia cases by months and years (Ankara province, Turkey).
Tablo 1. Tularemi olgularının aylar ve yıllara göre dağılımı (Ankara, Türkiye)

Months	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**Total**
January	1	56	13	0	0	3	18	0	1	**92**
February	5	98	34	0	0	1	14	1	2	**155**
March	8	41	23	0	0	0	23	3	1	**99**
April	7	18	5	1	0	0	4	2	0	**37**
May	5	10	1	1	0	1	5	0	0	**23**
June	1	9	4	0	0	2	2	1	0	**19**
July	2	25	2	0	0	2	3	1	0	**35**
August	2	10	0	0	0	1	6	1	0	**20**
September	17	15	1	0	0	2	1	3	0	**39**
October	10	8	0	0	0	0	1	2	0	**21**
November	39	45	2	0	0	1	2	2	0	**91**
December	32	14	2	0	0	10	7	0	0	**65**
Total	**129**	**349**	**87**	**2**	**0**	**23**	**86**	**16**	**4**	**696**

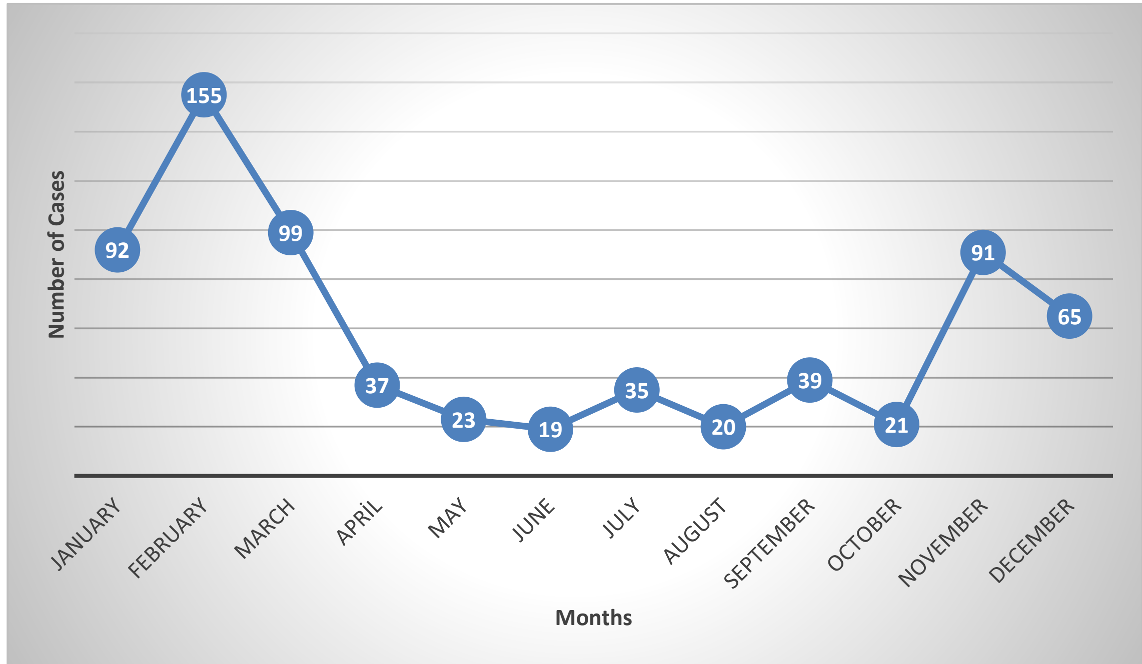

Figure 1. Distribution of tularemia cases by months between 2010-2018 (Ankara Province, Turkey).
Şekil 1. 2010- 2018 yılları arasındaki tularemi olgularının aylara göre dağılımı (Ankara, Türkiye)

Tularamia Cases (n=696)

1- Çubuk	(n=146)
2- Bala	(n=84)
3- Mamak	(n=50)
4- Haymana	(n=46)
5- Polatlı	(n=36)
6- Gölbaşı	(n=33)
7- Keçiören	(n=31)
8- Altındağ	(n=29)
9- Akyurt	(n=25)
10- Pursaklar	(n=25)
11- Beypazarı	(n=25)
12- Kalecik	(n=24)
13- Sincan	(n=22)
14- Elmadağ	(n=20)
15- Yenimahalle	(n=19)
16- Kızılcahamam	(n=14)
17- Çankaya	(n=13)
18- Etimesgut	(n=12)
19- Ayaş	(n=10)
20- Çamlıdere	(n=10)
21- Güdül	(n=8)
22- Nallıhan	(n=6)
23- Evren	(n=4)
24- Kahramankazan	(n=3)
25- Şereflikoçhisar	(n=1)

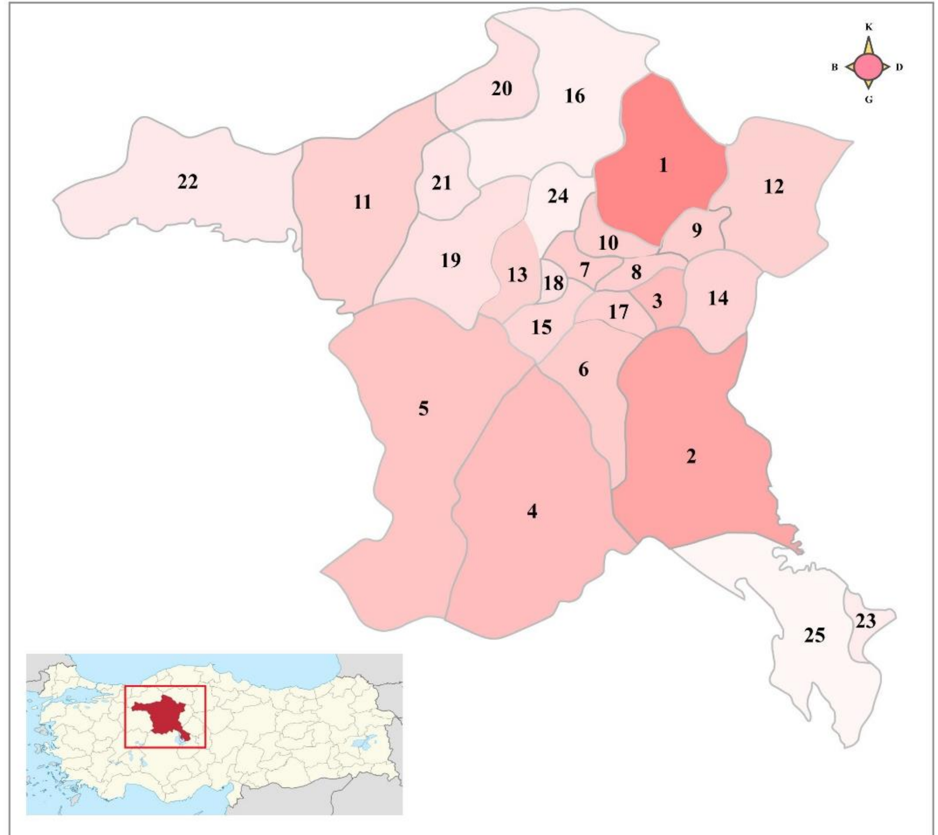

Figure 2. Place of residences of tularemia cases reported between 2010-2018 (Ankara Province, Turkey)
Şekil 2. 2010-2018 arasında bildirilen tularemi olgularının ikamet yerleri (Ankara, Türkiye)

Table 2. Distribution of tularemia cases by sex and age according to years.
Tablo 2. Tularemi olgularının yıllara göre cinsiyet ve yaş dağılımı

Characteristics	2010	2011	2012	2013	2015	2016	2017	2018	Total
	%	%	%	%	%	%	%	%	%
Sex									
Male (n=332)	48.8	47.3	47.1	50.0	43.5	47.7	62.5	25.0	47.7
Female (n=364)	51.2	52.7	52.9	50.0	56.5	52.3	37.5	75.0	52.3
Age									
0-10	4.7	6.9	8.0	0.0	0.0	2.3	6.3	0.0	5.7
11-20	14.7	10.0	11.5	0.0	8.7	15.1	12.5	75.0	11.6
21-30	22.5	19.5	12.6	0.0	26.1	12.8	6.3	0.0	18.5
31-40	20.2	16.9	18.4	0.0	8.7	16.3	43.8	0.0	17.8
41-50	21.7	15.2	10.3	0.0	21.7	17.4	18.8	25.0	16.4
51-60	7.8	14.3	20.7	100.0	26.1	18.6	6.3	0.0	14.8
61-70	8.5	12.6	9.2	0.0	0.0	12.8	6.3	0.0	10.8
71-80	0.0	4.3	6.9	0.0	8.7	2.3	0.0	0.0	3.6
81-90	0.0	0.3	2.3	0.0	0.0	2.3	0.0	0.0	0.7
Age (X±SD, Median, Min -Max)	35.01± 15.99 34.0 (5-69)	39.28±1 9.37 38.0 (1-81)	41.10±2 0.80 40.0 (2-82)	58.50± 0.71 58.5 (58-59)	41.70± 17.73 46.0 (12-74)	41.50± 18.84 42.5 (3-86)	36.19± 16.16 37.0 (22-68)	28.7± 8.26 25.5 (23-41)	38.99± 18.82 38.0 (1-86)
N of Cases	**129**	**349**	**87**	**2**	**23**	**86**	**16**	**4**	**696**

Table 3. Characteristics of tularemia cases reported between 2015-2018 (Ankara Province, Turkey
Tablo 3. 2015-2018 arasında bildirilen tularemi olgularının özellikleri (Ankara, Türkiye)

Characteristics	Yes (+) 2015		Yes (+) 2016		Yes (+) 2017		Yes (+) 2018		Yes (+) Total	
	N	%	N	%	N	%	N	%	N	%
History of raw vegetables / fruits eaten without washing	6	26.1	17	19.8	5	31,3	1	25.0	29	22.4
Is food open to animal contact	1	4.3	7	8.1	2	12.5	2	50.0	12	9.3
Has there been any rodent contact with food	2	8.7	3	3.5	0	0.0	0	0.0	5	3.9
Feeding any animal	4	17.4	24	27.9	3	18.8	0	0.0	31	24.0
There were tick around	0	0.0	10	11.6	3	18.8	0	0.0	13	10.1
History of contact with ticks or Mosquito / insect bite	0	0.0	6	7.0	5	31.3	0	0.0	11	8.5
Ever seen a rodent at home	0	0.0	7	8.1	0	0.0	0	0.0	7	5.4
There were rodents around	5	21.7	23	26.7	5	31.3	0	0.0	33	25.6
History of contact with hunting animals	1	4.3	4	4.7	0	0.0	0	0.0	5	3.9
Travel history in the last 1 month	6	26.1	30	34.9	9	56.3	3	75.0	48	37.2
Activity history in nature	5	21.7	24	27.9	11	68.8	0	0.0	40	31.0
Use of waterwork	11	47.8	47	54.7	12	75.0	4	100.0	74	66.7
Use of packaged water	5	21.7	30	34.9	4	25.0	3	75.0	42	32.6
Use of spring water	7	30.4	39	45.3	4	25.0	0	0.0	50	38.8
Use of public fountains	8	34.8	34	39.5	9	56.3	1	25.0	52	40.3
Use of well water	0	0.0	7	8.1	2	12.5	0	0.0	9	7.0
Use of lake / stream water	7	30.4	3	3.5	1	6.3	0	0.0	4	3.1
Clearance in water tank	2	8.7	7	8.1	1	6.3	0	0.0	10	7.8
Leaking from the water tank	1	4.3	4	4.7	1	6.3	0	0.0	6	4.7
Ever seen living / dead animals around the water tank	0	0.0	2	2.3	1	6.3	0	0.0	3	2.3
Is the water tank periodically cleaned	3	13.0	7	8.1	3	18.8	0	0.0	13	10.1
Does the chlorination device work in the water tank	3	13.0	7	8.1	7	43.8	0	0.0	17	13.2
Chlorine measurement in water tank	3	13.0	4	4.7	3	18.8	0	0.0	10	7.8
Is the water sample taken from the water source used for F. Tularensis analysis?	4	17.4	13	15.1	5	31.3	0	0.0	22	17.1
Is there space for animals to enter between the source and the water tank	0	0.0	9	10.5	1	6.3	0	0.0	10	7.8

Characteristics of tularemia cases reported between 2015-2018 are given in Table 3. A total of 22 water samples were collected and analyzed for *F. tularensis* in the field surveys between 2015-2018 and one of them was positive. The rate of activity in nature was 35.6% in women and 36.5% in men (p=0.918).

Discussion and Conclusion

Tularemia is known as a disease that can be seen in all age groups, and males in every age category and has a higher incidence in the world (6). In Turkey, the incidence of tularemia is higher in women than in men (3). In a study of 1091 tularemia cases seen in Turkey between 2005-2009, it was found that 54.7% of the cases were female and 45.3% were male (14). Korkmaz et al. evaluated tularemia cases in Eskişehir province, 46 (51.1%) of the cases with tularemia were reported as female and 44 (48.9%) as male (15). In our study, it was found that 52.3% of the cases with tularemia were female (n=364) and 47.7% were male (n=332). In other countries; the overrepresentation among males has been attributed to their more frequent outdoor professional and leisure activities As the consumption of spring water and non chlorinated drinking water is the main transmission route in Turkey, we thought that this difference in the distribution of the disease is due to the fact that women are more in contact with contaminated water and food in their home environment and that they are more exposed to reservoir animal extracts that carry the factor in their habitats. As a matter of fact in our study the rate of activity in nature was 35.6% in women and 36.5% in men (p=0.918).

When the distribution of tularemia cases according to age in Turkey is examined; the disease is mostly seen in adults over 30 years of age because adults have more risk group activities (3). According to Kılıç's study (14), 63.87% of the tularemia cases are seen over 30 years of age. In our study, 35.9% of the cases were seen between 0-30 years of age and 64.1% of the cases were over 30 years of age. Similarly, Christova et al. (7) found that tularemia disease affected all age groups but predominantly people of active age in Bulgaria.

The incidence of tularemia in countries where tularemia is endemic is the highest in late spring, summer and early spring months (6). In the outbreaks reported from Turkey, it is seen that tularemia cases usually occur in late autumn and winter periods, and they significantly decrease in the spring and summer months (1-3-14). Dikici et al. in their study that is covering the seasonal distribution of tularemia, it is has been seen in the most frequent in autumn in accordance with the general epidemiological characteristics (9). In our study, the most common cases of tularemia in Ankara were seen in winter in accordance with the general epidemiological characteristics of the seasonal distribution.

The World Health Organization's tularemia guide states that outbreaks of tularemia in humans often follow tularemia outbreaks in rodents, and the places where tularemia is endemic, antibodies to F. tularensis are frequently detected in sera of animals such as rats rats, beavers and field rats. The number of tularemia cases seen in the Novosibirsk region of the Russian Federation between 1956-2000 has been shown to be related to the density of the water vole population. A strong correlation has been reported in Sweden between peaks of hatchlings and rabbits and tularemia outbreaks (17). In various studies conducted in Turkey, a relationship has been established between tularemia epidemics and an increase in the population of field and water mice (5-11-16). In our study, it was found that 25.6% of patients who applied tularemia field research form had seen a rodent in their environment, 5.4% of them had seen a rodent in their homes and 3.9% of them had rodent contact with their food. This might be evidence of the rodents' role in tularemia disease.

Akalin et al. (1) outlined the findings that suggest water-borne epidemics as; limitation of the epidemics depends on the use of certain aqueducts in the same area, the lack of chlorination of the water system in some epidemic areas and PCR positivity for F. tularensis in water from epidemic areas (1). In our study, 40.3% of the cases examined in the field research form were found to use the public fountains. Çankaya et al. studied on the public fountains, 83% of the studied public fountains were found to be unsuitable for microbiological regulations (8). Therefore it was suggested that the fountains used by the public are frequently used and further, that the public fountains that could not be provided with clean and reliable water could be demolished or connected to the grid system.

As a result for the purpose of controlling tularemia, the control of well water, spring water, and public fountains should be ensured and the water tanks usage should be maintained and tanks should be cleaned at regular intervals. It is important that the water is chlorinated with automatic devices and the residual chlorine measurements are carried out without interruption, against microbiological contamination. Appropriate protection areas for water resources should be established and the contact with vector animals should be avoided. The training of health workers and the community on the subject should be carried out periodically.

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Case Report / *Olgu Sunumu*

Schistosomus (Fissura abdominalis), atresia ani and arthrogryposis in a Turkoman foal

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Summary: Schistosomus reflexus (SR) is a rare monstrosity. This fatal congenital anomaly is characterized by defect fusion that result in abdominal and sometimes thoracic viscera exposure (schistosomus), ventral curvature of the spine and curved chest walls as lateral (reflexus). The present study describes schistosomus (Fissura abdominalis) without reflexus in a Turkoman foal. The foal exhibited rupture of abdominal wall and the internal organs were exposed and outside the body. Arthrogryposis and atresia ani were other anomalies associated with SR. To the authors' knowledge, it seems the first report of SR with atresia ani and arthrogryposis in a foal.

Keywords: Atresia ani, foal, schistosoma reflexus.

The occurrence of inherited anomalies is economically considerable. Although the etiology of congenital anomalies remains unclear, the majority of these anomalies can be related to genetic factors (mutations, chromosomal anomalies), infectious agents, and environmental factors (25). Schistosomus reflexus (SR) is a rare monstrosity primarily in cattle (13, 23). This fatal congenital anomaly is characterized by defect fusion that result in abdominal and sometimes thoracic viscera exposure (schistosomus), ventral curvature of the spine and curved chest walls as lateral (reflexus) (22). This malformation has also been reported lambs (25), camels (6), dogs (20), cat (19), goat (24) and foal (10). This anomaly may be associated with other congenital defects. Congenital anomalies and lesser multiple anomalies are reported in domestic animals and some of them may be associated with obstetrical problems (2). The present study describes schistosomus (Fissura abdominalis), concurrent with atresia ani and arthrogryposis in a Turkoman foal.

A Turkoman foal was born from a six-year old mare with dystocia signs in Isfahan province, Iran in a small farm. The foal exhibited rupture of abdominal wall and the internal organs were exposed and outside the body. The fissure was continued to near the anus site. A part of the intestines was congested. No abnormality was observed in the formation of thoracic cavity. Ribs and costal cartilages were intact. Only partly open ventral body closure was determined. The animal was diagnosed as schistosomus with no reflexus. Arthrogryposis and atresia ani were other anomalies associated with schistosomus. The fore and hind limbs were observed to be fully developed, and to display normal bone structure but had arthrogryposis. Also, the head, eyes and nose were formed completely. The cervical, thoracic and lumbar vertebrae as well as the sacrum and costae were also determined to be fully developed. No findings were present about lordosis or dorsoflexion in this case.

Schistosomus reflexus (SR) is a birth defect that means inside out and bent backwards. This rare anomaly is reported primarily in cattle with about 1.3% of bovine dystocias (12). The exact etiology and the pathogenesis of SR are unknown. Role of environmental teratogens and genetic factors in development of this fetal condition are not demonstrated clearly (7). Genetic factor is suggested for occurrence of SR and some indications supporting this hypothesis. An autosomal recessive inheritance has been suggested in bovine SR that the same bull had sired affected calves (7, 14). Kovacs and Stranzinger (13) found no chromosomal defects in the somatic cells of a female calf affected to SR, but they observed a higher incidence of synaptic anomalies in meiocytes of calf in compared to normal bovine fetuses.

Figure 1. Schistosomus in Turkoman foal. Not fused abdominal wall and the exposed gastrointestinal tract outside the body.

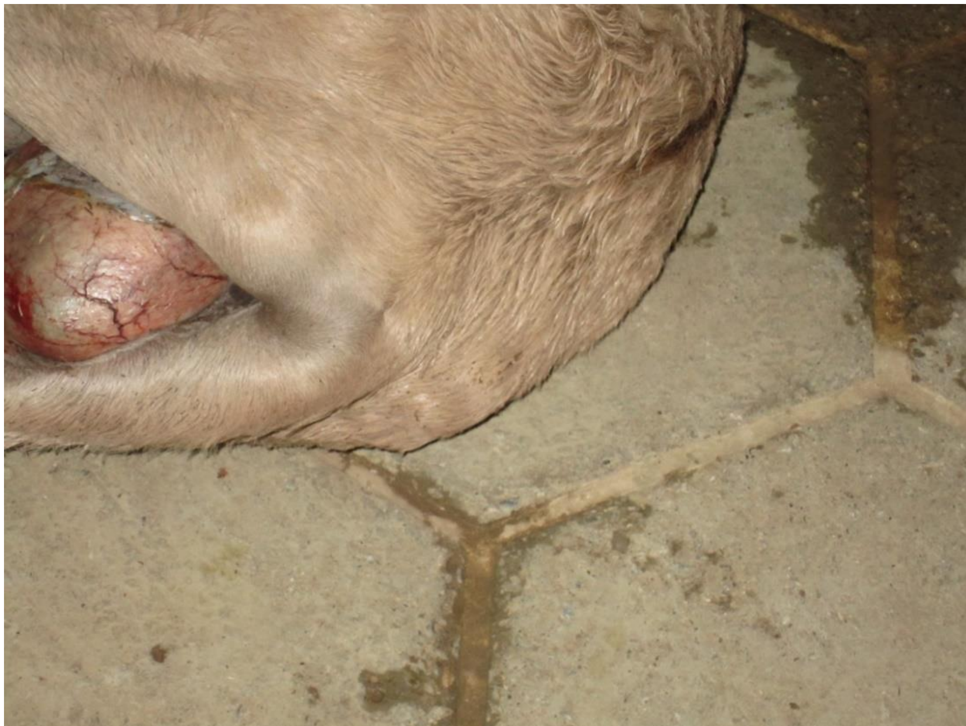

Figure 2. Atresia ani characterizes with agenesis of anal orifice**.**

The present foal was born from a mare with dystocia and displayed schistosomus (Fissura abdominalis) associated with atresia ani and arthrogryposis. Equine dystocia is a true emergency that threatens the survival of both dam and fetus. One of agents of dystocia is malformations like schistosomus reflexus. In the literature, the most reports of SR are documented in the calves (12, 13, 14). Few reports on schistosomus reflexus or schistosomus reflexus-like conditions have been reported in equids. Johnstone (10) reported equine

schistosomus that was born by caesarian section. Proctor (21) described foetal monstrosity resembling shistosomus reflexus in a Thoroughbred mare. The foal was born from a 6-year-old mare with dystocia. The case had marked dorsal angulation at the cervicothoracic junction and sternal cleft at its caudal extremity. The hind showed arthrogryposis. The diaphragm was complete. Ventral abdominal wall had closure defect. The kidneys were in a retropleural position within the thorax. The visceral organs were herniated from the ventral abdominal wall defect. Addo et al. (1) reported schistosomus reflexus-like syndrome in a twin foal. One of the twin displayed eventration of abdominal viscera and kyphoscoliosis in the thoracic spinal vertebrae**.** Ectrodactyly and ankyloses were observed in the right foreleg. Abdominal fissure was expanded from the xiphoid cartilage to the pubis. All viscera appeared normal but the liver was abnormally lobulated, cystic and fibrotic. Dubbin et al. (5) reported dystocia attributable to a fetal monster resembling schistosomus reflexus in a donkey.

SR uses for variable extension of visceral exposure and spinal inversion. Laughton et al. (14) stated that only cases show both exposed viscera and spinal inversion are considered as true SR. In the previous studies, the cases with only ventral clefts and no spinal inversion had been categorized as SR (9, 16).

A wide variety of other malformations reported in conjunction with SR are including thoracoschisis, extreme dorsal spinal flexion, limb arthrogryposis, positioning of the limbs near to the head, lung and diaphragm hypoplasia, skeletal anomalies (prognathia, scoliosis), urogenital anomalies, intestinal and anus atresia were with reported SR (14, 15). Atresia ani and a bifurcated scrotum has occurred in a conjoined twin lambs (5). Ozalp et al. (19) described SR in a cat. Abdominal wall was ruptured and the most internal organs protruded. No abnormality was observed in the formation of thoracic cavity and only a part of ventral body was opened. Lungs, liver and heart were hypoplastic. Atresia ani is characterized by imperforated anus at birth time that results in lack of defecation. This condition can occur individually, but may also be associated with other congenital abnormalities in different body systems such as distal spine, urogenital tract or colon atresia (3, 17).

The precise causative agent of the most congenital malformations is not clear because of the complex mechanisms in their development (11). Genetic causes are main risk factor, although toxic plants and some viral infections during the early stages of pregnancy can also been responsible in occurrence (18).

In conclusion, schistosoma is a rare anomaly in foal. To the authors' knowledge, it seems, this is the first case of this anomaly with atresia ani and arthrogryposis in a Turkoman foal.

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