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Research Article

The Effects of Fumaric Acid on *In Vitro* True Digestibility of Tea Wastes Produced with Different Cultivation Methods

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ABSTRACT

This study aims to determine the effects of adding different fumaric acid (FA) levels to tea factory wastes (TFW) produced by different cultivation methods on *in vitro* true digestibility. *In vitro* true digestibility of feed ($IVTD_{As\ feed}$), dry matter ($IVTD_{DM}$), organic matter ($IVTD_{OM}$), and neutral detergent fibre ($IVTD_{NDF}$) were performed with a Daisy Incubator. Fumaric acid did not add to the control group and added 0.1%, 0.2%, or 0.3% FA to the experimental groups. When the cultivation methods were compared (conventional and organic tea wastes), it was seen that FA made a significant difference ($P<0.05$) in conventional tea wastes at all levels. However, when comparing organic and conventional tea wastes themselves, there was no significant effect of FA level on digestibility parameters ($P>0.05$). There was a significant difference ($P<0.05$) for $IVTD_{As\ feed}$, $IVTD_{DM}$ and $IVTD_{OM}$ values in interaction between treatment and cultivation methods. The research results showed that the digestibility values of the conventional tea wastes were higher for ruminants than the tea waste produced by the organic method, and the use of 0.3% FA in organic tea wastes had a positive effect on IVTD values for ruminants.

Keywords: *By-product, feed, organic acid, ruminant, tea waste.*

Farklı Yetiştirme Yöntemleri İle Üretilen Çay Atıklarının *In Vitro* Gerçek Sindirilebilirliği Üzerine Fumarik Asitin Etkileri

ÖZET

Bu çalışma, farklı yetiştirme yöntemleri ile üretilen çay fabrikası atıklarına (TFW) farklı düzeylerde fumarik asit (FA) eklenmesinin *in vitro* gerçek sindirilebilirlik üzerindeki etkilerini belirlemeyi amaçlamaktadır. Çay fabrika atıklarının gerçek sindirilebilirlik (IVTDyem), kuru madde ($IVTD_{KM}$), organik madde ($IVTD_{OM}$) ve nötr deterjan lif ($IVTD_{NDF}$) in vitro gerçek sindirilebilirliği Daisy inkubatör sistemi ile gerçekleştirilmiştir. Kontrol grubuna FA ilave edilmezken deney gruplarına %0.1, %0.2 veya %0.3 FA eklenmiştir. Yetiştirme yöntemleri karşılaştırıldığında (konvansiyonel ve organik çay atıkları), FA'nın konvansiyonel çay atıklarında tüm seviyelerde önemli bir fark oluşturduğu ($P<0.05$) görülmüştür. Bununla birlikte, organik ve konvansiyonel çay atıklarının kendileri karşılaştırıldığında, FA seviyesinin sindirilebilirlik parametreleri üzerinde anlamlı bir etkisi olmamıştır($P>;0.05$). Fumarik asit düzeyleri ve yetiştirme yöntemleri arasındaki etkileşimde $IVTD_{yem}$, $IVTD_{KM}$ ve IVT_{DOM} değerleri için önemli fark ($P<0.05$) görülmüştür. Araştırma sonuçları konvansiyonel çay atıklarının sindirilebilirlik değerlerinin ruminantlar için organik yöntemle üretilen çay atıklarına göre daha yüksek olduğunu ve organik çay atıklarında %0.3 FA kullanımının ruminantlar için IVTD değerleri üzerinde olumlu etkisi olduğunu göstermiştir.

Anahtar Kelimeler: *Çay atığı, organik asit, ruminant, yan ürün, yem.*

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Introduction

The increase in feed prices in the field of animal nutrition adversely affects the economic livestock movements. Based on this situation, the interest in food wastes, plant wastes, and by-products used in different fields within alternative feed materials is increasing (Akram and Fırıncıoğlu, 2019). Tea Factory wastes obtained during the production phase of harvested tea are also evaluated within the scope of these alternative searches (Ozyılmaz and Genc, 2019). With the prohibition of antibiotics as growth factors with the European Union decision (EC Regulation No. 1831/20031) in 2006, the search for alternatives have been started in this field. Since this decision, many new natural and chemical substances have been used in this field. Organic acids, one of these substances, are classified as "generally recognized safe" by the USA Food and Drug Administration in animal nutrition. The effects of organic acids as growth promoters in ruminants occur by more than one mechanism (Carro and Ungerfeld, 2015). Their effects, such as being a very rapidly digestible substrate, providing rumen pH stabilization (Martin, 2004), decreasing methane emission (Bharathidhasan et al., 2016), and affecting NH_4 (Zhou et al., 2012) concentration, are taken into account within different metabolic mechanisms.

The effects of organic acids on rumen microecology and the tricarboxylic acid cycle in rumen fermentation have been focused on fumaric and malic acids (Castillo et al., 2004; Kolver and Aspin, 2006; Genc et al., 2020). Fumaric acid is an intermediate in the propionate formation mechanism formed in the rumen (Yang et al., 2012; Patra et al., 2017). With the increase of ruminal propionate, both the amount of hydrogen required for methanogenesis is reduced and the propionate absorbed from the rumen is transported to the liver to a large extent and becomes the main source of glucose for ruminants (Carro and Ungerfeld, 2015; Li and Guan, 2017).

Fumaric acid (FA) and its salts are prominent with *in vivo* and *in vitro* digestion trials as a growth factor for their bacteriostatic and bactericidal effects and also increase the population of beneficial microorganisms in the intestinal system (Angga et al., 2018).

This study is to determine the impact of different levels of fumaric acid on the *in vitro* digestion values of tea factory wastes produced by organic and conventional farming methods.

Materials and Methods

Since the rumen samples were only taken from slaughtered animals, no ethics committee approval was needed for this study. The samples (TFW) were obtained from several tea factories located in the Black Sea region in Turkey. The samples taken were obtained from the teas belonging to the first tea harvest in May. The FA (≥99% purity) was purchased from Sigma-Aldrich (St Louis, MO, USA).

In the study, tea factory wastes obtained from tea plants produced with conventional and organic techniques were used. Four different groups were formed, one of which was the control group, from the material representing each production technique. While no treatment was applied to the control groups, fumaric acid was added to the in vitro Daisy II incubator system jars of experimental groups at the level of 0.1%, 0.2% and 0.3% respectively. Tea factory waste samples were weighed and then dried at 65°C for 48h. The dried samples were ground in a mill with a 1 mm sieve to prepare them for chemical analysis. Each group sample's dry matter (DM) content was evaluated in an air circulation drying oven at 105°C for 4 hours. The dry material was burned in an ash oven at 550°C for 4 hours to determine the content of ash. The proportion of crude protein was calculated using the Kjeldahl technique (CP). AOAC (2006) methods were used for ether extract (EE) determination. Metabolizable energy determination was made according to Krichgessner et al. (1977). The neutral detergent fiber (NDF) and the acid detergent fiber (ADF) contents were analyzed in an ANKOM 200 Fiber Analyzer (ANKOM Technology Corp., USA) by the method reported by Van Soest et al. (1991). The methods offered in ANKOM Daisy Incubator (ANKOM Technology Corp., USA) performed all parameters of the *in vitro* true digestibility (IVTD) analysis with the ANKOM Daisy Incubator (ANKOM Technology Corp., USA) (2002). The rumen fluid was collected post-mortem from the rumen of three-year-old three Holstein cows slaughtered at a commercial slaughterhouse in Samsun Province, Turkey. Animals were fed with a diet containing grass hay and maize silage (60%) and concentrate feeds (40%). The rumen fluid was taken from each animal's rumen along with two handfuls of ruminal contents and carried in a thermos previously heated to 39°C and supplemented with CO_2. Samples were brought to the laboratory within 10 minutes. The rumen fluid was filtered through 4 layers of gauze. The F57 bags used in the study were soaked in 99.5% acetone for 3 minutes. All of the bags were dried for 2 hours at 105°C in a drying cabinet. The tare of the bags was weighed and 0.5 g of each samples (TFW) were transferred to separate F57 bags. According to ANKOM (2002) the buffer solution to be used in the analysis was prepared. In this experiment, four digestion units with a total volume of 2 L were used. The buffer solution was heated to 39°C and 1.6 L was given to each unit. Test units were closed by adding 400 ml of rumen fluid into the buffer solutions. Each digestion unit contained a total of 24 TFW samples, with six replicates prepared at the same time for each sample. The experimental groups received 0.1%, 0.2%, and 0.3% FA, respectively, whereas the control group received no FA. All samples were incubated for 48 hours. All liquids from the digestive units were removed after the incubation period, and the bags were rinsed under running distilled water. Washed bags were placed in the ANKOM Fiber Analyzer and were performed NDF analysis according to the method specified in ANKOM (2002). After NDF analysis, all bags were kept in an air-drying

cabinet at 105°C until they reached a constant weight. The IVTD values of all samples were calculated by the formula reported in ANKOM (2002).

A1= Weight of bag tare, A2= Weight of sample, A3= Final bag weight after *in vitro* process and sequential treatment, B1= Blank bag correction (final oven-dried weight/original blank bag weight)

Data were analyzed according to the factorial experimental design by the Completely Randomized Experimental Design (one-way classification), and the results were interpreted. The sample distribution of the data obtained in the study was determined by calculating the group of means and their standard error. In addition, the differences between the cases in which the differences between the means of the main effects were significant as a result of the statistical analysis were investigated with the Duncan multiple comparison test. The differences among the group combinations (interactions) were shown by the Tukey multiple comparison test. In the study, calculation of the sample distribution statistics of the data, descriptive statistics, and all statistical analyses were carried out in the (2009, SAS Institude) statistical program (P<0.05).

Results

It was determined that TFW's had similar values of nutrient (g/100g DM) and metabolisable energy (MJ/kg DM) (Table 1).Conventional wastes had a higher value in terms of crude protein (CP) and a lower value in terms of ether extract (EE). It was seen that different FA levels have a significant (P<0.05) effect on *in vitro* digestion parameters (C) of tea factory wastes according to the cultivation method (Table 2). Accordingly, all *in vitro* digestion parameters were found to be higher (*P*<0.05) in conventional tea wastes treated with 0.1% and 0.2% FA. In addition, when FA was used at the 0.3% level, $IVTD_{As\ feed}$, $IVTD_{DM}$, and $IVTD_{OM}$ values were higher (P<0.05), while $IVTD_{NDF}$ values were lower (P<0.05) in organic tea wastes. The *in vitro* digestion parameters evaluated within the groups were not significantly affected by the different FA levels (P>0.05).

It was determined that the addition of 0.3% FA level in organic tea wastes caused a numerically higher value (*P*>0.05) for all digestion parameters compared to the control and other experimental groups. It was observed that the increasing FA level in conventional tea wastes caused a numerical decrease (P>0.05) in all *in vitro* digestion parameters.

Regardless of the effect of FA levels applied to examine the interaction between the group and the cultivation method, $IVTD_{As\ feed}$, $IVTD_{DM}$, and $IVTD_{OM}$ values were found to be significantly higher (P<0.05) in the conventional production method when *in vitro* digestion parameters were examined in conventional and organic tea wastes.

$$\%IVTD\ (as\ received\ basis) = \frac{100 - [A3 - (A1xB1)]x100}{A2}$$

$$\%IVTD\ (DM\ basis) = \frac{100 - [A3 - (A1xB1)]x100}{(A2xDM)}$$

Discussion

In ruminant nutrition, the nutrient content of tea wastes as an alternative feed source varies. Ozyılmaz and Genc (2019) stated that the conventional tea factory wastes (CTFW) obtained in the July harvest had 95.80% DM, 12.67% CP, 4.1% ash, 0.30% EE, 52.52% NDF, and 47.98% ADF, while organic tea factory wastes (OTFW) had 95.93% DM, 10.20% CP, 3.92% ash, 0.76% EE, 57.23% NDF and 49.27% ADF levels, and these values vary depending on the organic or conventional growing and harvest periods of tea plants. Similar to these results, the effect of the cultivation method on the CP value coincides with the findings obtained in our research, and it is seen that the CP value is 17.8% higher for CTFW than OTFW. In an *in vitro* digestibility experiment (Genc et al., 2020) performed previously using a Daisy incubator, it was seen that CTFW nutrient values were 93.5% DM, 4.8% ash, 18.2% CP, 1.16% EE, 34.6% ADF, and 40.5% NDF. Nasehi et al. (2017) reported that in their study with CTFW, the values of DM, CP, CA, OM, EE, NDF, ADF and ME were 92.72%, 15.66%, 5.75%, 94.24%, 1.16%, 38.47%, 25.87% and 7.7 MJ/kg DM respectively. Another study (Angga et al., 2018) performed with Sumatra TFW reported that DM, OM, and CP values of CTFWs were 93.59%, 88.08%, and 19.63%, respectively. These results agree with the DM and OM values in our study.

Ozyilmaz and Genc (2019) reported that there was no difference (P<0.05) between gas volumes, $IVTD_{OM}$, and ME values in CTFW and OTFW. They emphasized that cultivation methods significantly affect these parameters.

Table 1. Nutrient composition (g/100g DM) and metabolizable energy (MJ/kg DM) of conventional and organic tea factory wastes for ruminants.

Cultivation	DM	Ash	CP	EE	NDF	ADF	OM	ME
Conventional	95.95	4.06	12.71	0.50	55.12	48.55	91.89	6.94
Organic	95.67	3.97	10.44	1.03	54.90	48.18	91.70	7.00

DM: Dry matter, CP: Crude protein, EE: Ether extract, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, OM: Organic matter, ME: Metabolizable energy (MJ/kg DM)

Table 2. Effects of the addition of different concentrations (%) of fumaric acid to tea factory wastes (n=6) on *in vitro* true digestibility values (Mean±SEM).

Parameters	Cultivation Control		Treatment				Total
			FA 0.1%	FA 0.2%	FA 0.3%		
$IVTD_{As\ feed}$	Conventional		54.07±0.46^{bc}	53.35±1.26^{c}	53.32±0.58^{c}	54.3±0.28^{bc}	53.76±0.28^{B}
	Organic		56.07±0.36^{a}	56.02±0.5^{a}	55.26±0.58^{ab}	53.63±0.15^{c}	55.24±0.28^{A}
	Total		55.07±0.39	54.69±0.39	54.29±0.39	53.96±0.39	54.5±0.25
	P value	C			0.0013		
		T			0.3087		
		CxT			0.0463		
$IVTD_{DM}$	Conventional		51.99±0.48^{bc}	51.24±1.32^{c}	51.21±0.61^{c}	52.23±0.3^{bc}	51.67±0.29^{B}
	Organic		54.21±0.37^{a}	54.16±0.52^{a}	53.37±0.61^{ab}	51.68±0.15^{c}	53.36±0.29^{A}
	Total		53.1±0.44	52.7±0.81	52.29±0.52	51.95±0.18	52.51±0.27
	P value	C			0.0006		
		T			0.3107		
		CxT			0.0468		
$IVTD_{OM}$	Conventional		53.15±0.46^{cd}	52.4±1.26^{d}	52.33±0.63^{d}	53.61±0.34^{bcd}	52.87±0.37^{B}
	Organic		55.48±0.4^{a}	55.17±0.54^{ab}	54.34±0.63^{ac}	52.66±0.17^{d}	54.41±0.31^{A}
	Total		54.31±0.45	53.79±0.78	53.33±0.52	53.14±0.23	53.64±0.27
	P value	C			0.0013		
		T			0.2635		
		CxT			0.0219		
$IVTD_{NDF}$	Conventional		12.55±0.87^{c}	12.86±1.16^{c}	11.12±1.11^{c}	12.98±0.54^{c}	12.38±0.47^{B}
	Organic		16.93±0.67^{a}	16.84±0.95^{a}	15.4±1.1^{ab}	13.27±0.99^{bc}	15.61±0.54^{A}
	Total		14.74±0.84	14.85±0.93	13.26±0.99	13.12±0.54	13.99±0.42
	P value	C			0.0001		
		T			0.1425		
		CxT			0.0500		

$^{a-d}$: There is no difference between cultivation treatment interactions with the same letter,$^{A-B}$: There is no difference between the main effects of cultivation and treatment with the same letter. $IVTD_{As\ feed}$: *In vitro* true digestibility as feed, $IVTD_{DM}$: *In vitro* true digestibility of dry matter; $IVTD_{OM}$: *In vitro* true digestibility of organic matter; $IVTD_{NDF}$: *In vitro* true digestibility of neutral detergent fiber; C: Cultivation; T: Treatment

In our current study, it was seen that the value of $IVTD_{OM}$ is significantly (P<0.05) different for CTFW compared to OTFW. These differences can be thought to be due to the difference in the cultivation method.

The results of the present study showed a significant difference (P<0.05) for $IVTD_{As\ feed}$, $IVTD_{DM,}$ and $IVTD_{OM}$ values in terms of interaction (CxT) between treatment and cultivation methods (conventional and organic). Organic acids improved the digestion and absorption of nutrients by affecting the rumen microbiota. As reported in the European Commission 2003 report, FA is one of the naturally occurring compounds involved in metabolism. Genc et al. (2020) reported that the statistically highest $IVTD_{OM}$ value was in the group with 0.1% FA in their *in vitro* digestion trial with conventional tea wastes and that $IVTD_{As\ feed}$, $IVTD_{DM}$, and $IVTD_{NDF}$ values increased mathematically with 0.1% and 0.2% FA application. In these study conducted with alternative roughage raw materials, it was observed that a 0.3% FA level in *Q. cerris* leaves significantly increased the values of $IVTD_{As\ feed}$, $IVTD_{DM}$, $IVTD_{OM}$, and $IVTD_{NDF}$. It seems that these findings are in harmony with our research results. Since both acids are organic dicarboxylic acids, it has been observed that they do not have a positive synergistic effect on IVTD parameters when used together, and similar findings were mentioned in an *in vivo* study (Ebrahimi et al., 2015). Li et al. (2018) reported that the rumen bacterial population and $IVTD_{DM}$ values were not affected by the FA level (P>0.05) in an *in vitro* digestion experiment in

which they used alfalfa hay with better quality nutrient content than tea wastes. Lopez et al. (1999) reported that sodium fumarate did not show a positive effect on rumen bacteria, and the bacterial population decreased ($P<0.01$) after prolonged incubation for more than 48 hours. Genc et al. (2020) reported that FA treatment had a positive effect on IVTD, but high-level (10 mM and 0.3%) FA supplementation caused a negative effect on the same parameter. Consistent with these data, it is seen that the digestion values were at the lowest level ($P>0.05$) in the CTFW group using 0.3% FA in our study. Sahoo and Jena (2014) explained this situation in relation to the anionic effects of organic acids that may adversely affect ruminal microbial life. In digestibility studies (Burner et al., 2008; Chen et al., 2011; Genc et al., 2020) on alternative feed properties of high tannin-containing vegetable raw materials, the effect of tannin and polyphenol fractions to prevent fermentation was mentioned, and it was reported that rumen digestibility of these raw materials could be higher if the tannin level was lowered. Yu et al. (2010) have also reported that organic acids do not show the expected effect in *in vitro* digestion trials in the presence of factors that adversely affect the degree of digestion.

Variable results in *in vitro* digestion studies using organic acids can be attributed to the different effects of these acids on rumen microbial life. It can be thought that situations such as Gram (+) and Gram (-) bacteria having different atomic numbers, being affected differently by different organic acids (Partanen, 2001), and being affected by the practices of microbial (bacteria-protozoa-archaea) life order can cause different effects on *in vitro* digestion values. In *in vitro* digestion experiments, how these microbial forms are affected by the practices should also be examined. The digestive properties of substrates in digestion experiments are affected by many factors (Kara et al., 2015). Condensed tannin density, organic acids, easy soluble nutrient profile, and microbiota population are the prominent factors (Kara et al., 2018). Kara et al. (2018), in a study on the *in vitro* digestive valence of organic acids, reported that formic acid reduced digestive values and pointed out that this may be due to the reduction of the total number of ruminal bacteria and Entodiniinae and Diplodiniinae ciliate protozoa due to the antimicrobial properties of organic acids. Li et al. (2009) reported that while fumarate does not affect $IVTD_{NDF}$ value, it affects $IVTD_{DM}$ value negatively. The similar effects of the increasing fumaric acid dose are also seen for the CTFW in the present study.

In a study on the digestibility effects of organic acids (Newbold et al., 2005), it was reported that disodium fumarate increased $IVTD_{DM}$ activity, but the same effect was not seen in the use of fumaric acid. In an *in vivo* study in which the acid form of fumaric acid was used instead of the salt form (Molano et al., 2008), it was reported that the feed consumption values of lambs decreased with the increasing fumaric acid dose. This data shows that the use of fumaric acid in different forms may have different results. In some studies (Bayaru et al., 2001; McGinn et al., 2004; Beauchemin and McGinn, 2006; Kolver and Aspin, 2006) where fumaric acid was used at doses of 12 to 50 g/kg DM, it was reported that it had no positive effect on DM digestion, energy, and fiber digestibility. Yu et al. (2010) associated the inability to see the expected effect of fumaric acid in *in vitro* digestibility trials with the lack of sufficient fermentable carbohydrates in the feed raw materials used.

In a study (Nasehi et al., 2017) on the use of black and green tea wastes in ruminant feeding, it was reported that tea wastes treated with polyethylene glycol (PEG) had a significant effect ($P<0.05$) on $IVTD_{DM}$, $IVTD_{OM}$, $IVTD_{ME}$, and $IVTD_{NEL}$ values. The researchers attributed this effect, as Kumar and Vaithiyanatha (1990) and Silanikove et al. (1996) reported, to the strong binding of PEG and the prevention of the binding of tannins to proteins, especially with the high level of condensed tannins possessed by tea wastes. Bryant (1973) reported that cellulolytic rumen bacteria could not benefit enough from ammonia that cannot release in this case. Based on this interpretation, future studies might consider using organic acids together with tannin binders such as PEG to demonstrate the effect of organic acids on digestive parameters of tannin-containing feeds. Even though the effects of organic acids on rumen metabolism are expressed by very complex mechanisms, they can be simply characterized by their effect of accelerating fermentation. However, according to the calculations in *in vitro* studies, it is seen that a significant part of the fumarate added to the system does not participate in fermentation (Carro and Ungerfeld, 2015). As can be seen in our research results, a possible explanation might be that although the dose used is increased, digestibility does not increase in direct proportion. In the light of this information, it should be considered whether the amount of organic acid used in *in vitro* digestibility tests is effective or not. As a matter of fact, as Li et al. (2018) also reported in their research, it is known that the metabolism of fumaric acid is not fully understood in terms of bacterial population activity, especially fumarate-utilizing bacteria.

Conclusion

It is observed that the *in vitro* digestion parameters of tea factory wastes produced by the conventional method are higher with the application of fumaric acid compared to the wastes produced by the organic method. However, considering the levels of fumaric acid used, it suggests that the use of organic acids in tea factory wastes with additives such as tannin binders may yield more meaningful results due to higher *in vitro* digestion values in control groups. It was concluded that there is a need for different *in vivo* and *in vitro* studies considering the effects of antinutritional factors contained in tea factory wastes and their effects on ruminal microbiota.

Conflict of interest

The authors declare that they have no conflict of interest in this study.

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Research Article

Investigation of Plasma Lactate Concentration in Anemic Dogs

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ABSTRACT

In many clinical situations, the importance of determining plasma lactate level is emphasised, while the effect of type and severity of anemia on plasma lactate concentration is not fully known. It aimed to evaluate the impact of anemia type and severity on plasma lactate concentrations in dogs with anemia in this study. A total of 48 dogs (36 anemic, 12 healthy) of different breeds, ages, and sexes were included in the study. Dogs with anemia were classified according to the severity and type of anemia. Hematologic evaluations included RBC, HGB, HCT, and MCV measurements. Plasma lactate concentrations were colorimetrically tested on a point-of-care analyser. Plasma lactate levels were significantly (P<0.05) higher in dogs with anemia than in healthy dogs. Plasma lactate levels were significantly (P<0.05) higher in dogs with severe anemia than in healthy dogs. Plasma lactate concentrations of dogs with regenerative anemia were significantly higher than healthy dogs, but there was no significant difference between regenerative and nonregenerative anemia groups for plasma lactate concentrations. This study concluded that the type and severity of anemia affect plasma lactate concentrations in dogs with anemia.

Keywords: *Anemia, dog, lactate.*

Anemili Köpeklerde Plazma Laktat Konsantrasyonunun İncelenmesi

ÖZET

Birçok klinik durumda, plazma laktat düzeyinin belirlenmesinin önemi vurgulanırken aneminin tipi ve şiddetinin plazma laktat konsantrasyonuna etkisi tam anlamıyla bilinmemektedir. Bu çalışmada anemili köpeklerde aneminin tipi ve şiddetinin plazma laktat konsantrasyonlarına etkisinin değerlendirilmesi amaçlandı. Farklı ırk, yaş ve her iki cinsiyetten 36 anemik, 12 sağlıklı, toplam 48 köpek çalışmaya dahil edildi. Anemili köpekler aneminin şiddetine ve tipine göre sınıflandırıldı. Hematolojik değerlendirmeler RBC, HGB, HCT ve MCV ölçümlerini kapsadı. Plazma laktat konsantrasyonları hasta başı analizörü ile kolorimetrik olarak test edildi. Anemili köpeklerde plazma laktat değerleri sağlıklı köpeklere göre önemli (P<0,05) düzeyde yüksek bulundu. Şiddetli anemili köpeklerde, plazma laktat değerleri, sağlıklı köpeklere göre önemli (P<0,05) düzeyde yüksek belirlendi. Rejeneratif anemili köpeklerin plazma laktat konsantrasyonları sağlıklı köpeklerden önemli ölçüde daha yüksekti, ancak plazma laktat konsantrasyonları açısından rejeneratif ve nonrejeneratif anemi grupları arasında anlamlı bir fark yoktu. Bu çalışmada anemili köpeklerde aneminin tipi ve şiddetinin plazma laktat konsantrasyonlarını etkilediği sonucuna varılmıştır.

Anahtar kelimeler: *Anemi, laktat, köpek*

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Introduction

Lactate is an important biomarker whose level changes at various pathological processes produced by mammals. Measurement of blood lactate level has proven to be a helpful parameter in diagnosing, monitoring, and prognosis of different clinical syndromes in humans. Its clinical use is also increasing in small animals, and some studies show its role, especially in intensive care (Pang and Boysen, 2007; Saint-Pierre et al., 2022).

Lactate is the end product of anaerobic glycolysis. Lactate production rate increases in some conditions, such as hypoxia (Hood, 2005). Erythrocytes, skeletal muscle cells, brain, skin, and renal medulla produce lactate. While the liver removes most of the lactate produced, some are eliminated by the heart and kidney (Dugdale, 2010). Lactate is transported to the liver, which is primarily essential for its metabolism. If lactate production exceeds the liver's metabolization ability, hyperlactatemia occurs (Nel et al., 2004). Studies have shown that plasma lactate is a good predictor of treatment response and prognosis In addition, serial lactate measurements have been reliable in evaluating the response to treatment (Blutinger et al., 2021; Saint-Pierre et al., 2022). Blood lactate value is measured to determine tissue perfusion and prognosis and diagnose some disease groups in feline and canine medicine (Saint-Pierre et al., 2022).

The reference value of lactate in dogs is between 0.3-2.5 mmol/L (Hughes, 2000). 2 mmol/L plasma lactate concentration has been indicated as a target measure for veterinary and human patients (Pritte, 2006). It is graded as slight (3-5 mmol/L), moderate (5-8 mmol/L), and severe increase (>8 mmol/L) in dogs. The benefit of serial lactate monitoring has been demonstrated in multiple studies in veterinary medicine. It has increased significantly in many disease processes, such as septic peritonitis, immune-mediated hemolytic anemia, babesiosis, trauma, gastric dilatation-volvulus, and intracranial disease (Di Mauro et al., 2016; Rosenstein et al. 2018; Blutinger et al., 2021).

Anemia is not a disease but a symptom of many diseases' courses and outcomes. The laboratory finding of anemia is characterised by a decrease in the erythrocyte count or hematocrit (HCT) and hemoglobin (HGB) concentration below the physiological lower limit (Furman et al., 2014). It develops due to disrupting the balance between erythrocyte production and destruction or loss. Anemia is a common and sometimes life-threatening symptom in dogs. It can be clinically determined when the degree is moderate or severe. On the other hand, mild anemia can only be revealed with laboratory findings (Tvedten, 2010).

Anemias are classified as pathophysiologically regenerative and nonregenerative anemias. Classification schemes are based on reticulocyte count, erythrocyte indices, and pathogenesis. The response of reticulocytes to chronic hemorrhage is variable, and iron deficiency develops, with indices showing microcytosis and hyperchromasia. Nonregenerative anemias are normocytic normochromic. The pathology of regenerative anemias includes internal or external bleeding and intravascular or extravascular hemolysis. Nonregenerative anemia is due to causes such as chronic diseases, chronic kidney failure, and primary bone marrow disease (Thrall, 2012).

The primary function of erythrocytes is reoxygenation (Mohanty et al., 2014). Anemias from different etiologies cause oxidative stress by various mechanisms, and the developing oxidative stress shortens the lifetime of erythrocytes and decreases their oxygen-carrying capacity (Nagababu et al., 2008; Harvey, 2010; Iuchi, 2012). Severe anemia may produce mild to moderate hyperlactatemia without hypoperfusion, especially if the anemia is initially acute. Experimental studies of euvolemic hemodilution anemia required less than 15% PCV to increase plasma lactate. When the rate of lactate production in hypoxic tissue exceeds the rate of lactate metabolism in the body, blood lactate concentration increases. Hyperlactatemia in dogs with immune hemolytic anemia (IMHA); may result from decreased oxygen delivery to tissues due to severe anemia or systemic hypoperfusion. In this case, dogs with severe anemia, tissue hypoxia, and high lactate concentration are expected to have an increased risk of multiple organ failure, followed by death (Holahan et al., 2010).

While the importance of determining the lactate level is emphasised in many clinical situations, the effect of the type and severity of anemia on plasma lactate concentration is not fully known in anemic conditions. Therefore, this study aimed to evaluate the impact of the severity and type of anemia on plasma lactate concentrations in anemic dogs.

Materials and Methods

This study was carried out with the approval of the ethics committee decision numbered 64583101/2017/080 from the Animal Experiments Local Ethics Committee of Aydın Adnan Menderes University. The animal material of this study was composed of a total of 48 dogs, including 36 anemia dogs of different breeds, ages, and genders brought to Aydın Adnan Menderes University Veterinary Faculty Animal Hospital Polyclinics. Twelve healthy dogs were brought for general control or vaccination. Dogs that did not receive any treatment protocol before were included in the study. All dogs' descriptions, medical histories, anamnesis, physical examination findings, and laboratory analysis results were recorded. All dogs were included in the study voluntarily by informing the patient owners. Blood samples were taken from vena cephalica antebrachial tubes with EDTA (2 ml) for hemogram parameters and Heparin (2 ml) for plasma lactate determination. Care was taken to ensure that the tourniquet applied during blood collection lasted 15 seconds. Patients whose tourniquet duration exceeded 15 seconds were excluded from the study. Complete blood counts of blood samples taken into tubes containing EDTA were performed with an automatic blood count device (Abacus Junior Vet 5, Diatron, Hungary) immediately after blood collection.

The dogs were examined in two main groups healthy (control) and anemia. Dogs with anemia were grouped as mild, moderate, severe, and very severe anemic patients according to the criteria reported by Tvedten (2010). Blood samples taken into heparinised tubes were centrifuged at 3500 rpm for 10 minutes immediately after blood collection. Plasma samples obtained after centrifugation were immediately tested colorimetrically in the Euro Lyser Solo (Euro Lyser, Austria) device with the lactate test kit (Euro Lyser, Austria) according to the procedure specified by the manufacturer.

The blood samples taken from the tubes with EDTA were mixed with the same amount of New Methylene Blue dye. A thin smear was prepared from the mixture after waiting for 15 minutes at room temperature. CX31 and reticulocyte percentages were found by counting at least 1000 erythrocytes and reticulocytes in these optical fields under the microscope (Olympus, Japan). According to their reticulocyte percentages, anemia dogs were classified as regenerative and nonregenerative anemia dogs (Cowgill et al., 2003).

The arithmetic means standard error and minimal-maximal values of the parameters at the sampling time were calculated in the groups. The distribution of numerical data was evaluated using the Shapiro-Wilk test. For parameters with

normal distribution, normal distribution after transformation (logarithmic or square root), and homogeneous variance, one-way analysis of variance (ANOVA) was used in a comparison of more than two groups, differences between groups using the Tukey test in post-hoc comparison and from which group or groups the determined difference originated were tested. The Kruskal-Wallis test and posthoc comparisons were performed using the paired method for the parameters (MCV) determined not to show the parametric test assumptions. In comparing two independent groups, the Mann-Whitney U Test was used for the parameters that did not show the t-Test for the independent groups (MCV) for the normally distributed and homogeneous groups. Probability (P-value) P<0.05 was considered significant in all analyses. SPSS 22.0 (Statistical Package for the Social Sciences, IBM SPSS Statistics, Chicago, IL, USA) program was used for statistical evaluations.

Results

Statistical evaluations of red blood cell (RBC), HGB, HCT, and mean corpuscular volume (MCV) values in healthy and anemic dogs are presented in Table 1. RBC, HGB, HCT, and MCV values in dogs with anemia (n=36) were significantly lower (P<0.001) compared to healthy dogs (n=12). These hemogram parameters (HCT, RBC, HGB, MCV) and statistical results of dogs with anemia grouped according to severity are shown in Table 2. Since no dog could be found with a hematocrit value below 13%, a very severe anemia group was not formed. There were significant (P<0.001) differences in HCT value, RBC count, and HGB concentrations between mild, moderate, and severe groups in dogs according to the severity of anemia. It was determined that as the degree of anemia increased in dogs with mild, moderate, and severe anemia, the levels of HCT, RBC, and HGB decreased significantly. There was no significant difference between the mild and moderate anemia groups regarding MCV value. The MCV value was significantly lower (P<0.01) in dogs with severe anemia compared to healthy dogs.

Some hemogram parameters (HCT, RBC, HGB, MCV) and statistical results of dogs grouped according to the type of anemia are shown in Table 3. There was no significant difference between regenerative (n=20) and nonregenerative (n=16) anemia groups for HCT value, RBC count, and HGB concentrations. HCT, RBC, HGB and MCV values of regenerative and nonregenerative anemic dogs were significantly lower than those of healthy dogs.

Plasma lactate values and statistical results of healthy and anemic dogs, regardless of severity and type, are shown in Table 4. Plasma lactate concentrations in dogs with anemia were significantly higher (P<0.05) compared to healthy dogs.

Table 1. Some hematological findings (Mean±Standard Deviation, Minimum-Maximum) of healthy and anemic dogs.

Parameter	Healthy (n=12)	Anemic (n=36)	P
RBC	6.83±0.13 (6.22-7.86)	4.34±0.16 (2.32-5.82)	0.0001
HGB	13.92±0.43 (11.10-17.00)	8.38±0.34 (4.20-12.20)	0.0001
HCT	44.50±1.03 (38.48-51.24)	26.49±1.06 (14.36-36.63)	0.0001
MCV	65.08±0.74 (60.00-69.00)	61.08±0.84 (47.00-80.00)	0.001

Table 2. Some hemogram parameters (Mean±Standard deviation, Minimum-Maximum) of healthy dogs and anemic dogs grouped according to the severity of anemia.

Parameter		Severity of anemia (n=36)			
	Healthy (n=12)	Mild (n=12)	Moderate (n=12)	Severe (n=12)	P
RBC	6.83±0.13^{a} (6.22-7.86)	5.36±0.10^{b} (4.53-5.82)	4.46±0.14^{c} (3.85-5.20)	3.21±0.14^{d} (2.32-4.01)	0.0001
HGB	13.92±0.43^{a} (11.10-17.00)	10.33±0.39^{b} (8.40-12.20)	8.66±0.23^{c} (7.50-10.10)	6.16±0.30^{d} (4.20-8.20)	0.0001
HCT	44.50±1.03^{a} (38.48-51.24)	33.33±0.65^{b} (30.43-36.63)	27.20±0.69^{c} (24.19-32.16)	18.95±0.63^{d} (14.36-24.20)	0.0001
MCV	65.08±0.74^{a} (60.00-69.00)	62.25±1.11^{ab} (56.00-69.00)	61.16±0.78^{ab} (57.00-65.00)	59.83±2.15^{b} (47.00-80.00)	0.002

a, b, c, d: The difference between groups containing different letters on the same line is significant.

Table 3. Some hemogram parameters (Mean±Standard deviation, Minimum-Maximum) of healthy dogs and anemic dogs grouped according to the type of anemia.

Parameter	Healthy (n=12)	Type of Anemia (n=36)		P
		Regenerative (n= 20)	Nonregenerative (n=16)	
RBC	6.83±0.13[a] (6.22-7.86)	4.16±0.23[b] (2.32-5.68)	4.56±0.22[b] (2.47-5.82)	0.0001
HGB	13.92±0.43[a] (11.10-17.00)	8.15±0.46[b] (5.20-12.20)	8.68±0.50[b] (4.20-12.10)	0.0001
HCT	44.50±1.03[a] (38.48-51.24)	25.70±1.50[b] (17.50-36.63)	27.48±1.49[b] (14.36-35.40)	0.0001
MCV	65.08±0.74[a] (60.00-69.00)	61.90±1.23[b] (55.00-80.00)	60.06±1.08[b] (47.00-65.00)	0.003

a, b: The difference between groups containing different letters on the same line is significant.

Table 4. Plasma lactate concentrations (Mean±Standard deviation, Minimum-Maximum) of healthy and anemic dogs.

Parameter	Healthy (n=12)	Anemic (n=36)	P
Lactate	2.80±0.15 (2.18-4.10)	3.83±0.20 (0-99-7.07)	0.023

Table 5. Plasma lactate concentrations (Mean±Standard deviation, Minimum-Maximum) of healthy dogs and anemic dogs grouped according to the severity of anemia

Parameter	Healthy (n=12)	Severity of Anemia (n=36)			P
		Mild (n=12)	Moderate (n=12)	Severe (n=12)	
Lactate	2.80±0.15[b] (2.18-4.10)	4.01±0.30[ab] (2.81-6.18)	3.37±0.47[ab] (0.99-7.07)	4.10±0.25[a] (2.54-5.51)	0.021

a, b: The difference between groups containing different letters on the same line is significant.

Table 6. Plasma lactate concentrations (Mean±Standart deviation, Minimum-Maximum) of healthy dogs and anemic dogs grouped according to the type of anemia.

Parameter	Healthy (n=12)	Type of Anemia (n=36)		P
		Regenerative (n=20)	Nonregenerative (n=16)	
Lactate	2.80±0.15[b] (2.18-4.10)	3.88±0.26[a] (1.78-6.18)	3.76±0.34[ab] (0.99-7.07)	0.032

a, b: The difference between groups containing different letters on the same line is significant.

Statistical values of plasma lactate concentrations of dogs with anemia grouped according to severity are given in Table 5. According to the severity of anemia, there were no significant differences in plasma lactate concentrations between mild, moderate, and severe groups in dogs. In dogs with severe anemia, the plasma lactate value was significantly higher ($P<0.05$) than in healthy dogs.

Statistical results of plasma lactate concentrations of dogs grouped according to the type of anemia are given in Table 6. The plasma lactate concentrations of the dogs grouped as regenerative anemia according to the type of anemia were significantly ($P<0.05$) higher than the healthy dogs. Plasma lactate concentrations were not significantly different between the regenerative (n=20) and nonregenerative (n=16) anemia groups.

Discussion

Lactate measurement has become common in veterinary medicine due to its clinical utility and the increasing number of lactate analysers (Mackenzie et al., 2010). It has been reported that when the lactate production rate in hypoxic tissue exceeds the lactate metabolism rate in the body, blood lactate concentration increases (Holahan et al., 2010). While the importance of determining the lactate level in many disease states and symptoms is emphasised, the effect of the type and severity of anemia on plasma lactate concentration in anemic patients is not fully known. According to our knowledge, this is the first study to evaluate the severity and type of anemia effect on plasma lactate concentrations in anemic dogs.

Lactate concentrations can be measured from whole blood, plasma, or serum (Rosenstain et al., 2018). The term plasma lactate only refers to the lactate concentration in the plasma fraction. In contrast, whole blood lactate refers to the mean concentration of intraerythrocytic and plasma lactate fractions following red blood cell lysis (Hughes et al., 1999). It is reported that whether the blood sample taken from the patient is venous, arterial, or capillary affects the lactate value (Gallagher et al., 1997). The difference in lactate level between arterial and venous blood was determined as 0.18-0.22 mmol/L. It is reported that there is a high correlation (mean difference, 0.08 mmol/L) between venous and arterial blood lactate measurements. (Middleton et al., 2006). Researchers reported that lactate levels in venous and arterial blood samples were similar and acceptable. Gillespie et al. (2017) report that venous blood may be preferred because it is easier and less painful. Venous blood samples from dogs were selected for this study.

For accurate plasma lactate measurement, it is necessary to minimise the short vessel occlusion and the struggle for restraint (Gillespie et al., 2017). The effort affects plasma lactate concentrations, possibly depending on muscle activity (Rosenstain et al., 2018). Various studies have shown that struggle affects plasma lactate levels in healthy cats (Rand et al., 2002). It has been reported that the plasma lactate level increased rapidly and significantly in cats that applied a spray bath for 5 minutes before blood collection (Rand et al., 2002). In a smaller study of 21 cats, lactate levels of only 3 cats were found to be higher than 2.5 mmol/L (Redavid et al., 2012). A study on humans reported that blood draws by applying a tourniquet for a long time significantly increased the plasma lactate level, but temporary tourniquet applications for routine vein puncture did not significantly (Dede, 2016; Gillespie et al., 2017).

Anemia, a result of hematological and non-hematological diseases, is a crucial symptom. The most common causes are blood loss, decreased erythrocyte production, or increased erythrocyte consumption (Vuckovic and Allegreti 2015; Ray & Hemphill, 2016). The rate of lactate production increases in hypoxia (Hood, 2005). Common causes of tissue hypoxia resulting from oxygen supply/demand imbalance include hypovolemia, cardiogenic and septic shock, anemia, hypoxemia, and hypermetabolism (Torata and Raper, 1997; Zipes et al., 2005). The primary way of energy production in tissues is aerobic metabolism using oxygen. However, there is no oxygen storage system in the tissues, and convective and diffuse mechanisms provide oxygen delivery to the cells. By carrying oxygen, blood contributes to convection, that is, to delivering oxygen to cells (Leach and Treacher, 1998). The amount of oxygen reaching all cells in the body is called "oxygen delivery (DO_2)". The oxygen consumed in mitochondria is known as "oxygen consumption (VO_2)". Oxygen delivery is the partial pressure of oxygen in the blood reaching the cell. If it continues, consumption is also affected after a point called the "point where oxygen consumption becomes delivery dependent" or "critical DO_2". This is where tissue oxygenation begins to deteriorate (Rolland, 2011). An experimental model showed that the persistence of the critical level of DO_2 in the absence of treatment resulted in death within a maximum of 3 hours (Fontana et al., 1995). When the decrease in oxygen supply to the tissues exceeds a critical level, the oxidative mechanism in the cells is interrupted, and anaerobic metabolism begins.

The cardiac rate, the haemoglobin level in the blood, and the degree of saturation of hemoglobin with oxygen (SaO_2) determine the oxygen reaching the cell. A reduction in any of these is the cause of hypoxia (Zander, 1990). The decrease in hemoglobin can be called "anemic hypoxia" (Meier et al., 2012). Meier et al. (2004) reported in an experimental study that in subjects with hemodilution, and hemodynamic decompensation, an increase in lactate and catecholamine levels was observed when the critical hemoglobin level was reached and that the subjects died within 3 hours in the absence of blood transfusion. Cain (1977) reported that hyperlactatemia occurs with a decrease in HCT in dogs. In our study, plasma lactate value was significantly higher ($P<0.05$) in all dogs with anemia compared to healthy dogs. This situation is related to hypoxemia due to low oxygen transport capacity in patients with anemia.

The symptoms of anemia depend on the severity and cause of the anemia, the rate of occurrence, and the patient's age. Symptoms are usually due to hypovolemia and decreased oxygen delivery to tissues in acute anemia, such as bleeding. Signs and symptoms are more severe in considerable blood loss and acute hemolysis cases. The emergence of symptoms may be delayed until the hemoglobin concentration falls below 5 g/dl with the activation of compensatory mechanisms in chronically developing anemia. It has been shown that when the hematocrit value decreases, there is an increase in the lactate level (Fink, 2002; Dixon et al., 2003; Von Heymann et al., 2006; Huybregts et al., 2009; Garcia-Alvarez et al., 2014). However, Dixon et al. (2003) reported no correlation between increased lactate levels and hematocrit values in the intraoperative and postoperative periods. There were no significant differences in plasma lactate concentrations between mild and moderate groups in anemic dogs compared to healthy dogs in this study. However, in dogs with severe anemia, plasma lactate levels were significantly higher ($P<0.05$) than in healthy dogs. This is related to the low oxygen-carrying capacity due to anemia. However, since a very severe anemia group could not be formed, the effect of anemia at values below the critical level on plasma lactate levels could not be thoroughly evaluated.

The correlation between anemia and hyperlactatemia is highly dependent on the chronicity of the disease (Holahan et al, 2010). Clinically significant hyperlactatemia may develop in animals that become acutely anemic with secondary hemorrhage or hemolysis. In contrast, animals with chronic, severe anemia may have plasma lactate concentrations in the reference range (Holahan et al., 2010). In one study, hyperlactatemia did not develop in dogs with dilutional anemia until the hematocrit fell below 15% (Cain, 1965). Hyperlactatemia due to hypoxemia is rare in veterinary medicine, as PaO_2 values must be 25–40

mmHg before lactate concentrations start to increase (Cain, 1965; Cilley et al., 1991; Rosenstain et al., 2018). Different tissues have different tolerance to anemia. Blood flow changes because of the tissues' oxygen requirements and blood redistribution (Van Woerkens, 1992; Fan et al., 1980). Lauscher et al. (2013) reported that the determination of global oxygen supply and consumption is insufficient to determine each tissue's anemia tolerance. For example, kidney and skeletal muscle tissue show tissue hypoxia when hemoglobin is 6-7 g/dl. In this study, the plasma lactate concentrations of dogs with regenerative anemia were significantly ($P<0.05$) higher than those of healthy dogs. However, there was no significant difference between the groups with regenerative and nonregenerative anemia. As Du Pont Thibodeau et al. (2014) stated that the critical hemoglobin concentration, the point at which tissue oxygenation begins to deteriorate, and the oxygen usage conditions may be related to the variation according to the tissue and the individual.

Conclusion

While there is still much to learn about lactate measurement, it is an inexpensive and easy-to-do test that provides quick results to assist veterinarians in diagnosing and managing critically ill patients. Anemia in dogs is a life-threatening symptom, when severe, occurring as a course or consequence of many diseases. Effective and rational treatment of this symptom is possible by identifying and eliminating the disease or condition that causes anemia. The presence of concomitant tissue hypoxia in the presence of anemia is of vital importance in terms of early resuscitation and prognosis of patients. According to the results obtained from this study, it was determined that the plasma lactate level in dogs with anemia was significantly higher than in healthy dogs. In addition, it has been revealed that the type and severity of anemia may play a role in plasma lactate levels. In addition, it is thought that the data obtained can be used as a reference for wider and more comprehensive studies on dogs.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Research Article

Determination of Levels of Some Acute Phase Proteins, Tumor Necrosis Factor-α, Interleukin-1 and Interleukin-6 in Cattle with Trichophythosis

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A B S T R A C T

Our aim in the study was to determine the levels of some acute phase proteins (APP) and proinflammatory cytokines in cattle with trichophytosis. The animal material of the study consisted of total 30 cattle, 15 with trichophytosis and 15 healthy. The blood samples taken from the *Vena jugularis* of the cattle in the study into tubes without anticoagulant. In the obtained serum samples, haptoglobin, serum amyloid A (SAA), ceruloplasmin, albumin, total protein, tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL-6) analyzes were performed. It was determined that haptoglobin, SAA, TNF-α, IL-1, IL-6 and ceruloplasmin levels were increased in cattle with trichophytosis compared to the control group. In addition, albumin, total protein and globulin values were lower than the control group, but the difference between the groups was statistically insignificant. As a result, it is thought that trichophytosis causes changes in biochemical parameters in cattle, the use of biochemical parameters, especially APPs, will contribute to the diagnosis of trichophytosis.

Keywords: *Acute phase proteins, cattle, proinflammatory cytokine, trichophytosis.*

Trikofitozisli Sığırlarda Bazı Akut Faz Proteinleri, Tümör Nekrozis Faktör-α, İnterlökin-1 ve İnterlökin-6 Düzeylerinin Belirlenmesi

Ö Z E T

Çalışmadaki amacımız trikofitozisli sığırlarda bazı akut faz proteinler (AFP)'in ve proinflamatuar sitokin düzeylerinin belirlenmesidir. Çalışmanın hayvan materyalini 15 trikofitozisli ve 15 adet sağlıklı olmak üzere toplam 30 adet sığır oluşturdu. Çalışmada yer alan sığırların *Vena jugularis*'inden antikoagulansız tüplere alındı. Elde edilen serum örneklerinde haptoglobin, serum amiloid A (SAA), seruloplazmin, albümin, total protein, tümör nekrozis faktör-α (TNF-α), interlökin-1 (IL-1) ve interlökin-6 (IL-6) analizleri yapıldı. Trikofitozisli sığırlarda haptoglobin, SAA, TNF-α, IL-1, IL-6 ve seruloplazmin düzeylerinin kontrol grubuna göre yükseldiği belirlendi. Bunun yanı sıra albümin, total protein ve globulin değerlerinin ise kontrol grubuna göre düşmekle beraber gruplar arası fark istatistik olarak anlamsız olarak belirlendi. Sonuç olarak, trikofitozisin sığırlarda biyokimyasal parametrelerde değişime neden olduğu, biyokimyasal parametrelerden özellikle de AFP'lerin kullanımının trikofitozisin teşhisine katkı sağlayacağı düşünülmektedir.

Anahtar Kelimeler: *Akut faz protein, sığır, proinflamatuvar sitokin, trikofitozis.*

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Introduction

Trichophytosis is an enzootic and zoonotic skin disease characterized by hair loss, dandruff and keratinized crusting of the skin caused by fungi such as *Epidermophyton, Microsporium* and *Trichophyton*. It causes economic losses such as slowdown in animal growth, loss of live weight, deterioration of leather quality, ban on export of sick animals (Gökçe et al., 1999; Papini et al., 2009). *Trichophyton verrucosum* and *Trichophyton mentagrophytes* have been reported as the most important causative agents of the disease in cattle (Balıkçı and Gazioğlu, 2017). The disease generally shows symptoms in the form of itchy, painless, round, chalk dust/asbestos-like lesions in the head, neck and inguinal region of animals (Yılmazer and Aslan, 2010).

Acute phase proteins (APP) are known as proteins synthesized by the liver in inflammation, infection, tissue damage, neoplastic developments and some immunological diseases (Petersen et al., 2004; Murata et al., 2004; Gökçe and Bozukluhan, 2009). It is important because it is a good marker in the formation of inflammatory process and disease diagnosis, as well as being used in separating clinical diseases from subclinical and in the follow-up of treatment (Bozukluhan and Merhan, 2022; Merhan and Bozukluhan, 2022). The aim of the study is to determine the levels of some APP and proinflammatory cytokines in cattle with trichophytosis.

Material and Methods

The study was carried out with the approval of the Kafkas University Animal Experiments Local Ethics Committee (KAU-HADYEK) Ethics Committee numbered 2021/023. The animal material of the study consists of cattle of different breeds (8 montofon crosses and 22 simental crosses), both sexes (8 females and 22 males), and aged between 5-18 months, obtained from livestock farms in Kars province Digor district. A total of 30 cattle, 15 with trichophytosis and 15 healthy cattle in the control group, were formed. The diagnosis of the disease was made according to clinical and microscopic findings. The samples taken from the *Vena jugularis* of the cows in the study into tubes without anticoagulant were centrifuged at 3000 rpm for 15 minutes and their serums were obtained. Serums were stored at -20°C until analysis.

Ceruloplasmin was measured by the Colombo and Richterich (1964) chemical method, haptoglobin (Cat. No:TP-801), serum amyloid A (SAA, Cat. No:TP-802) (Tridelta Phase, Ireland) and TNF-α (Cat. No:E0019Bo), IL-1 (Cat. No:E0197Bo), IL-6 (Cat. No:E0001Bo, BT LAB, China) were measured with the ELISA kit, and albumin, total protein (Biolabo, France) was measured using a commercial test kit. Globulin value was calculated by subtracting albumin values from total protein.

Statistical Analysis: Independent Sample T-test was used to compare the groups in the statistical package for social sciences (SPSS) 20.0 package program.

Results

Haptoglobin, SAA, TNF-α, IL-1, IL-6 ($P<0.001$) and ceruloplasmin ($P<0.05$) levels were found to be statistically higher in cattle with trichophytosis compared to the control group. Although albumin, total protein and globulin values were lower than the control group, the difference between the groups was statistically insignificant ($P>0.05$) (Table 1). In the clinical examination of animals with trichophytosis, round, chalk dust/asbestos-like lesions were detected in the head, neck and inguinal region. When the samples taken from cattle were examined microscopically, it was determined that all of the factors were *Trichopyton verrucosum*.

Discussion

Trichophytosis is an enzootic and zoonotic skin disease in calves and young cattle characterized by hair loss, dandruff and keratinized crusting of the skin caused by fungi such as *Epidermophyton, Microsporium* and *Trichophyton*. It causes economic losses such as slowdown in animal growth, loss of live weight, deterioration of leather quality, ban on export of sick animals (Özkanlar

Table 1. Some acute phase proteins, tumor necrosis factor-α, interleukin-1 and interleukin-6 parameters in clinically healthy cattle with trichophytosis. Data are presented as mean±standard error (X±SEM).

Parameters	Control	Infected	P
Haptoglobin (g/L)	0.079±0.003	0.362±0.022	0.0001
Serum Amyloid A (µg/mL)	11.93±0.90	91.80±6.46	0.0001
Ceruloplasmin (mg/dL)	11.63±0.48	17.48±2.05	0.010
Albumin (g/L)	2.97±0.05	2.95±0.06	NS
Total Protein (g/L)	6.89±0.15	6.86±0.13	NS
Globulin (g/dL)	3.92±0.16	3.91±0.13	NS
TNF-α (pg/mL)	77.26±11.47	165.34±5.29	0.0001
IL-1 (pg/mL)	38.44±4.53	124.75±10.70	0.0001
IL-6 (pg/mL)	88.73±5.96	154.41±5.52	0.0001

NS: Non Significant

et al., 2009; Papini et al., 2009; Bozukluhan, 2014). As reported in the studies (Kabu and Sayın, 2016; Constable et al., 2017), in the clinical examination of animals with trichophytosis, round, chalk dust/asbestos-like lesions were detected in the head, neck and inguinal region. When the lesions were examined, it was determined that the causative agent was *Trichopyton verrucosum*.

Fungi produce different proteolytic enzymes and metabolic products, especially keratinases (Muhsin et al., 1997; Schaufuss and Steller, 2003). Following the infection, the organism reacts to the metabolic products that pass from the skin to the bloodstream (Atakisi et al., 2006; Apaydin Yildirim, 2020). As a result of this reaction, APP is produced from the liver. APP levels can change in conditions such as inflammation or infection, stress (Murata et al., 2004; Merhan and Bozukluhan, 2022).

Along with the activation of mononuclear cells such as monocytes and granulocytes in the inflammatory region, cytokines such as TNF-α, IL-1 and IL-6 are released. Cytokines are substances in peptide or glycoprotein structure that initiate and regulate inflammation as well as immunity (Ceciliani et al., 2002; Merhan and Bozukluhan, 2022). In a study conducted on buffaloes infected with *Fasciola gigantica*, it was reported that IL-6 and IL-8 levels were increased (Molina, 2005). In another study conducted in Anatolian buffaloes with trichophytosis, they reported that the cytokine level was higher than the control group (Kabu and Sayın, 2016). In the study, it was determined that the proinflammatory cytokine level was higher in cattle with trichophytosis compared to the control group. This increase may be due to the inflammatory response to trichophytosis.

Secreted proinflammatory cytokines are transported to the liver through the blood and stimulate the production of APP. Haptoglobin, SAA and ceruloplasmin are important APPs in cattle (Petersen et al., 2004; Merhan and Bozukluhan, 2022). Haptoglobin, which is found at very low levels in the serum of healthy ruminants, increases significantly in its concentration following tissue damage and inflammation (Petersen et al., 2004; Tothova et al., 2014). Haptoglobin, an important APP in cattle, has been reported to increase in diseases such as hypodermosis, pneumonia, enteritis, peritonitis, endocarditis, and traumatic reticuloperitonitis (Bozukluhan and Gökçe, 2007; Merhan et al., 2016; Merhan et al., 2017a; Bozukluhan et al., 2021).

Serum amyloid A is a positive APP used in determining the prognosis and severity of inflammation in complex with high-density lipoprotein (HDL) (Witkowska-Pilaszewicz et al., 2019; Merhan and Bozukluhan, 2022). SAA, which can increase over 1000 times in the circulation in inflammatory conditions, has functions such as preventing antibody formation by lymphocytes, inducing collagenase, and increasing leukocyte adhesion to endothelial cells (Petersen et al., 2004; Murata et al., 2004). The levels of SAA increases in bacterial (Merhan et al., 2017b), viral (Merhan et al., 2017c), and parasitic infections (Bozukluhan et al., 2017). In addition, serum levels increase in ketosis (Brodzki et al., 2021), after operations (Bozukluhan and Gökçe, 2007), and in fasting for more than 3 days (Katoh et al., 2002).

Ceruloplasmin, which is of moderate importance in cattle, has a molecular weight of about 151 kDa and a half-life of 5-7 days (Hellman and Gitlin, 2002). Ceruloplasmin, which has oxidase activity for many polyamine and polyphenol substrates, is an oxido-reductase and plays a role in the activity of ferroxidase, which is necessary for the oxidation of Fe^{+2} to Fe^{+3} (Hellman and Gitlin 2002; Merhan and Bozukluhan, 2022). In a study conducted with dogs with different diseases, they reported that APP concentration increased significantly in dogs with skin problems (Ulutaş et al., 2007). Studies on trichophytosis in cattle are limited in number, and in a study they conducted in Anatolian buffaloes infected with trichophytosis, they reported that haptoglobin and SAA levels increased statistically significant (Kabu and Sayın, 2016).

In other studies, it was reported that haptoglobin, SAA and ceruloplasmin levels increased before the treatment and decreased after the treatment when compared with the control group (Balıkçı and Gazioğlu, 2017; Şeliman, 2018). In the study, it is thought that haptoglobin, SAA and ceruloplasmin concentrations increased compared to the control group, and this increase is probably related to tissue damage and inflammation due to trichophytosis.

Biochemical changes in the blood are used in the diagnosis of many diseases. Studies have reported that trichophytosis causes changes in the blood biochemistry of animals (Atakisi et al., 2006; Arslan et al., 2007; Karapehlivan et al., 2007). Atakisi et al., (2006) reported that the liver enzymes gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH), alanine amino transferase (ALT), aspartate amino transferase (AST), and adenosine deaminase activities increased in cattle with trichophytosis compared to the healthy group. Arslan et al. (2007) reported that there was no change in blood urea nitrogen (BUN), creatinine levels, AST and ALT activities in cattle with trichophytosis. In addition, Kumar and Khurana (2002) reported in another study that AST and ALT enzyme activity was not statistically significant when compared to the healthy group. In a study by Kabu and Koca (2018), they reported that serum ALT and AST activities increased, BUN and creatinine levels decreased compared to the control group in buffaloes with trichophytosis. There was no statistical difference between the groups in GGT activity, levels of total protein, albumin, and total bilirubin. In studies conducted in ruminants with trichophytosis (Karapehlivan et al., 2007; Yıldırım et al., 2010), they reported that there was no significant difference between the groups in total protein and albumin levels. In the study, it was determined that albumin, total protein and globulin levels decreased when compared to the control group, but this decrease was not statistically significant.

Conclusion

As a result, it is thought that trichophytosis causes changes in biochemical parameters in cattle, the use of biochemical parameters, especially APPs, will contribute to the diagnosis of trichophytosis and more detailed studies should be done on this subject.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Research Article

A Study on Prof. Dr. Osman Kaya's Life and Scientific Studies

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ABSTRACT

Prof. Dr. Osman Kaya was born on May 20, 1960, in the Akşehir district of Konya. He graduated from Ankara University Faculty of Veterinary Medicine in 1985, which he entered in 1980. He started his scientific studies in 1987 at Selcuk University, Institute of Health Sciences. He received the title of microbiology doctor on March 25, 1991 with his thesis titled "Studies on the Diagnosis of Sheep and Goat Brucellosis with Allergic Skin Tests". Continuing his scientific studies at the same university, Kaya was appointed as Assistant Professor in the Faculty of Veterinary Medicine Microbiology Department on April 14, 1992. He won a scholarship from the Ministry of National Education and participated in research in various departments for two months at the Budapest Veterinary Faculty in 1992. Still, in 1994, with the support of TÜBİTAK, he conducted research and investigations at Edinburgh Veterinary Faculty and Moredun Research Institute for one month. Kaya was assigned as a transplant to Adnan Menderes University Faculty of Veterinary Medicine Department of Microbiology as Assistant Professor Dr. on the 3rd of July, 1995. On the 5th of October,1995, he received the title of Associate Professor with his chief work titled "Serological and Bacteriological Studies on *Haemophilus somnus* Infection in Cattle". Also, in 2001, he received the title of Professor at Adnan Menderes University. Kaya, who passed away on the 3rd of February, 2017, in his 30 years of academic life, has managed 22 theses and 13 projects, 7 of which are master's and 15 are doctoral dissertations. He has signed a total of 112 scientific studies, including 84 articles, 19 of which are in international peer-reviewed journals, 65 of which are in national peer-reviewed journals, as well as 28 papers presented at scientific events and meetings. Kaya, who was also the vice-rector, held administrative positions at various levels of Adnan Menderes University and contributed to the development of various faculties and colleges, especially Adnan Menderes University's Faculty of Veterinary Medicine, in addition to his academic contribution.

Keywords: *ADU Faculty of Veterinary Medicine, History of Veterinary Medicine, Prof. Dr. Osman Kaya, Veterinary Microbiology.*

Prof. Dr. Osman Kaya'nın Hayatı ve Bilimsel Çalışmaları Üzerine Bir Araştırma

ÖZET

Prof. Dr. Osman Kaya, 20.05.1960 tarihinde Konya'nın Akşehir ilçesinde doğdu. 1980 yılında başladığı Ankara Üniversitesi Veteriner Fakültesinden 1985 yılında mezun oldu. Bilimsel çalışmalarına 1987 yılında, Selçuk Üniversitesi, Sağlık Bilimleri Enstitüsünde başladı. "Koyun ve Keçi Brusellozisi'nin Allerjik Deri Testleri ile Teşhisi Üzerinde Çalışmalar" başlıklı teziyle 25 Mart 1991 tarihinde mikrobiyoloji doktoru unvanını aldı. Çalışmalarına aynı üniversitede devam eden Kaya, 14 Nisan 1992 tarihinde Veteriner Fakültesi Mikrobiyoloji Anabilim Dalında Yardımcı Doçent kadrosuna atandı. Milli Eğitim Bakanlığı yurt dışı bursunu kazanarak, 1992 yılında Budapeşte Veteriner Fakültesi'nde 2 ay süre ile çeşitli bölümlerde araştırmalara katıldı. 1994 yılında ise TÜBİTAK desteği ile 1 ay süreyle Edinburgh Veteriner Fakültesi ve Moredun Araştırma Enstitüsü'nde araştırma ve incelemelerde bulundu. Kaya, 03.07.1995 tarihinde Adnan Menderes Üniversitesi Veteriner Fakültesi Mikrobiyoloji Anabilim Dalı'na Yrd. Doç. Dr. olarak naklen atandı. 05.10.1995 tarihinde "Sığırlarda *Haemophilus somnus* Enfeksiyonu Üzerine Serolojik ve Bakteriyolojik Çalışmalar" isimli baş eseri ile doçentlik unvanını aldı. 2001 yılında da Adnan Menderes Üniversitesinde Profesör unvanını aldı. 03.02.2017 yılında hayata gözlerini yuman Kaya, 30 yıllık akademik yaşamında; 7'si yüksek lisans, 15'si doktora tezi olmak üzere 22 tez ve 13 proje yönetti. 19'u Uluslararası hakemli dergilerde, 65'i Ulusal hakemli dergilerde olmak üzere 84 makale ve ayrıca bilimsel etkinlikler ve toplantılarda sunduğu 28 bildiri olmak üzere toplamda 112 bilimsel çalışmaya imza attı. Başta rektör yardımcılığı olmak üzere, Adnan Menderes Üniversitesinin çeşitli kademelerinde idari görevlerde bulunarak, akademik katkısının yanı sıra başta Adnan Menderes Üniversitesi Veteriner Fakültesi olmak üzere çeşitli fakülte ve yüksekokulların gelişimine de katkıda bulundu.

Anahtar Kelimeler: *ADÜ Veteriner Fakültesi, ADÜ Veteriner Fakültesi, Prof.Dr. Osman Kaya, Veteriner Hekimliği Tarihi, Veteriner Mikrobiyoloji*

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Introduction

Biography studies are also included in the research studies in the history of veterinary medicine.

It is precious in terms of the formation of the profession's history that people who are known for their contributions to the veterinary medical profession in the process they live in are written down and presented in a tidy article. Many biographical books (Bekman, 1945; Erk and Dinçer, 1970; Sinmez and Yaşar, 2011) and articles (Yaşar, 1997; Yaşar, 1998a, 1998b; Yaşar et al., 2010; Sinmez et al., 2012; Yaşar et al., 2020) have been published since it has an important and valuable place in Veterinary History research. Similarly, it seems that it has become a tradition to write biographies of faculty members who retired or died to establish the history of Aydın Adnan Menderes University Veterinary Faculty (Arslan, 1999; Arslan, 2004; Arslan, 2008a, 2008b).

In this respect, writing the biography of Prof. Dr. Osman Kaya is also seen as an essential debt of loyalty to the history of the profession.

Osman Kaya, who provided not only academic contributions but also support and services at the point of development and structuring of the university, also served as the Vice Rector of Aydın Adnan Menderes University. While carrying out his academic and administrative duties, Kaya could not get rid of his cancer disease, which was still under treatment, and was sent off on his last journey on February 03, 2017.

This study aims to reveal Professor Osman Kaya's contributions to the world of science by examining his national and international studies and administrative duties in veterinary microbiology. In addition, it is aimed to contribute in terms of bibliography by giving his works in order and collectively.

Materials and Methods

The materials of this study were composed of primary sources in the ADU Rectorate Archive, his private file in the Dean's Office, the Department of Microbiology Archive, and the Department of History of Veterinary Medicine and Deontology Archive. The study was supported by documents provided by Dr. Osman Kaya's older sister Sevil Kaya[1].

The information obtained from examining the documents has been transcribed following the chronology with a retrospective approach. The original records are mentioned in the endnotes.

In this study, Prof. Dr. Osman Kaya's scientific studies are given chronologically by type. The Turkish articles and journal titles have yet to be translated into English for easy access and use by researchers in the field of microbiology.

[1] Interviews and letters with Prof. Dr. Osman Kaya's older sister Sevil Kaya.

T.C.
ÜNİVERSİTELERARASI KURUL

Doçentlik Belgesi

2547 Sayılı Yükseköğretim Kanununun ilgili maddeleri ve Doçentlik Sınav Yönetmeliği hükümleri uyarınca girdiği Doçentlik Sınavını başaran Dr. Osman Kaya'ya 5.10.1995 tarihinde Mikrobiyoloji (Veteriner) Ana Bilim Dalında Üniversite Doçenti ünvan ve yetkisi verilmiştir.

Prof. Dr. Mehmet YÜCESOY
ÜNİVERSİTELERARASI KURUL
BAŞKANI

Figure 1. Prof. Dr. Osman Kaya's Associate Professorship Certificate.

Results

Professional and Scientific Life

Prof. Dr. Osman Kaya was born on 20.05.1960 in the Akşehir district of Konya. He completed his primary and secondary education in Ankara. Kaya graduated from Ankara University Faculty of Veterinary Medicine in 1985, which he entered in 1980. He started his scientific studies in 1985 as a research assistant at Selcuk University, Faculty of Veterinary Medicine. Prof. Kaya began his doctorate at Selçuk University, Institute of Health Sciences in 1987 (Anonymous, 2014).

Kaya completed his doctoral studies in "Veterinary Microbiology" with his doctoral thesis titled "Studies on the Diagnosis of Sheep and Goat Brucellosis with Allergic Skin Tests" and received the title of microbiology doctor on March 25, 1991. Kaya was appointed to the staff of Assistant Professor in the Microbiology Department of the Faculty of Veterinary Medicine on April 14, 1992 (Anonymous, 2014).

Kaya was reassigned by transferred to Aydın Adnan Menderes University, Faculty of Veterinary Medicine, Department of Microbiology on, July 3, 1995, as an Assistant Professor. He received the title of associate professor with his "chief work" named "Serological and Bacteriological Studies on *Haemophilus Somnus* Infection in Cattle" on October 5, 1995 (Figure 1). And then, Osman Kaya was appointed to the professorship in 2001 by the Adnan Menderes University Appointment and Promotion Regulations (Anonymous,1995; Anonymous, 2014) (Figure 2). Throughout his academic life, Kaya managed seven master's theses, 15 doctoral theses, and 13 scientific projects. He has 84 published articles, 19 in international peer-reviewed journals, and 65 in national peer-reviewed journals. Prof. Dr. Kaya has 29 papers presented in national and international symposiums on various dates.

Kaya speaks English and has been involved in 12 scientific research throughout his academic life. As of 2010, Osman Kaya's total number of citations is 52. Unable to recover from the cancer he caught, Prof. Dr. Osman Kaya left us on February 3, 2017 (Anonymous, 2017).

Figure 2. Osman Kaya with his colleagues during his professorship (Anonymous, 2022).

Prof. Dr. Kaya, in 1992, won the overseas scholarship opened by the Ministry of National Education and participated in research in various departments at the Budapest Veterinary Faculty in Hungary for two months to work on Pasteurellosis. With the support of TÜBİTAK, he went to Scotland in 1994 and conducted research and investigations for a month at the Edinburgh Faculty of Veterinary Medicine and Moredun Research Institute (Anonymous,1995).

Education and Training Activities

Kaya, who started his academic life at Selcuk University, Faculty of Veterinary Medicine, Department of Microbiology, attended applications microbiology classes as an assistant (Figure 3). He also gave postgraduate courses to graduate and doctorate students at Aydın

Figure 3. Prof. Dr. Osman Kaya during his laboratory work.

Adnan Menderes University Health Sciences Institute and advised their theses.

After being appointed Assistant Professor Doctor, he gave undergraduate courses in microbiology. He again taught undergraduate microbiology courses in the Department of Microbiology at Aydın Adnan Menderes University, Faculty of Veterinary Medicine, where he continued his academic life.

Master's and Doctoral Theses Supervised

Master's Theses Supervised

1. Koyun ve Kuzu Akciğerlerinden Mycoplasma İzolasyonu (Murathan GÜLAL -1999, Master Thesis, Adnan Menderes University Institute of Health Sciences).

2. Aydın Yöresindeki Konjunktivisli Ev Köpeklerinden Etken İzolasyonu ve Duyarlı Antibiyotiklerin Saptanması (Birgül ÜNAL- 1999, Master Thesis, Adnan Menderes University Institute of Health Sciences).

3. Aydın Bölgesinde İneklerde Mastitislere Neden Olan Koagulaz negatif ve pozitif Stafilokokların Biyotiplendirilmesi (Levent ŞAHİN -2001, Master Thesis, Adnan Menderes University Institute of Health Sciences).

4. Tavuklarda Mycoplasma gallisepticum'a karşı oluşan antikorların çeşitli serolojik yöntemlerle (Rapıd Sera Aglutınatıon-rsa ve Enzym Lınked Immuno Sorment Assay- Elısa) saptanması ve sonuçlarının karşılaştırılması. (Gülay ÖZKAYNAK- 2004, Master Thesis, Adnan Menderes University Institute of Health Sciences).

5. Siirt İlinde Hizmet Veren Değişik Birimlerden (Lokanta, Kafeterya gibi) Alınan Örneklerden Patojen Mikroorganizmaların Aranması (Melih SAYIN- 2007, Master Thesis, Adnan Menderes University Institute of Health Sciences).

6. Otitis Eksternalı Kedilerden Bakteriyel Etkenlerin İzolasyonu ve Antibiyotiklere Duyarlılıklarının Belirlenmesi (M. Erkan ÖZENER- 2010, Master Thesis, Adnan Menderes University Institute of Health Sciences).

7. Aşılı Broiler Sürülerinde Newcastle ve İnfeksiyöz Bronşitis Viruslarına Karşı Oluşan Aşı Etkinliğinin ELISA Metodu ile Saptanması (S. Belgin AYDIN- 2010, Master Thesis, Adnan Menderes University Institute of Health Sciences).

Doctoral Theses Supervised

1. İshalli Buzağılar ve Septisemili Tavuklardan İzole Edilen E. coli'lerin Adhezinleri (K99, K88, F41, 987P ve TİP1 Fimbria) ve Enterotoksinleri (Sta ve VT) Üzerinde Çalışmalar (Doktora Tezi, S.Ü.) (Muhammet Aksın, 1994, PhD Thesis, Selçuk University Institute of Health Sciences).

2. Aydın Yöresinde Koyunların Solunum Sisteminde İnfeksiyon Nedeni Mannheimia (Pasteurella) Haemolytica'nın Biyotip ve Serotip Tayini, Elektroforez ve PCR ile tanısı. (Şükrü Kırkan – 2003, PhD Thesis, Adnan Menderes University Institute of Health Sciences).

3. Kedi ve Köpek Orijinli Acinetobacter Türlerinin İzolasyonu, İdentifikasyonu ve Antibiyotik duyarlılıklarının belirlenmesi (Öznur Kaan Yılmaz- 2005, PhD Thesis, Adnan Menderes University Institute of Health Sciences).

4. Değişik Kaynaklardan İzole Edilen Pseudomonas Aeruginosa Suşlarının Antibiyotik Duyarlılıklarının Tespiti ve Biofilm Oluşumunun Araştırılması (Birgül Ünal-2005, PhD Thesis, Adnan Menderes University Institute of Health Sciences).

5. Gökkuşağı Alabalıklarında Enterik Kızıl Ağız Hastalığına Karşı Yersinia Ruckeri Serotip I ve Saha Suşlarından Hazırlanan Monovalan Ve Polivalan Aşıların Bağışıklık Güçlerinin Karşılaştırmalı İncelenmeleri, (Yeşim Tıravoğlu Demirtaş, 2006, PhD Thesis, Adnan Menderes University Institute of Health Sciences).

6. Aydın ve İzmir Bölgesindeki Sığırlardan Pasteurella Multocida'nın İzolasyonu, Tiplendirilmesi ve Antibiyotiklere Duyarlılıkları, (Göksel Erbaş-2007, PhD Thesis, Adnan Menderes University Institute of Health Sciences).

7. Broiler Piliçlerden Escherichia Coli O157:H7 Serotipinin İdentifikasyonu ve Antibiyotik Duyarlılıklarının Belirlenmesi, (Sinem Gökçe SEKMEN- 2008, PhD Thesis, Adnan Menderes University Institute of Health Sciences).

8. Kerevit Vebasının İleri Tanı Yöntemleri ile Araştırılması (Lütfi AVSEVER- 2008, PhD Thesis, Adnan Menderes University Institute of Health Sciences).

9. Birinci Basamak Sağlık Kuruluşuna Başvuran Hastalarda Tetanoz İmmünitesi, (Selcen ÖNCÜ-2008, PhD Thesis, Adnan Menderes University Institute of Health Sciences)

10. Köpeklerde Sistemik Mantar Enfeksiyonu Oluşturan Aspergillus Fumigatus ve bazı Patojen Candida Türlerinin Nested PCR ile Saptanması, (Serten TEKBIYIK-2009, PhD Thesis, Adnan Menderes University Institute of Health Sciences).

11. Broiler piliçlerde İmmun Kompleks İnfeksiyöz Bursal Hastalık (Gumboro) Aşısının Etkinliğinin ELISA ile Araştırılması (Meral Meltem YILMAZLAR-2010, PhD Thesis, Adnan Menderes University Institute of Health Sciences).

12. Aydın İli Mezbahalarında E. coli O157:H7 ve Listeria Monocytogenes Varlığının Araştırılması (Cemalettin YEŞİLYURT-2010, PhD Thesis, Adnan Menderes University Institute of Health Sciences).

13. Staphylococcus Aureus Suşlarında Panton-Valentine Lökosidin (PVL) Genlerinin Araştırılması (Şebnur HAZIMOĞLU-2011, PhD Thesis, Adnan Menderes University Institute of Health Sciences).

14. Sığır Koyun ve Keçi Sürülerinde Coxiella Burnetti Yayılımının Saptanması. (Uğur Parın-2011, PhD Thesis, Adnan Menderes University Institute of Health Sciences).

15. Aydın İlinde Bulunan Yumurtacı Tavuklarda İndikatör Bakterilerin İdentifikasyonu ve Antibiyotik Duyarlılıklarının Belirlenmesi (Gökhan Ege-2016, PhD Thesis, Adnan Menderes University Institute of Health Sciences).

Research Projects Conducted

1. Aydın'da Üretilen Dondurmalarda Patogen Mikroorganizmaların İncelenmesi. ADÜ Araştırma Fonu (VTF-96001).

2. Aydın Bölgesinde Sığır Brucellozis'inin Serolojik Testlerle Belirlenmesi. ADÜ Araştırma Fonu (VTF-97006).

3. Koyun Pnömonik Pasteurella İnfeksiyonlarına Karşı Aşı Geliştirme Çalışmaları. Tübitak VHAG-997 nolu projesi.

4. Koyun ve Kuzu Akciğerlerinden Mycoplasma İzolasyonu. ADÜ Araştırma Fonu (SAE 98001).

5. Aydın Bölgesindeki Sağlıklı ve Pnömoni Şüpheli Koyunlardan Pasteurella Haemolytica'nın İzolasyonu, Biyotip Tayini ve Antibiyotiklere Duyarlılıkları. ADÜ Araştırma Fonu (SAE 98003).

6. Aydın Yöresindeki İneklerde Klinik Mastitis'e Neden olan Mikroorganizmaların Saptanması ve Bunların Antibiyotiklere Duyarlılıklarının İncelenmesi. ADÜ Araştırma Fonu (SAE 98004).

7. Aydın Bölgesinde İneklerde Mastitislere Neden Olan Koagulaz Negatif ve Pozitif Stafilokok'ların Biyotiplendirilmesi. ADÜ Araştırma Fonu (SAE 99001).

8. Değişik Kaynaklardan Plesiomonas shigelloides Suşlarının İzolasyonu ve Bu İzolatların Antibiyotiklere Duyarlılıklarının Belirlenmesi. ADÜ Araştırma Fonu (VTF 99008).

9. Aydın ve İzmir Bölgesindeki Sığırlardan Pasteurella Multocida'nın İzolasyonu, Tiplendirilmesi ve Antibiyotiklere Duyarlılıkları (Doktora Tezi, ADÜ BAP VTF 06007).

10. Kerevit Vebasının İleri Tanı Yöntemleri ile Araştırılması (Doktora Tezi, ADÜ BAP VTF-07019).

11. Broiler Piliçlerden Escherichia Coli O157:H7 Serotipinin İdentifikasyonu ve Antibiyotik Duyarlılıklarının Belirlenmesi (Doktora Tezi, ADÜ BAP VTF-07021) Akademik ve Bilimsel Çalışmaları.

12. Staphylococcus Aureus Suşlarında Panton-Valentine Lökosidin (PVL) Genlerinin Araştırılması (Doktora Tezi, ADÜ BAP).

13. Aydın İlinde Bulunan Yumurtacı Tavuklarda İndikatör Bakterilerin İdentifikasyonu ve Antibiyotik Duyarlılıklarının Belirlenmesi (ADÜ BAP VTF-15007).

Scientific Publications

Articles published in international peer-reviewed journals:

1. Erganiş, O., Çorlu, M., **Kaya, O.**, Ateş, M. (1988). A Study Rapid Diagnosis of E. coli Strains with K99 Antigen. Livestock Adviser. 13(11), 47-50.

2. Erganiş, O., Çorlu, M., **Kaya, O.**, Ateş, M. (1988). Isolation of Acinetobacter Calcoaceticus from Septicaemic Hens. Vet. Rec. 123,374.

3. Erganiş, O., **Kaya, O.**, Çorlu, M., İstanbulluoğlu, E. (1989). Hemaglutination Hydrophobicity, Enterotoxigenicity and Drug-Resistance Characteristics of Avian Escherichia Coli. Avian Dis. 33, 631-635.

4. Kaya, O., Ateş, M., Erganiş, O., Çorlu, M., Şanlıoğlu, S. (1989). Isolation of Acinetobacter Lwoffi from Hens with Septicemia. J. Vet. Med. B. 36, 157-158.

5. Diker, S., Akan, M., **Kaya, O.** (1999). Serotypes and Electrohoretic Protein Profiles of Pasteurella Haemolytica Isolated from Pneumonic Ovine Lungs. Dtsch. Tierärztl. Wschr., 106, 189-228.

6. Diker, K.S, Akan, M, **Kaya, O.** (2000). Evalution of Immunogenicity of Pasteurella Haemolytica Serotypes in Experimantal Models. Türk J. Vet. Anim. Sci., 24, 139-143.

7. Kaya, O., Kırkan, Ş., Ünal, B. (2000) Isolation of Dermatophilus Congolensis from a Cat. J. Vet. Med. B, 47, 155-157.

8. Erganiş, O., **Kaya, O.**, Hadimli, H.H., Güler, L. (2002). Rapid diagnosis of ovine Brucella, Campylobacter and Salmonella Infections from Fetal Stomach Contents by Coagglutination Test. Small Ruminant Research, 2188, 1-5.

9. Kirkan, S., Göksoy, E.O., **Kaya, O.** (2003). Isolation and antimicrobial susceptibility of Aeromonas Salmonicida in Rainbow Trout (Oncorhynchus mykiss) in Turkey Hatchery Farms. J. Vet. Med. B, 50, 339-342.

10. Kirkan, S., **Kaya, O.** (2005). Serotyping of Mannheimia Haemolytica strains isolated from pneumonic lungs of sheep in Aydin region of Turkey. Turk. J. Vet. Anim. Sci., 29, 491-494 .

11. Kirkan, S., Göksoy, E.O., **Kaya, O.** (2005). Identification of and antimicrobial susceptibility to Staphylococcus Aureus and Coagulase Negative Staphylococci from bovine mastitis in the Aydin region of Turkey. Turk. J. Vet. Anim. Sci., 29, 791-796.

12. Türkyılmaz, S., **Kaya, O.**, (2005). Detection of Antibodies Produced Against Ornithobacterium Rhinotracheale and Bordetella Avium by Enzyme-Linked Immunosorbent Assay in Hens and Turkeys in Aydın Province, Turkey. Turk. J. Vet. Anim. Sci., 29, 897-902.

13. Türkyılmaz, S., **Kaya, O.**, (2006). Determination of Some Virulence Factors in Staphylococcus spp. Isolated from Various Clinical Samples. Turk. J. Vet. Anim. Sci., 30, 127-132.

14. Türkyılmaz, S., Türkyılmaz, M., **Kaya, O.** (2006). A Comparative Study of Detection of Bordetella Avium Antibodies in Turkeys by ELISA, SPAT, and AGID Test. Turk. J. Vet. Anim. Sci., 30, 165-169.

15. Goksoy, E.O., Kirkan, S., **Kaya, O.** (2006). Comparison of Polymerase Chain Reaction and Conventional Methods for the Diagnosis of Listeria Monocytogenes in Stuffed Mussels. Turk. J. Vet. Anim. Sci., 30, 229-234.

16. Kirkan, S., Goksoy, E.O., **Kaya, O.**, Tekbıyık, S. (2006). In-vitro Antimicrobial Susceptibility of Pathogenic Bacteria in Rainbow Trout (Oncorhynchus mykiss, Walbaum). Turk. J. Vet. Anim. Sci., 30, 337-341.

17. Kirkan, S., Göksoy, E.O., **Kaya, O.** (2006). Detection of Listeria Monocytogenes by using PCR in Helix pomatia. Turk. J. Vet. Anim. Sci., 30, 375-380.

18. Kirkan, S., **Kaya, O.** Tekbiyik, S., and Parin, U. (2008). Detection of Coxiella Burnetii in Cattle by PCR. Turk. J. Vet. Anim. Sci., 32, 215-220.

19. Savasan, S., **Kaya, O.**, Kırkan, Ş., Çiftci, A, (2008). Balık kökenli Enterococcus Faecalis Suşlarının Antibiyotik Dirençlilikleri, A Ü Vet Fak Derg, 55(2):107-110.

Articles published in national peer-reviewed journals

1. Aslan, V., Sezen, Y., Erganiş, O., Tiftik, A., **Kaya, O.** (1987) Buzağılarda Eksperimental Kolibasillozisin ESHA Calvasid 60, ESHA Calvasid ve Cholostral Suplement ile Tedavisi Üzerinde Araştırmalar. S.Ü. Vet. Fak. Derg. 3 (1), 133-144.

2. Erganiş, O., Çorlu, M., **Kaya, O**, Ateş, M., İstanbulluoğlu, E. (1987). The Use of Simmon's Citrate Agar Supplemented with Adonitol for Selection of E. coli strains with K99 Antigen. Doğa, Tr. J. Veterinary and Animal Sciences, 13 (3),185-190.

3. Erganiş, O., Ateş, M., Çorlu, M., **Kaya, O.**, Ateş, M., İstanbulluoğlu, E. (1988). İshalli Buzağılardan İzole Edilen E. coli Suşlarında K99 Fimbriyanın Varlığı Üzerinde Bir Çalışma. Doğa, Tr. J. Veterinary and Animal Sciences, 12 (3),185-190.

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5. Erganiş, O., **Kaya, O.** (1988). Tavuk Tifosu. Hasad Derg. 52, 23-24.

6. Erganiş, O., Ateş, M., **Kaya, O.**, Çorlu, M., (1989) Konya Bölgesindeki İshalli Buzağılardan İzole Edilen *E.coli*'lerin Biyokimyasal, Hemaglütinasyon, Mannoz Rezistan Hemaglütinasyon ve Enteropatojenik Özellikleri Üzerinde Araştırmalar. Doğa, Tr. J. Veterinary and Animal Sciences, 13 (2),109-122.

7. Kaya, O., Kıran, M., Erganiş, O., Çorlu, M., Ateş, M., (1989). Pnömonili bir Kuzudan Kingella Denitrificans izolasyonu. Etlik Vet. Mikrob. Derg. 6 (4), 101-106.

8. Çorlu, M., Ateş, M., Erganiş, O., **Kaya, O.** (1990). Konya Bölgesindeki İshalli Kuzulardan İzole Edilen E. coli Suşlarının Çeşitli Özellikleri Üzerinde İncelemeler. Doğa, Tr. J. Veterinary and Animal Sciences, 14 (1),126-133.

9. Erer, H., Ateş, M., **Kaya, O.**, Kıran, M., Berkin, S. (1990). Koyun Mastitisleri Üzerinde Patolojik ve Bakteriyolojik İncelemeler. Etlik Vet. Mikrob. Derg. 6 (6), 79-97.

10. Çoker, A., Mete, K., **Kaya, O.** (1990). Brucella Mellitensis ile Enfekte Koyun Kan Serumlarında Rivanol Pleyt Testin Diğer Yardımcı Testlerle Karşılaştırılması. Pendik Hay. Hast. Araş. Enst. Derg. 21 (2). 17-22.

11. Erganiş, O., **Kaya, O.** (1990). Cryptosporidiozis Infection in Layer Type Chickens in Turkey. Veterinarium. 1 (1), 24-25.

12. Kenar, B., Erganiþ, O., **Kaya, O.** , Güler, L. (1990) Konya Bölgesinde Koyunlarda Atıklara Sebep Olan

Brucella, Campylobacter, Salmonella ve Chlamidia'ların Bakteriyolojik ve Serolojik İncelenmesi. Veterinarium. 1 (1), 17-19.

13. Erganiş, O., **Kaya, O.**, Ateş, M., İstanbulluoğlu, E. (1990). Konya EBK Kombinasında Kesilen Koyunlardaki Abseli Lenf Yumruları Üzerinde Mikrobiyolojik ve Serolojik İncelemeler. Veterinarium. 1 (1), 8-11.

14. Erganiş, O., **Kaya, O.**, Sezen, Y. (1990). Civcivlerde Mycoplasma Gallisepticum ile Kombine bazı İnfeksiyonlara Karşı Baytril ve Tri-Alplusin'in Etkinliği Üzerinde Bilimsel Bir Çalışma. Veterinarium. 1 (2), 20-23.

15. Çoker, A., Mete, K., **Kaya, O.** (1990). Brucella Mellitensis Rev 1 Aşısının Fermentörde Sıvı Vasatta Üretilmesi. Pendik Hay. Hast. Araş. Enst. Derg. 21 (2). 5-9.

16. Kaya, O. , Erganiş, O., Türütoğlu, H. (1991). Brucella Mellitensis Rev 1 Aşısı ile Aşılı Koyunlarda Brucella Allerjik Deri Testinin Güvenilirliği. Veterinarium. 2 (3-4), 22-25.

17. Kaya, O. (1991) Hindilerde Rastlanılan Shigella İnfeksiyonu. Hasad Derg. 63,28.

18. Aştı, R., Çelik, I., **Kaya, O.** (1991). Laktasyon ve Kuru Dönemdeki Meme Dokusunun Sellüler ve Humoral Savunma Sistemi Üzerindeki Işık Mikroskobik Çalışmalar. Veterinarium. 2 (3-4), 17-21.

19. Aslan, V., Turgut, K., **Kaya, O.**, Sevinç, M. (1991). Sığırlarda Listeriozis Olgusu. Hayv. Araş. Derg. 1 (1), 37-39.

20. Kaya, O., Erganiş, O. (1991). Koyun ve Kuzu Pnömonileri Üzerinde Etiyolojik Survey. Veterinarium. 2 (3-4), 27-29.

21. Diker, S.K., Aydın, F., Sertel, M., Yurdaydın, N., Daşkın, A., **Kaya, O.** (1991). Prevalence and Causes of Ram Epididymitis in Turkey. Doğa, Tr. J. Veterinary and Animal Sciences, 15, 65-71.

22. Başoğlu, A., Turgut, K., Maden, M., **Kaya, O.** (1992). İshalli Buzağılarda Cryptosporidium'ların Önemi Üzerinde Araştırmalar. Hayv. Araş. Derg., 2 (1), 40-41.

23. Erganiş, O., Orhan, G., **Kaya, O.**, Uçan, S., Kuyucuoğlu, Y. (1992). Kolibasillozisli Tavuklardan İzole Edilen Escherichia Coli'lerde Type 1 Pylus Tiplendirilmesi. Verterinarium. 3 (2), 7-12.

24. Erganiş, O., **Kaya, O.**, Güler, L., Kenar, B. (1992). Koyun Brusellozisinin Sahada Koaglütinasyon Testi ile Teşhisi. Veterinarium. 3 (1), 11-13.

25. Kaya, O. (1992) Koyun ve Keçi Brusellozis'inin Allerjik Deri Testleri ile Teşhisi Üzerinde Çalışmalar. Veterinarium, 3 (1), 13-18.

26. Ataoğlu, T., Erganiş, O., **Kaya, O.**, Kesim, S. (1993). Kemikiçi Dental İmplant Sulkuler Mikroflorasında Periodent Apatojen Bakterilerin Varlığı. G.Ü. Diş Hek. Fak. Derg., X (1), 105-112.

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29. Kaya, O., Orhan, G., Erganiş, O., Güler, L., Kuyucuoğlu, Y., Kesler, K. (1993). Broiller Civcivlerinde Sekal Anaerob Flora ve Karbonhidratların Salmonella typhimurium'un Barsak Epiteline Kolonizasyonu Üzerine Etkisi. Veterinarium. 4 (2), 27-32.

30. Kıran, M., Berkin, B., **Kaya, O.**, Dinçer, Z. (1993). Konya Bölgesi Koyun Pnömonilerinde Patolojik ve Etiyolojik Araştırmalar. S.Ü. Vet. Fak. Derg. 9 (1), 3-9.

31. Kaya, O., Erganiş, O., Boynukara, B. (1993) Koyun, Kuzu ve Buzağı Pnömonilerinde Bakteriyel Etiyoloji ve Antibiyogram. Türk Vet. Hek. Derg. 5 (2), 57-60.

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35. Kaya, O., Arıkan, S., Özlem, M.B. (1996). Köpeklerin Vagina ve Prepisyum Bakteriyel Florası Üzerine Çalışmalar. Bornova Vet. Kont. Araş. Enst. Md. Derg., 21(35), 9-14.

36. Erer, H., Ateş, M., Kıran, M.M., Çiftçi, M.K., **Kaya, O.** (1996). İneklerde Mastitislerin Patolojik ve Bakteriyolojik İncelenmesi. Vet. Bil. Derg., 12 (1), 123-133.

37. Güler, L., Baysal, T., Gündüz, K., Erganiş, O., **Kaya, O.**, Orhan, G. (1996). Koyun ve Keçilerden İzole Edilen Pasteurella Haemolytica Suşlarının Biyotip ve Serotiplendirilmesi. Veterinarium, 7 (1-2), 5-12.

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41. Özlem, M. B., **Kaya, O.**, Akar, F., Arıkan, S. (1997). Buzağı Pnömonilerinde Pasteurella Haemolytica'nın Özelliklerinin Belirlenmesi ve Tedavisi. Bornova Vet. Kont. Araşt. Enst. Müd. Derg., 22, 7-14.

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Figure 4. Prof. Dr. Osman Kaya shortly before his decase.

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Administrative Duties

Prof. Dr. Osman Kaya carried out administrative duties at Aydın Adnan Menderes University (ADU) on various dates and contributed to the development and social and administrative structure of the university.

In addition to the Vice-Rector position, he was appointed on 18.11.2002. He was appointed by proxy to the Department of Physical Education and Sports (2002-2006), Fine Arts and Informatics Department (2002-2006), and again to the determination commission of ADU Construction (2002-2006), and to the Responsible Supervisor of Head of the Health, Culture, and Sports Department (2005). Kaya, during his vice rectorship, Kaya also served as ÖSYM Aydın Provincial Coordinator (2002-2006), ADU Çine Vocational School Board Member (2002-2006), and ADU Bozdoğan Vocational School Board Member (2002-2006).

Prof. Dr. Osman Kaya was appointed by Prof. Dr. Cavit Bircan, who served as the rector of ADU between 2014-2018, and as Advisor to the Rector (2015). Moreover, he was appointed by Prof. Dr. Cavit Bircan as a Member of the Board and as a Member of the Faculty Board of the Faculty of Dentistry (2016-2017) (Figure 4).

Prof. Dr. Osman Kaya was a member of the "Society of Veterinarians Microbiology" and "The Association of ADU Employees".

Kaya, who speaks English, was the father of 1 daughter.

Discussion

Kaya, who was born on May 20, 1960 in the Akşehir district of Konya, passed away on February 02, 2017 and was bid farewell to his last journey with a ceremony held at Atatürk Congress Center Meandros Hall on Friday, February 03, 2017 (ADU). His funeral was removed from the Adnan Menderes Mosque after the Friday Prayer and was buried in the Kemer Cemetery of Aydın-Efeler district.

The rector of the period (2014-2019) Prof. Dr. Cavit Bircan; "Expressing that Prof. Dr. Osman Kaya is a well-liked academician, he never lost his faith in humanity even during his illness, and he always thought of the best for our university", and he expressed his gratitude for his contributions to our university and the academic world.

As a result, in his short 57-year life Prof. Dr. Osman Kaya devoted 30 years (1987-2017) to academic life, and he has conducted many scientific studies. He has trained academicians in the field of Veterinary Microbiology and made many contributions with his scientific studies. It is obvious that his contributions to the veterinary microbiology discipline with his publications and presentations in scientific meetings are significant.

Again, successfully fulfilling his responsibilities regarding the administrative duty assigned to him and gaining an appreciation for his disciplined attitude and with their behavior throughout this responsibility, Kaya has always been loved by his colleagues and students for witty personality that has always made a name for himself.

For Osman Kaya, his older sister Sevil Kaya, whose information was consulted after his decase; "He had a well-behaved childhood and chose to become a veterinarian because of his interest in animals. With his very hasty and hyperactive character, he had a structure that tried to carry out the decision he made immediately in life, but got angry when he could not implement it." in his words, he expressed the well-known characteristics of Kaya.

Conclusion

Prof. Dr. Osman Kaya's services and works still make a name for himself today. He will always be remembered with gratitude on behalf of the entire scientific community and the history of veterinary medicine in his spiritual presence.

On this occasion, the tradition of "writing the biographies of retired or deceased faculty members and recording the history of the faculty" initiated at Aydın Adnan Menderes University Veterinary Faculty will be fulfilled.

We commemorate him with professional and personal respect and mercy.

Acknowledgement

For their support and assistance in collecting the data for the study, Prof. Dr. Osman Kaya's older sister Miss. Sevil Kaya, Faculty Members of ADU Faculty of Veterinary Medicine, Department of Microbiology; Mr. Prof. Dr. Şükrü Kırkan and Mr. Assoc. Prof. Göksel Erbaş and ADU Faculty of Veterinary Medicine Department of Microbiology staff we would like to thank.

Conflict of Interest

As the undersigned authors; We declare and undertake that the submitted manuscript is original, has not been published in another journal, has not been evaluated for publication in another journal, and the order of authors is as above and there is no conflict of interest between us.

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Research Article

The Investigation Protective Effect of *Tarantula Cubensis* Extract in Rats Induced Experimental Gentamicin Nephrotoxicity

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ABSTRACT

Gentamicin (GM), which is used in the treatment of infections caused by gram-negative bacteria, has limited clinical use due to its serious nephrotoxic side effects. *Tarantula cubensis* extract (TCE) is a homeopathic product that is widely used and proven to be effective in veterinary medicine to benefit from its regeneration, demarcation, antiphlogistic and resorptive effects. The aim of the study was to investigate the possible protective effects of TCE against these negative effects of Gentamicin, which is one of the drugs that trigger the formation of free radicals in the body, in terms of oxidative stress, apoptosis and antioxidant parameters. In this study, rats were divided into four equal groups. Groups; Control, GM, TCE, GM+TCE. Blood and kidney tissue samples were taken 24 hours after the last injection. Urea and creatinine analysis were performed in serum, MDA, SOD and TAS analysis were performed in kidney and serum samples. Bcl-2 and Bax analyzes and histopathological evaluations were performed in the kidney tissue. An increase in MDA, creatinine and urea levels, and a decrease in TAS and SOD levels were observed in the GM group compared to the control group. On the other hand, in the GM+TCE group, a decrease was observed in the parameters that increased compared to the GM group, and an increase in TAS and SOD levels was observed. In the histopathological and immunohistochemical examination of kidney tissue, it was determined that pathological disorders and increased apoptosis (decrease in Bcl-2, increase in Bax) decreased in the GM+TCE group. In conclusion, in the light of the data in this study, we believe that high-dose gentamicin causes side effects in the kidneys, while TCE may have antioxidant, antiapoptotic, protective and curative effects. However, additional studies are needed to confirm this assumption.

Keywords: *Antioxidant, apoptosis, gentamicin, nephrotoxicity, Tarantula cubensis extract.*

Deneysel Gentamisin Nefrotoksisitesi Oluşturulan Ratlarda *Tarantula Cubensis* Ekstraktının Koruyucu Etkisinin Araştırılması

ÖZET

Gram negatif bakterilerin neden olduğu enfeksiyonların tedavisinde kullanılan gentamisinin (GM), ciddi nefrotoksik yan etkileri nedeniyle klinik kullanımı sınırlıdır. *Tarantula cubensis* ekstraktı (TCE), rejenerasyon, demarkasyon, antiflojistik ve rezorptif etkilerinden yararlanmak için veteriner hekimlikte yaygın olarak kullanılan ve etkinliği kanıtlanmış homeopatik bir üründür. Bu çalışmanın amacı, vücutta serbest radikal oluşumunu tetikleyen ilaçlardan biri olan gentamisinin bu olumsuz etkilerine karşı TCE'nin olası koruyucu etkilerinin oksidatif stres, apoptoz ve antioksidan parametreler açısından araştırılmasıdır. Bu çalışmada sıçanlar dört eşit gruba ayrıldı. Gruplar; Kontrol, GM, TCE, GM+TCE'dir. Son enjeksiyondan 24 saat sonra kan ve böbrek doku örnekleri alındı. Serumda üre ve kreatinin, böbrek ve serum örneklerinde MDA, SOD ve TAS analizleri yapıldı. Böbrek dokusunda Bcl-2 ve Bax analizleri ile histopatolojik değerlendirmeler yapıldı. GM grubunda kontrol grubuna göre MDA, kreatinin ve üre düzeylerinde artış, TAS ve SOD düzeylerinde azalma gözlendi. GM+TCE grubunda ise GM grubuna göre artan parametrelerde azalma, TAS ve SOD düzeylerinde ise artış gözlendi. Böbrek dokusunun histopatolojik ve immunohistokimyasal incelemesinde, GM+TCE grubunda patolojik bozuklukların ve artan apoptozun (Bcl-2'de azalma, Bax'ta artış) azaldığı belirlendi. Sonuç olarak, bu çalışmadaki veriler ışığında, yüksek doz gentamisin böbreklerde yan etkilere neden olurken, TCE'nin antioksidan, antiapoptotik, koruyucu ve iyileştirici etkilerinin olabileceği kanısındayız. Ancak, bu varsayımı doğrulamak için ek çalışmalara ihtiyaç vardır.

Anahtar kelimeler: *Antioksidan, apoptoz, gentamisin, nefrotoksisite, Tarantula cubensis ekstraktı.*

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Introduction

Gentamicin (GM) is an aminoglycoside derivative broad-spectrum antibiotic used in the treatment of infections caused by gram-negative bacteria, and its clinical use is quite common due to its economic cost (Hodiamont et al., 2022). However, nephrotoxicity, which is a serious side effect of Gentamicin, limits its clinical use (Secilmis et al., 2005). It is known that 10-20% of cases with acute renal failure are caused by GM (Rizwana et al., 2022).

Gentamicin is eliminated from the body entirely by glomerular filtration from the kidneys and accumulates in lysosomes in the proximal tubules after being filtered by glomerular filtration (Li et al., 2008). The resulting tissue damage; basement membrane erosions, proximal tubule swelling, tubular atrophy or dilatation, interstitial inflammatory cell infiltration and reduction of basolateral membrane remnants (Volpini et al., 2006). The molecular mechanism of kidney failure due to GM has not been fully explained in the studies, but it has been suggested that it causes changes in different enzyme activities. It is thought that these mechanisms may cause nephrotoxicity by accumulation of superoxide anions, lysosomal enzyme changes and microsomal protein synthesis inhibition (Sharma, 2004; Secilmis et al., 2005; Leonard et al., 2023).

Homeopathy is a treatment system in which harmless and natural methods are used for diagnosis or treatment according to anamnesis. It is a treatment model that receives successful feedback in cases where chronic or modern medicine is insufficient in diagnosis and treatment. Mixtures used in homeopathic treatment are obtained from plants, metals, animal secretions or infected people (Basar, 2014). Homeopathic treatment is less known than modern medicine and it is an area open to development of new methods. It is an alternative system to modern medicine (Ilhan, 2018). Tarantula cubensis extract (TCE), which is used in homeopathic medicine, is obtained from the Tarantula cubensis spider. The extract from this spider was diluted in alcohol. It has been proven in different studies that this drug has regeneration, anti-inflammatory and antioxidant effects (Lotfollahzadeh et al., 2012; Karabacak et al., 2015).

Antioxidant defense mechanisms undertake the task of protecting the organism from the harmful effects of ROS. Most cells can tolerate low levels of oxidative stress. Repair systems find damaged molecules and remove them from the cell (Puppel et al., 2015; Akbari et al., 2022). The organism tries to counteract oxidative stress with enzymatic and/or non-enzymatic antioxidants. These are catalase, GSH and SOD, which are called natural antioxidants. The physiological function of SOD is to protect oxygen-using cells against the damage of superoxide radicals (Memisogullari, 2005).

Antioxidants are a combination of complex systems that protect tissues from the harmful effects of ROS and balance oxidative stress. The greatest contribution to TAS in the organism comes from the antioxidants in the plasma. In the antioxidant defense system, individual antioxidants can act together to protect organs against oxidative damage. For this reason, it is more accurate to measure the TAS level to evaluate the antioxidant defense system (Vural et al., 2007).

MDA is formed as a result of peroxidation of lipids and is widely used in the detection of oxidative stress levels. It can be analyzed in both urine and blood. Although there is no specific indicator for the peroxidation of lipids, it correlates well with the degree of peroxidation. For this reason, the detection of MDA is used as an indicator of lipid peroxide levels (Dimova et al., 2022).

In advanced healthy organisms, the increased number of cells by cell division is kept in balance by cell death. Cells that are not needed in the organism activate intracellular communication systems and initiate the process of death or suicide. This process is called “Apoptosis” or “Programmed Cell Death” (Mak, 2003; Aksit and Bildik, 2008). Apoptosis is a physiological requirement for the development of the organism, aging, as well as for the organism to maintain its balance. In addition, it acts as a protective mechanism in the organism against diseases or harmful agents (Vaux and Flavell, 2000; Elmore, 2007). Mitochondrial/Intrinsic pathway triggers mitochondrial degradation when sufficient levels of cytochrome-c are released (Bratton et al., 2000). This released cytochrome-c plays an important role in the activation of caspase-3 enzymes in apoptosis (Dirican et al., 2023). This pathway is regulated by Bcl-2 proteins. Antiapoptotic genes; c-myc, p-53 and bcl-2 family and the proteins they produce with the same name (Vervliet et al., 2023). Proapoptotic genes are Bax, Bad and Bid (Agena et al., 2023).

In this study, it was aimed to determine whether TCE has a protective effect on oxidative stress, apoptosis, antioxidant parameters and kidneys by experimentally induced renal toxicity with gentamicin, by biochemical, histopathological and immunochemical methods. In this study, it is aimed to fill an important literature deficiency in this field.

Materials and Methods

Drugs and chemicals

Gentamicin sulphate was purchased from Goldbio (Cas:1405-41-0). TCE (Theranekron®, alcoholic extract (1:100) of Tarentula cubensis in alcoholic solution 1 mg/ml) was obtained from Richter Pharma (Welss/Australia). TAS Assay kit was obtained from Rel Assay Diagnostics (Turkey). Urea (A2332) and creatinine (A2162) kits were obtained from Archem (İstanbul, Turkey). Antibodies against Bcl-2 (E1904), and Bax (G0104) were purchased from Santa Cruz Biotechnology (CA, USA). Seconder antibody (E0431) was obtained from Dako Cytomation (Glostrup, Denmark). All other chemicals of analytical grade were purchased from Merck (Darmstadt, Germany) or Sigma Chemical Co (St. Louis, MO, USA).

Animals

40 male Sprague Dawley rats were used. Rats were obtained from Balikesir University Experimental Animals Production, Care, Application and Research Center, Balikesir, Turkey. Standard commercial pellet food and water were provided ad libitum. All tests and procedures were performed according to the European Economic Community Guidelines for the care and use of laboratory animals and were proved by the Local Ethics Committee of Balikesir University (Ethics Committee Approval Decision No: BAU-HADYEK 2019/2-3).

Experimental procedure

Rats were acclimatized for one week prior to the study. 40 male Sprague Dawley rats were divided into four equal groups randomly. Groups; Control (0.5 ml isotonic saline, ip for 8 days), GM (100 mg/kg ip in isotonic saline for 8 days), TCE (200 µl/kg/day, sc for 14 days), GM (100 mg/kg ip for 8 days) + TCE (200 µl/kg/day sc, a total of 14 days, 3 days before and 3 days after GM application). GM dose was chosen based on the previous studies (Kalkan et al., 2012; Khaskari et al., 2021). TCE dose was chosen based on the study by Karabacak et al. 2015. 24 hours after the last application, blood samples were taken, serum was separated and stored at -80 °C. Kidney tissues were immediately dissected after sacrificed under isoflurane anesthesia, rinsed from blood in isotonic saline and then half of them was stored at -80 °C until analysis, the other half of the tissues were fixated in 10% phosphate buffered formalin for histopathological and immunohistochemical examinations.

Measurement of biochemical parameters

The serum creatinine and urea levels were measured using the biochemical autoanalyzer (Sinnowa D280, Nanjing, China) and commercially available diagnostic kits (Archem, İstanbul, Türkiye).

Kidney tissues were weighed and homogenized in ice-cold 1.15% Potassium chloride to prepare a 10% (w/v) tissue homogenate at 1300 rpm for 3 min with homogenizator (Stuart SHM 1, UK). The half of homogenate was used for the determination of MDA described by Yoshioka et al. 1979. The other half of homogenate was centrifuged at 5000 g for 60 min at 4°C and supernatant samples were separated for the determination of SOD and TAS analysis. Total protein level of the tissues homogenate and supernatant was analyzed using the Lowry method (Lowry et al., 1951). The levels of SOD in tissue supernatant were assessed following the methods of Sun et al. 1988. The levels of TAS were measured using commercially available kit according to procedure in supernatants. Serum MDA, SOD and TAS analyzes were also performed according to the same procedures.

Histopathological examination

Kidney tissues samples in formalin fixation for 72 hours were blocked by passage through alcohol, xylol and were embedded in paraffin block. 3 µm sections were taken in the microtome (Leica 2245, Nussloch, Germany) and then stained with hematoxlin and eosin (H&E). The preparations were closed with entellan. All slides of each group were examined under a light microscope. Histopathological evaluation scored as follows in kidney tissues: Tubulointerstitial inflammatory infiltrates, (tubulointerstitial inflammatory infiltrates: none = 0, leukocytes confined within the interstitium = +1, and leukocytes infiltrating the interstitium and tubular epithelial cells = +2). Pathologic Proteinous Casts, (no damage (0), mild (1, unicellular, patchy isolated damage), moderate (2, damage less than 50%), severe (3, damage more than 50%), The degree of medullary congestion no congestion (0), mild (1, vascular congestion with identification of erythrocytes by ×400 magnification), moderate (2, vascular congestion with identification of erythrocytes by ×200 magnification), severe (3, vascular magnification with identification of erythrocytes by ×100 magnification). Tubular necrosis (0 = normal cortex, 1 = small and one or two areas of tubule damage, 2 = tubular damage up to 50% of the cortex, 3 = tubular damage covering more than 50% of the cortex) (Said, 2011; Kader et al., 2017).

Immunohistochemical examination

Following the follow up and blocking procedures 3 µm sections were taken in polylysine coated slides. Immunohistochemistry was performed on sections using avidin biotin peroxidase complex (ABC, Daco Cytomation, Denmark) method according to manufacturer recommendation.

The sections were deparaffinized, rehydrated and blocked with 3% hydrogen peroxide. And then incubated with Bax (dilution: 1:100, Santa Cruz, CA, USA) or Bcl-2 (dilution: 1:100, Santa Cruz, CA, USA) antibodies. Sections were incubated with biotinylated goat anti-rabbit secondary antibody (Dako Corporation, Carpinteria, USA) and streptavidin peroxidase complex (ABC; Dako Corporation, Carpinteria, USA). The slides were treated with 3,3-diaminobenzidine (DAB) in Phosphate buffered saline (PBS) (0.5 mg DAB/ml) containing hydrogen peroxide 30% v/v. The Bax and Bcl-2 positive cells were examined semi-quantitatively under a light microscope and then photographed.

Statistical analysis

All data were expressed as mean and standard error (as mean ± SE) in each group. Statistical analysis of differences between groups was performed using ANOVA. Post hoc multiple comparisons were performed using Duncan's test. If the obtained data were not normally distributed (Histopathological examination), non-parametric Kruskal–Wallis data analysis was applied for the comparative test. All analyses were performed using the SPSS (Version 17.0, Chicago, IL, USA) software program. The difference between the groups in terms of the parameters examined was considered significant at the $P<0.05$ level.

Results

Biochemical results

The results of this study showed that serum urea, creatinine, and tissue and serum MDA levels increased with GM administration, while SOD and TAS levels were significantly decreased compared to the control group. Serum urea and creatinine, tissue and serum MDA levels decreased, TAS and SOD levels were increased in GM+TCE group compared to GM group (Table 1 and Table 2). The levels of serum and tissue kidney parameters of rats in all groups are presented in Table 1, 2.

Histopathological findings and immunohistochemical evaluation

Bax and Bcl-2 were assessed by immunohistochemically. Histopathological alterations in kidney tissues were examined (HxE). Immunohistochemical examination showed that apoptosis induced (up regulation of Bax and down regulation of Bcl-2) in kidney tissue in GM group. Bcl-2 activity was increased, Bax positive intensity of kidney tissue was decreased in GM+TCE group compared to GM group. The immunohistochemical findings showed that slight apoptotic activity in control and TCE groups when compared with GM and GM+TCE (Figure 1 and 2).

Histopathological alteration in kidney tissues of all study groups are shown in Table 3, Figure 3. Microscopical

Table 1. Serum biochemical parameters in study groups (Mean±SE)

Parameters	Control (n=10)	TCE (n=10)	GM (n=10)	GM+TCE (n=10)
MDA (µmol/L)	20.40±0.77^{c}	21.59±0.50^{c}	36.95±1.45^{a}	27.55±0.35^{b}
SOD (U/L)	6.94±0.07^{a}	6.90±0.05^{a}	3.43±0.08^{c}	4.91±0.09^{b}
TAS (mmol trolox equiv./L)	2.35±0.07^{a}	2.31±0.06^{a}	1.51±0.03^{c}	1.83±0.03^{b}
Urea (mg/dl)	35.40±0.85^{c}	35.00±1.23^{c}	72.70±2.17^{a}	58.70±1.02^{b}
Creatinine (mg/dl)	0.56±0.01^{c}	0.49±0.02^{c}	1.39±0.05^{a}	0.88±0.03^{b}

$^{a, b, c}$: Between groups with different letters in the same row mean difference is significant (P<0.05). TCE: *Tarantula cubensis* extract, GM: Gentamicin.

Table 2. Biochemical parameters of kidney tissue samples in study groups (Mean±SE)

Parameters	Control (n=10)	TCE (n=10)	GM (n=10)	GM+TCE (n=10)
MDA (µmol/mg protein)	9.26±0.21^{c}	10.01±0.30^{c}	17.61±0.34^{a}	13.58±0.32^{b}
SOD (U/mg protein)	5.70±0.17^{a}	5.37±0.19^{a}	2.95±0.15^{c}	4.27±0.11^{b}
TAS (mmol trolox equiv./mg protein)	2.39±0.04^{a}	2.46±0.05^{a}	1.58±0.05^{c}	2.03±0.02^{b}
Urea (mg/dl)	35.40±0.85^{c}	35.00±1.23^{c}	72.70±2.17^{a}	58.70±1.02^{b}
Creatinine (mg/dl)	0.56±0.01^{c}	0.49±0.02^{c}	1.39±0.05^{a}	0.88±0.03^{b}

$^{a, b, c}$: Between groups with different letters in the same row mean difference is significant (P<0.05). TCE: *Tarantula cubensis* extract, GM: Gentamicin.

Table 3. Histopathological alteration in kidney tissue of study groups (H&E) (Mean ± SE)

Parameters	Control (n=10)	TCE (n=10)	GM (n=10)	GM+TCE (n=10)	P
Pathologic Proteinous Casts	0.50±0.22^{b}	0.70±0.21^{b}	1.80±0.33^{a}	1.30±0.30^{a,b}	0.014
Tubulointerstitial inflammatory infiltrates	0.20±0.13^{b,c}	0.10±0.10^{c}	0.80±0.29^{a,b}	1.20±0.29^{a}	0.009
Tubular necrosis	0.40±0.16^{b,c}	0.10±0.10^{c}	1.20±0.29^{a}	0.90±0.23^{a,b}	0.005
The degree of medullary congestion	0.80±0.25^{b}	1.10±0.31^{a,b}	1.80±0.20^{a}	1.40±0.16^{a,b}	0.040

$^{a, b, c}$: Between groups with different letters in the same row mean difference is significant. P value is from Asymp. Sig. Kruskal-Wallis test. TCE: *Tarantula cubensis* extract, GM: Gentamicin.

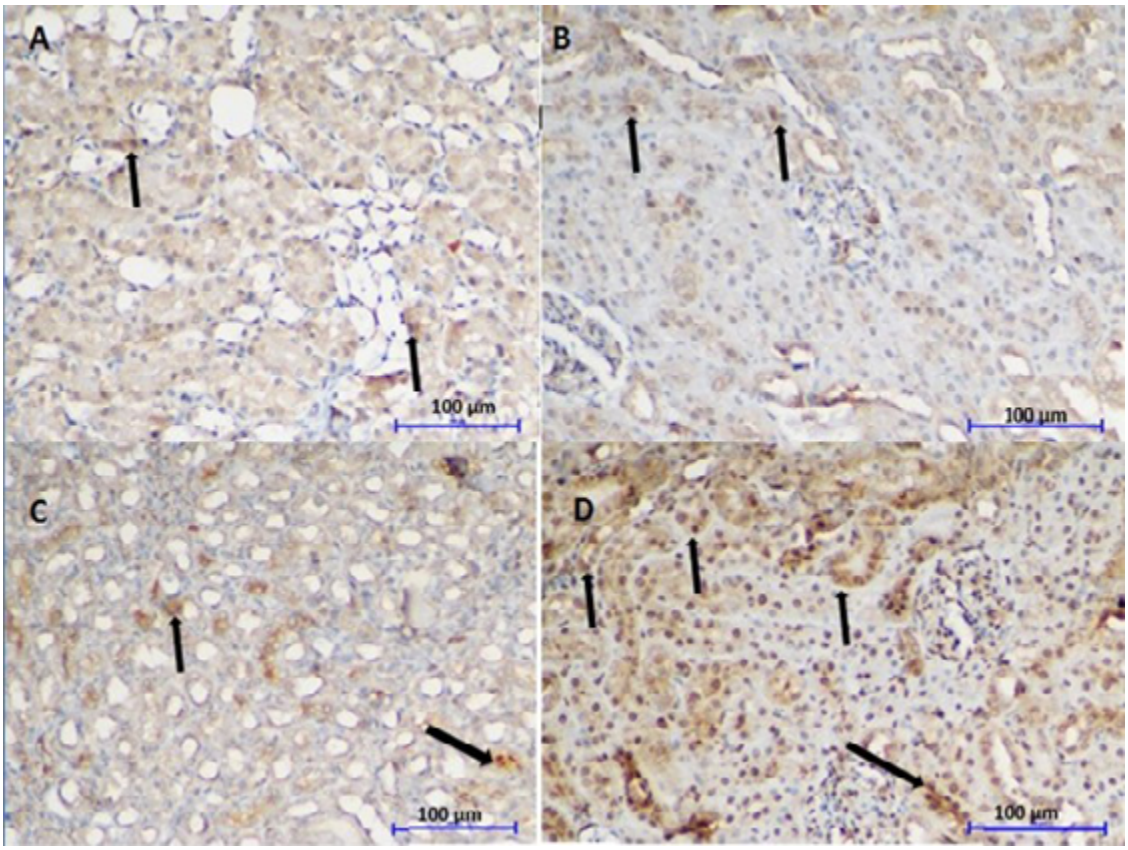

Figure 1. Immunohistochemical photomicrographs of Bcl 2 in rat kidney tissue. Control group (A), TCE group (B), GM group (C), GM+TCE group (D), arrow marks Bcl-2 positive cells, (200x).

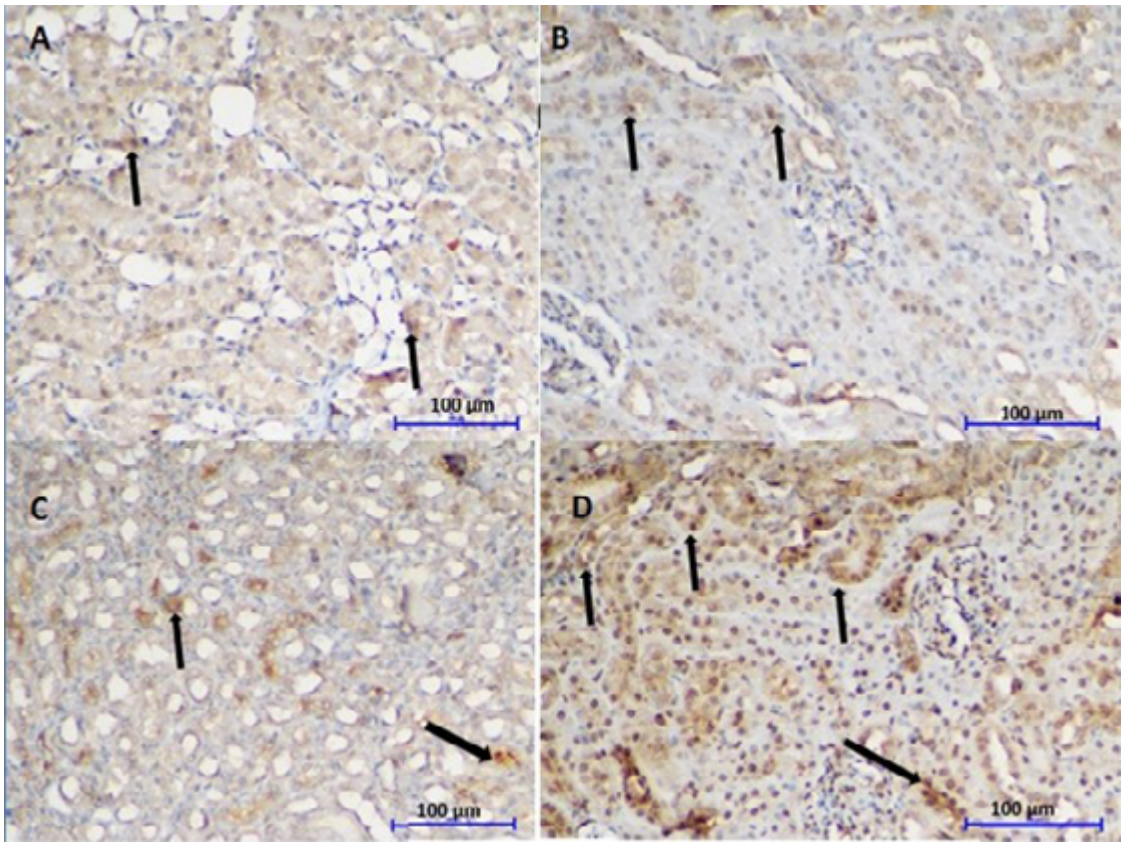

Figure 2. Immunohistochemical photomicrographs of Bax in rat kidney tissue. Control group (A), TCE group (B), GM group (C), GM+TCE group (D), arrow marks Bax positive cells, (200x).

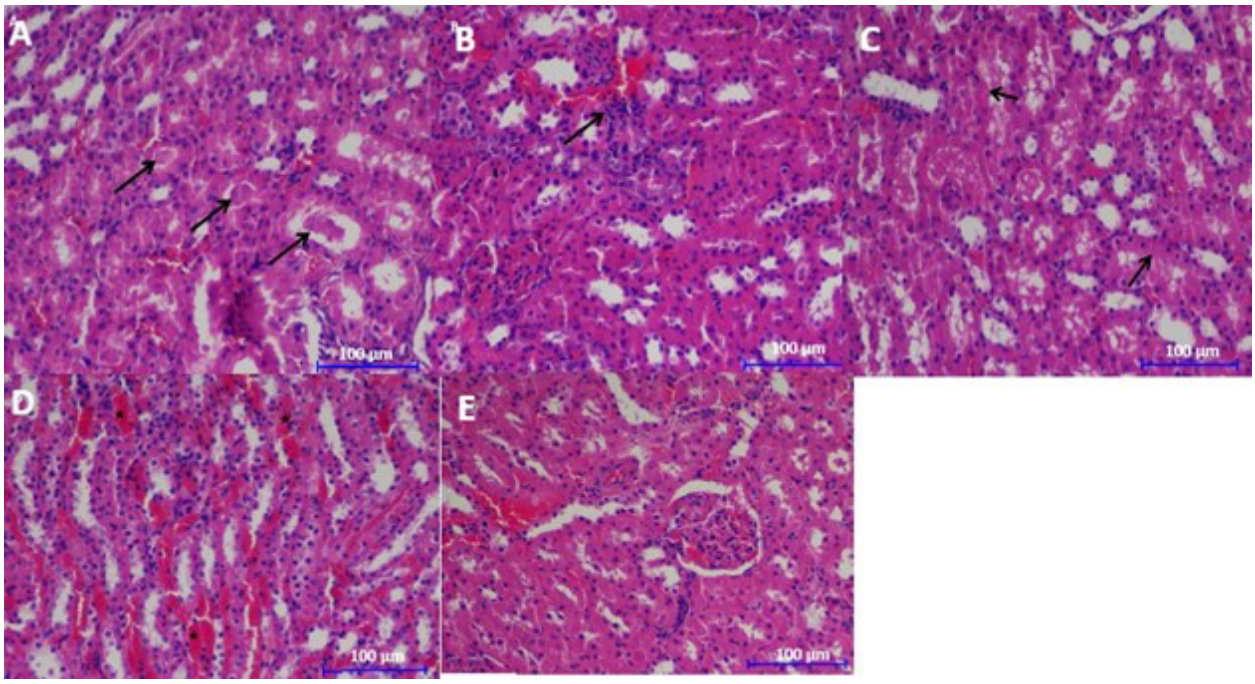

Figure 3. Renal histopathological disorders and normal kidney tissue determined in the study groups; Pathologic Proteinous Casts, arrow marks (A), Tubulointerstitial inflammatory infiltrates, arrow marks (B), Tubular necrosis, arrow marks (C) Medullary congestion, star marks (D), Normal kidney tissue of control group (E) (H&E), (200x)kidney tissue of control group (E) (H&E), (200x).

evaluation of kidney tissue was found severe pathologic proteinous casts, tubulointerstitial inflammatory infiltrates, medullary congestion and tubular necrosis in the GM group compare to control and TCE groups. Control and alone TCE treated groups showed normal tissue morphology. Significant reduction in some histopathological scores was found in GM group administrated with TCE (Table 3).

Discussion

Nephrotoxicity is among the most common side effects of GM, which is frequently used in clinics due to its wide spectrum of action and rapid effects (Rizwana et al., 2022). Although the nephrotoxic mechanism of action due to the use of GM has not yet been determined, many factors are thought to play a role, especially the accumulation of oxygen radicals (Sakr and Kamel, 2023). It has been reported that chemical agents generally used in the treatment of diseases cause complications and oxidative stress (Li et al., 2023). ROS affects intracellular proteins, nucleic acids and membrane lipids, causing changes in their structures and functions (Wang et al., 2023).

TCE, which is a homeopathic agent, has been observed in veterinary medicine to provide very rapid healing in foot diseases, ulcers, abscesses, all kinds of inflammatory and necrotic cases, even with a single dose. It is stated that TCE also has regeneration, demarcation, resorption and antiphlogistic effects (Kacar et al., 2007).

In many studies in experimental animals, GM is used to cause acute kidney injury and the effects of different substances are investigated in order to reduce the severity of the nephrotoxic effect (Abdelzaher et al., 2023; Elkhoely, 2023). Most agents thought to have antioxidant properties, especially extracts from different plants and animals, have been used in experimental GM nephrotoxicity (Aldahmash et al., 2016; Yarjani et al., 2016; Yılmaz et al., 2018; Ozsayın, 2019; Samy et al., 2023).

Some researchers reported that GM cause reactive oxygen metabolites. Increased ROS production leads to lipid peroxidation and oxidative damage. Under normal conditions the antioxidant-oxidant system is in balance, while there is an increase in MDA and a decrease in SOD and TAS during oxidative stress. SOD enzyme is a major antioxidant defense component (Yaman and Balikci, 2010). ROS plays a vital role as an inducer of apoptosis. Apoptosis is a form of programmed cell dead resulting in interconnected intracellular caspase proteins (Tanyeli et al., 2019).

The increase in MDA levels in GM-induced nephrotoxicity has been shown in many studies (Ulutas et al., 2006 ; Wijayanti et al., 2023). In different studies, it has been determined that gentamicin impairs redox reactions by reducing antioxidant enzyme activities in the organism, such as SOD. It has been reported in studies that ROS cause peroxidation of lipids (Yadav et al., 2017; Leonard et al., 2023). In terms of these parameters, this study is compatible with the literature.

Ozsayin (2019) reported that nephrotoxicity was created with gentamicin in rats, oil, water and ethanol extracts of the Cyclotrichium niveum plant were applied and it was determined that there was a significant decrease in MDA, serum urea and creatinine levels in all extracts. In addition, in the histopathological examination, it

was determined that the disorders in the tissues were restored. In another study by Karabulut (2016), it was determined that the increased MDA and oxidative stress levels in GM nephrotoxicity induced in rats decreased in the GM+L-arginine combination, while the decreased SOD level increased. As a result of the findings obtained in this study, it was determined that the increase in MDA, urea, creatinine levels and histopathological damage obtained as a result of GM nephrotoxicity, and the decrease in SOD level changed in the opposite direction when TCE was used, and the findings are consistent with the literature.

Karabacak et al. (2015) investigated the effects of TCE on the toxic effects of aflatoxin in kidney, liver and other organs in a study conducted in rats. They observed that MDA levels increased in the kidney tissue of rats given aflatoxin, and a decrease in MDA levels with TCE administration. In a study conducted by Ozbek (2019), it was determined that TCE provided histopathological improvement in renal ischemic reperfusion. However, they found that TCE reduced the effects of ischemic reperfusion-induced oxidative stress and inflammation. In this study, it was determined that the MDA level increased in kidney toxicity caused by GM and decreased with TCE administration, which is consistent with the literature.

Bai et al. (2023) reported that blood urea and creatinine levels increased significantly when they administered 100 mg/kg ip GM to experimental animals for 10 days. Renal tubules showed necrosis and vacuolation with occasional desquamation appeared on epithelial cells of the proximal convoluted tubules. Mild inflammatory cell infiltration, edema and extravasated red blood corpuscle were determined histopathologically in the interstitium. The Bax protein expression decreased and the Bcl-2 protein expression increased with the applications of antioxidant substance *C. deserticola* in GM-induced nephrotoxicity in rats significantly. When the current study is evaluated in terms of these parameters, it is compatible with the literature.

Geshnigani et al. (2023) determined that concentrations of BUN, creatinine and malondialdehyde were significantly increased. But, the level of glutathione and activities of glutathione peroxidase, and superoxide dismutase decreased during treatment with gentamicin. On the other hand, the concentrations of creatinine, BUN, nitric oxide, malondialdehyde, were significantly reduced, and the glutathione level, activities of glutathione peroxidase were significantly increased via co-administration with antioxidant activities of diosmin. Veljkovic et al. (2016) reported that GM disrupts the kidney morphology, thus causing necrosis in the kidney tubules, degenerations in the cytoplasm and causing interstitial inflammation. Yilmaz et al. (2018) observed atrophy in the histopathological examination of the renal tissues of experimental animals administered GM. They stated that there are degenerative changes in tubule epithelial cells and capsule. In this study, in the histopathological examination of renal tissues, it was observed that pathological proteinaceous casts were formed in the GM group, increases in tubulointerstitial inflammatory infiltrates and medullary congestion were formed. In the group in which the GM+TCE combination was used, these pathological findings were observed to be alleviated. But, there is no significant difference between the GM and the GM+TCE groups in some pathological disorders.

Nadeem et al. (2023) reported GM provoked an upsurge in the relative kidney weight and serum level of urea and creatinine. The MDA level was markedly boosted, with a decline in the level of TAS, SOD, and Nrf2 expression in the renal tissue. Additionally, caspase-3 and Bax expression were elevated, whereas the Bcl-2 level was reduced. Furthermore, histological examination revealed inflammation, degradation, and necrosis. GM-provoked pathological abnormalities were reversed by antioxidant treatment, which restored normal kidney architecture. When this study was evaluated in terms of these parameters, similar results were obtained.

In this study, in the nephrotoxicity model induced by GM, an increase was observed in the MDA level due to oxidative stress in the GM group, however, it was determined that the MDA level decreased in the GM+TCE group and decreased the oxidative stress. A significant decrease was detected in the SOD level, which is another evaluation criterion of this study, in the group given GM, however, in the group given the GM+TCE combination, the SOD level increased and the ROS in the cells were removed. However, while increases in serum creatinine and urea levels, which are markers of renal impairment, were observed in the GM group, a significant decrease was observed in serum urea and creatinine levels in the GM+TCE combination group. It was determined that in GM-induced nephrotoxicity, there was a decrease in antiapoptotic Bcl-2 gene expression in the GM group, and an increase in apoptosis-inducing Bax. An increase in Bcl-2 gene expression and a significant decrease in Bax were observed in the GM+TCE group. Differences were determined in the cells in the cortex and medulla layer of the kidney tissue. In the cortex layer, it was determined histopathologically that tubule necrosis was high in areas with tubule damage, and low apoptosis was determined immunohistochemically. On the contrary, it was observed that the number of apoptotic cells was higher in the medulla.

Conclusion

In this study, GM was used to induce nephrotoxicity. The findings support that GM administration at the administered dose causes renal damage and that oxidative stress contributes to this damage. Although different agents thought to be antioxidant effective were used in GM-induced nephrotoxicity studies, TCE application was tried for the first time. In the study, the protective effect of TCE against kidney damage induced by GM was

investigated biochemically, immunohistochemically and histopathologically. As a result, it was concluded that GM-induced kidney damage can be prevented by the antioxidant and anti-apoptotic effects of TCE, and these findings should be supported by further studies. This is the first study to examine the protective effect of TCE on GM-induced nephrotoxicity and will be a reference for future studies.

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Conflict of Interest

No conflict of interests was declared by the authors.

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Research Article

Detection of Tick Borne Zoonotic Bacteria by PCR in Dogs

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ABSTRACT

The prevalence of some tick-borne zoonotic bacteria (*Borrelia* sp., *Coxiella burnetii*, and *Rickettsia* sp.) in dog blood samples were examined using by PCR. A total of 200 dog blood samples were collected from dogs. Three pathogens for dogs, *Borrelia burgdorferi*, *C. burnetii* and *Rickettsia* sp. of were detected in dog blood samples. A single step PCR was performed for the *C. burnetii* and multiplex PCR was applied for the *Rickettsiae* spp. and *Borrelia burgdorferi*. At the end of PCR study, *Borrelia burgdorferi*, *C. burnetii* and *Rickettsiae* sp. target pathogens were identified at the rate of 55.0% from all blood samples. The percentage distributions according to identified species were at the rate of *C. burnetii* 1.5%, *Borrelia burgdorferi* sensu lato 2.0% and *Rickettsiae* spp. 51.5%. The results indicated that dogs may play a role in disseminating tick-borne zoonotic bacteria, thereby posing a potential health hazard to humans.

Keywords: *Tick-borne zoonotic bacteria, identification, multiplex PCR.*

Köpeklerde Kene Kaynaklı Zoonotik Bakterilerin PCR Yöntemiyle Belirlenmesi

ÖZET

Araştırmamızda, köpek kan örneklerinde bazı kene kökenli zoonotik bakterilerin prevalansı PCR metodu kullanılarak araştırıldı. Araştırmamızda 200 adet köpek kan örneği kullanıldı. Köpekler için patojen oldukları bilinen *Borrelia burgdorferi*, *C. burnetii* ve *Rickettsiae* sp. varlığı köpek kan örneklerinde araştırıldı. *C. burnetii için tek adımlı PCR ve Rickettsiae* sp. ile *Borrelia burgdorferi* için multipleks PCR uygulandı. PCR işlemi sonucunda, tüm kan örneklerinin %55.0'inden *Borrelia burgdorferi*, *C. burnetii* and *Rickettsiae* spp. identifiye edildi. İdentifiye edilen türlerin %1,5'i *C. burnetii*, %2'si *Borrelia burgdorferi* sensu lato ve %51,5'i *Rickettsiae* spp. olarak dağılım gösterdiği saptandı. Araştırmanın sonuçları, köpeklerin kene kökenli zoonotik bakterilerin yayılımında bir risk faktörü olabileceğini göstermektedir. Kene kökenli zoonotik bakteriler insan sağlığı açısından bir tehlike oluşturabilmektedir.

Anahtar Kelimeler: *Kene kaynaklı zoonotik bakteriler, identifikasyon, multipleks PCR.*

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Introduction

Ticks are the most of great importance vectors of pathogenic bacteria, viruses, and protozoa and causing diseases in animals and humans worldwide. Most of those agents are accepted as both bioterrorism (such as *C. burnetii*) and vector-borne pathogens (Azad and Radulovic, 2003). In addition, *Rickettsia* and some *Borrelia* species also cause zoonotic diseases reported in both animals and humans (Piesman and Gern, 2004; Parola et al., 2005).

Ticks are the most important vectors of microorganisms, and can transmit a much larger number of pathogenic microorganisms to both animals and humans than any other arthropod group. Ticks can adapt to climate changes caused by global warming and related regions. Ticks are epidemiological markers for the infectious agent (Psaroulaki et al., 2006).

By sticking to their hosts, ticks ensure that infectious agents are effectively transmitted easily and that microorganisms spread to different geographical habitats through the pets they travel on. Pathogens that are ingested by ticks can spread interstellar or interovary. Because female ticks are highly fertile and this accelerates the spread of infectious agents in pet dogs and their owners. However, a small number of tick species can transmit infectious agents to pet dogs and their owners (Hillyard, 1996).

Tick borne infectious diseases in dogs are babesiosis and ehrlichiosis. However, *Borrelia burgdorferi* and *Rickettsia conorii* generally cause subclinically infections, and in this case, it is quite difficult to assess their relationship with clinical disease in dogs (Levy and Magnarelli, 1992). Dogs are responsive to infection by *Coxiella burnetii* (Weissenbock and Holtzmann, 1996). The natural cycle of the transmission of this agent the dog is play a role in infected dogs (Senneville et al., 1991).

Ticks can a reservoirs of spotted fever group (SFG) rickettsiae. Pathogens of the SFG rickettsiae are usually associated with ixodid ticks. The ixodid ticks are transfer SFG ricketsiae to vertebrates via salivary secretions both transtadially and transovarially (Raoult and Roux, 1997)

Coxiella burnetii is obligate intracellular bacteria and they live in the phagolysosomes of the host cell. The ticks carry *C. burnetii* both transovarially and transtadially and are a reservoir for this pathogen. It has been reported that the role of ticks in the transmission of *C. burnetii*, which is found in several tick species, to humans is minimal. However, it is stated that the excrement of ticks carrying the *C. burnetii* agent is much more important in transmitting the agent to humans (Parola and Raoult, 2001).

Borrelia burgdorferi sensu lato is caused canine borreliosis and it's transmitted by ticks of the genus Ixodes. (Filipuzzi-Jenny et al., 1993). The distribution of borreliosis is increasing rapidly in Northern Europe and it is reported that *I. ricinus* ticks transmit the agent to humans in urban areas (Talleklint and Jaenson, 1998; Junttila et al., 1999). There is significant genetic heterogeneity between European and North American isolates of *B. burgdorferi* (Lovrich et al., 1994). *B. burgdorferi* sensu lato, *B. japonica*, *B. afzelii*, *B. garinii* genotypes cause diseases in humans in the northern hemisphere (Filipuzzi-Jenny et al., 1993). The extent to which *B. japonica*, *B. afzelii*, *B. garinii* contribute to dogs' infection is unclear (Azuma et al., 1994).

PCR methods are widely used as rapid and effective tools for the identification of tick-borne pathogens. Sensitivity-specificity is significant in identifying tick-borne infections with PCR-based methods (Rijpkema et al., 1995; Schouls et al., 1999).

The aim of this study was to evaluate the prevalence of zoonotic tick-borne pathogens in dogs Western Türkiye.

Materials and Methods

Sampling

Dog blood samples (n=200) were collected from dogs

Table 1. The sampling provinces and distribution of dog breed.

Dog Breeds	Sampling Provinces		
	Aydın	**İzmir**	**Muğla**
Mongrel (n=73)	42	24	7
Kangal (n=35)	14	8	13
Setter (n=22)	6	7	9
Terrier (n=20)	5	6	9
Pointer (n=20)	5	7	8
German Shepherd (n=18)	6	4	8
Doberman (n=12)	2	4	6
Total	**80**	**60**	**60**

DNA Extraction from dog blood samples

located in western Türkiye. The numerical distribution of the dog blood samples is shown in Table 1. For the dog blood samples, the researchers were taken permission from Adnan Menderes University Local Ethical Committee of Animal Experiments (Document No: 2009/54).

DNA isolation from dog blood samples was done with a DNA extraction kit. (Fermentas®) as recommended by the manufacturer. DNA's were stored at -20°C until PCR studies.

Primers and PCR

Coxiella burnetii (Trans 1-Trans 2), SFG Rickettsiae (Rr190.70p-Rr190.602n), *Borrelia* sp. (BORF-16S) and *B. burgdorferi* sensu lato (23SN2-5SCB) primers were designed which informed as Barandika et al. (2007).

PCR conditions of *C. burnetii*, SFG Rickettsiae and *Borrelia* sp. were given by Barandika et al. (2007).

For the *C. burnetii* the PCR was performed according to Barandika et al. (2007). The reference strain of *C. burnetii* Nine Mile Strain Phase I was used as positive control and *Escherichia coli* was also used to negative control for the PCR.

For the detection of SFG Rickettsiae and *Borrelia* spp., the multiplex PCR was examined according to Barandika et al. (2007).

Detection of the Amplified Products

PCR amplicon were detected by agarose gel electrophoresis and were visualised using the Vilber Lourmat Gel Documentation System (Vilber Lourmat, Germany).

Results

In this study, a total of 200 dogs blood samples collected from Aydın, İzmir and Muğla provinces were examined by using PCR for the diagnosis of Rickettsiosis, Borreliosis and Q Fever infections from tick-borne diseases in dogs. DNA samples were extracted from the blood samples and then PCR was performed on all DNA samples. Because of the differences between the binding scores in the PCR process, the studies are step-by-step.

In the single step PCR for the *C. burnetii* were found to be positive 3 (1.5%) samples obtained from 200 different dog breeds blood samples. It was determined that *C. burnetii* was positive in 1 Kangal dog in Izmir province and 2 Pointer dogs in Muğla province. *C. burnetii* positivity was not found in 80 dog blood samples taken from Aydın province (Figure 1).

As a result of the multiplex PCR for *Rickettsiae* sp. and *Borrelia* sp., a total of 4 (2.0%) samples were found to be positive for the *Borrelia* sp. and 103 (51.5%) samples were found to be positive for the *Rickettsiae* sp. The distribution of *Rickettsiae* sp. and *Borrelia* sp.

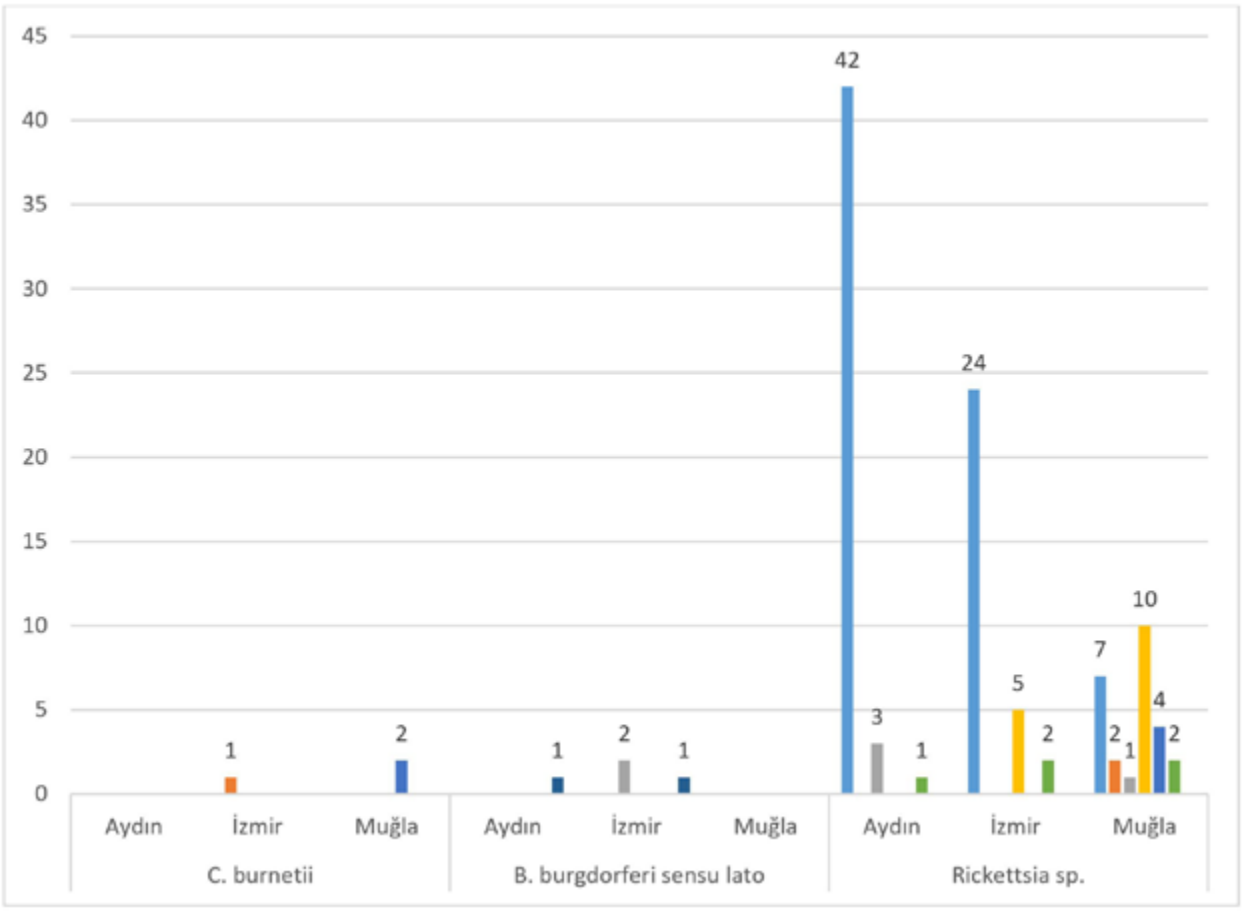

Figure 1. Distribution of PCR positive tick-borne zoonotic bacteria by dog breeds and regions

positive samples by dog breeds is shown in Figure 1. It is noteworthy that all blood samples of 73 Mongrel dogs collected in our study were *Rickettsia* sp. positive.

In this study, PCR tests with DNA from 200 blood samples taken from dogs resulted in 1.5% *C. burnetii*, 2.0% *B. burgdorferi* sensu lato and 51.5% *Rickettsiae* sp. positivity were determined (Figure 1).

In this study, *Rickettsia* sp. was found to be PCR positive at different rates in all regions, while *C. burnetii* in Aydın and *Borrelia burgdorferi* sensu lato in Muğla were not found to be PCR positive.

Discussion

Investigations in different parts of the world have found a close relationship between ticks and tick-borne pathogens (Magnarelli et al., 1995; Lane, 1996; Kramer et al., 1999; Kosik-Bogacka et al., 2004; Wielinga et al., 2006). To take into consideration the geographical and climatic conditions of Türkiye, the various types of ticks within our borders continue their existence. The ticks have completed a part of their life cycle in dogs. During this process, it is thought that dogs carry tick-borne bacterial zoonotic infections and infect people in various ways. Diseases such as Borreliosis and Ricketsiosis that were found to be among tick-borne infections have been reported to be subclinical form in dogs and the dogs are susceptible to *Coxiella burnetii* infections. Especially, it is argued that dogs play a role in the transmission of these infections to humans (Levy and Magnerelly, 1992; Mumcuoglu et al., 1993).

In our study, it was aimed to investigate the presence of *C. burnetii*, *B. burgdorferi* sensu lato and *Rickettsiae* sp. zoonotic bacteria in dogs, which are particularly threatening to human health. Our research materials consist of 200 blood samples taken from dogs.

Lyme Borreliosis is very common in the northern hemisphere. There are 60,000 cases reported in Europe and 15,000 cases reported in the United States each year (Steere, 2000; Hayes and Piesman, 2003). In some countries in Europe, the incidence of the disease is calculated to be 155 out of every 100,000 cases due to regional variability and diversity (Stanek et al., 2012). In some studies (Barral et al., 2002; Gil et al., 2005; Barandika et al., 2008), the presence of *B. burgdorferi* was also detected at low rates. In our study, 2% *B. burgdorferi* sensu lato was isolated in dogs.

Despite the fact that there are many species of tick in the world, it is seen that the rate of transmission of natural *C. burnetti* infection and humans is low (Maurin and Raoult, 1999). *Coxiella burnetii* is also isolated from several species of ticks in Europe (Rehacek et al., 1994; Psaroulaki et al., 2006; Smetanova et al., 2006). In our study, dog blood samples collected from western regions of our country were used and 1.5% of *C. burnetii* were found in dogs by PCR.

Rickettsia sp. was isolated from tick-borne infections frequently. In a survey of prevalence in South Brazil, *Rickettsia* sp. reported a positive rate of 33.7% in dogs (Saito et al., 2008) and reported a positive rate of 81.3% in Brazil's Minas Gerais area (Vianna et al., 2008). In a research conducted in northern Greece, the presence of *Rickettsia* sp. was demonstrated locally (Psaroulaki et al., 2006). In our study, *Rickettsia* sp. positivity was detected in 103 (51.5%) of 200 blood samples taken from dogs.

Conclusion

In conclusion, considering the density of tick populations in Türkiye, the importance of tick-borne bacterial zoonoses and their adaptation to the field conditions is becoming important. In addition to conventional methods, the development of fast and safe methods will ensure the detection of diseases in a short time and contribute to the country's economy with treatment and prophylactic measures to be made as an early diagnosis. In addition, infection of tick-borne bacterial zoonoses will be prevented and community health will be preserved.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Research Article

Investigation of Some Heavy Metal Levels of Carp Fish (*Cyprinus carpio*) Which is Hunted in Büyük Menderes River

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ABSTRACT

In this study, the heavy metal accumulation lithium, beryllium, boron, chromium, manganese, iron, cobalt, nickel, copper, zinc, rubidium, lead, strontium, cadmium, arsenic, vanadium, and uranium (Li, Be, B, Cr, Mn, Fe, Co, Ni, Cu, Zn, Rb, Pb, Sr, Cd. As, V, and U) in the muscle and liver tissues of *Cyprinus carpio* fish caught from three different regions of Büyük Menderes River were investigated. A total of 30 *Cyprinus carpio* were caught from three different regions of the Büyük Menderes River. Muscle and liver tissues of the fish samples were dissected and were solubilized in a microwave oven. The heavy metal levels were performed by ICP-MS. Average concentrations of heavy metals in fish muscle tissue samples collected from each region investigated were found as Li: 0.25±0.01, Be: 1.56±0.05, B: 1.70±0.08, Cr: 1.33±0.09, Mn: 0.32±0.02, Fe: 23.42±1.55, Co: 0.09±0.028, Ni: 0.66±0.06, Cu: 1.43±0.25, Zn: 12.51±1.16, Rb: 1.47±0.22, Pb: 0.45±0.02, Sr: 1.18±0.58, Cd: 0.09±0.01, As: 0.03±0.01, V: 26.04±1.05, U: 0.22±0.07 µg/g. The levels of Li, Be, B, Cr, Co, Rb, Cd, V, and U were higher than the limit levels. Li, Cr, Mn, Fe, Ni, Zn, Pb, Cd, V, and U heavy metals in liver; Be, B, Co, Rb, Sr and As heavy metals were found to be higher in muscle tissue. Results demonstrate that the third region has the highest levels of heavy metal pollution among the three regions investigated. In addition to that, the source of the heavy metal toxicity determined in the second region was found as the third region. According to obtained results, human consumption of fish caught in all three regions may be risky to health.

Keywords: *Bioaccumulation, Cyprinus carpio, heavy metal, pollution.*

Büyük Menderes Nehri'nde Avlanan Sazan Balıklarının (*Cyprinus carpio*) Bazı Ağır Metal Düzeylerinin Araştırılması

ÖZET

Bu çalışmada, Büyük Menderes Nehri'nin üç farklı bölgesinden avlanan *Cyprinus carpio* türüne ait balıkların kas ve karaciğer dokularındaki lityum, berilyum, bor, krom, manganez, demir, kobalt, nikel, bakır, çinko, rubidyum, kurşun, stronsiyum, kadmiyum, arsenik, vanadyum ve uranyum (Li, Be, B, Cr, Mn, Fe, Co, Ni, Cu, Zn, Rb, Pb, Sr, Cd. As, V ve U) ağır metal birikimleri incelenmiştir. Büyük Menderes Nehri'nin üç farklı bölgesinden toplam 30 adet *Cyprinus carpio* yakalanmıştır. Balık örneklerinin kas ve karaciğer dokuları disekte edilerek mikrodalga fırında çözündürülmüştür. Ağır metal seviyelerinin ölçümü ICP-MS ile yapılmıştır. Analiz edilen üç farklı bölgeye ait balıklarda ortalama ağır metal düzeyleri kas dokuda Li: 0,25±0,01, Be: 1,56±0,05, B: 1,70±0,08, Cr: 1,33±0,09, Mn: 0,32±0.02, Fe: 23,42±1,55, Co: 0,09±0,028, Ni: 0,66±0,06, Cu: 1,43±0,25, Zn: 12,51±1,16, Rb: 1,47±0,22, Pb: 0,45±0,02, Sr: 1,18±0,58, Cd: 0,09±0,01, As: 0,03±0,01, V: 26,04±1,05, U: 0,22±0,07 µg/g olarak bulunmuştur. Li, Be, B, Cr, Co, Rb, Cd, V ve U düzeylerinin sınır değerlerin üstünde olduğu görülmüştür. Li, Cr, Mn, Fe, Ni, Zn, Pb, Cd, V ve U ağır metalleri karaciğerde; Be, B, Co, Rb, Sr ve As ağır metalleri kas dokusunda daha yüksek düzeyde bulunmuştur. İncelenen üç bölge arasında, ağır metal kirliliğinin en çok ikinci bölgede olduğu, üçüncü bölgenin kirliliğinin temel nedeninin, ikinci bölge olduğu görülmüştür. Her üç bölgeden avlanan balıkların insanlar tarafından tüketilmesinin sağlık açısından riskli olabileceği düşünülmektedir.

Anahtar kelimeler: *Ağır metal, biyoakümülasyon, Cyprinus carpio, kirlilik.*

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Introduction

The Büyük Menderes River is the most extended river in Western Anatolia, and it covers a vast area of cities such as Aydın, Denizli, Afyon, and Uşak. For centuries, the population living in this region has thrived because of the river basin, which provides the main source of water for agricultural lands. However, due to migration, the increased population led to urbanization and industrialization, which are the primary causes of environmental pollution and ecological problems.

Metals, which are capable of forming cations by losing electrons and exhibit high electrical conductivity, are naturally occurring substances in the ecosystem. These substances can be found in different amounts in various locations in nature, such as the atmosphere, the earth's crust, and water bodies. Additionally, they can accumulate within living organisms (Järup, 2003). Out of the 35 naturally occurring metals, 23 have a specific density greater than 5 g/cm^3 (Davis, 1984). Typically the specific density of these metals is approximately 6 g/cm^3, and their atomic weight exceeds 40.04 (the atomic weight of Ca) (Duffus, 2002). These metals are generally referred to as heavy metals (Ming-Ho, 2005). Heavy metals are defined in Health Sciences Literature as metals with potentially toxic properties, regardless of their atomic weight (Ozbolat and Tuli, 2016). The US EPA (United States Environmental Protection Agency) (1986) and IARC (International Agency for Research on Cancer) (2019) classify heavy metals as human carcinogens. Although heavy metals are naturally present in all ecosystems, their concentrations can vary greatly due to both natural and human-induced factors. This can lead to differing levels of heavy metals across various environments.

There are many factors that can impact how heavy metals accumulate and spread in the environment. These include natural processes like the erosion of the earth's crust, as well as human activities such as mining, soil degradation, and the inappropriate disposal of industrial and urban waste. The use of harmful insecticides and pesticides, as well as air, water, and soil pollution, is also among the various factors that can affect the accumulation and distribution of heavy metals in the environment (Ming-Ho, 2005). Therefore, heavy metals have been classified as environmental pollutants (Tiller, 1989). These pollutants persist and can be found in various industrial wastes, and they cannot be decomposed or neutralized through natural processes. Heavy metals enter the ecosystem and subsequently move up the food chain, impacting various living organisms. This pollution process typically begins with industrial activities and affects the atmosphere, soil, water, plants, animals, and ultimately, humans (Dittmar, 2011).

Several factors can affect the extent of harm caused by heavy metals, including the amount and method of exposure, the specific type of metal, as well as individual characteristics like age, gender, genetic makeup, and nutritional status. Certain heavy metals, such as arsenic, cadmium, chromium, lead, and mercury, are particularly toxic and can damage various organs even at low levels of exposure. Additionally, the accumulation of heavy metals in the environment can increase their toxicity and have negative effects on living organisms (Piskorova et al., 2003).

Animals can suffer from severe illnesses when exposed to heavy metals in a contaminated environment, as the levels of heavy metals in their tissues and milk can quickly rise (Kottferova and Korenekova, 1998). This can result in various health issues, including reduced vitality, reproductive problems, weakened immunity, and the development of cancer and teratogenic diseases (Houpert et al., 1997). Numerous local studies have demonstrated that soil and vegetables may contain toxic levels of heavy metals such as cadmium, lead, copper, zinc, iron, chromium, and manganese (Bires et al., 1995). Animal feed grown on polluted soils can also contain heavy metals, which may accumulate in the tissues of the animals that consume them (Qadir et al., 2000). Furthermore, fish with high nutritional value living in aquatic ecosystems can also be impacted by heavy metal pollution. Animal-based food products, such as meat and milk, can contain heavy metals, which can significantly affect human health if consumed (Licata et al., 2004).

Heavy metal pollution is a major environmental issue in aquatic ecosystems, and the amount of heavy metals present depends on the soil and rock structure of the area (Tunca, 2012). The concentration of heavy metals present in these ecosystems is influenced by the soil and rock composition of the region, and human activities like mining, agriculture, and industrial processes further exacerbate this issue.

In this study, heavy metals as bioaccumulative pollutants which are the major environmental pollution source in the aquatic environment are aimed to be studied. Carps fish (*Cyprinus carpio*) tissue samples in three different sampling sites in Büyük Menderes River were used to determine heavy metal pollution levels.

Materials and Methods

Cyprinus carpio fishes, were hunted in three regions determined in Büyük Menderes River. The first region includes parts of the river basin in the provinces of Afyon, Uşak and Denizli. The second region includes the areas of the river basin in districts of Aydın province (except for Söke district). Third Region included part of the river basin in Söke district. Between February 2018 and March 2018, when fishing was not prohibited. Samples were collected from local fishermen. Study materials consisted of the muscle and liver tissues of 30 *Cyprinus carpio* fish that were dissected from three distinct regions of the Büyük Menderes River. Liver and muscle tissue samples taken from *Cyprinus carpio* were dissolved in microwave oven (Cem Mars 5). Teflon tubes were used to hold approximately 0.5 g of each sample.

To facilitate their dissolution, the tubes were subjected to microwave heating for a duration of 20 minutes, following the addition of 5 ml of 65% HNO_3 and 2 ml of 37% HCl. The heating process was done at 210°C, with 80% power and approximately 1 atm of pressure. After heating, the tubes were allowed to cool for 90 minutes. Once cooled, the samples were transferred to 25 ml volumetric flasks and made ready for ICP-MS analysis by adding deionized water to reach the 25 ml final volume (Aktaş, 2013).

A multi-standard stock solution (10 ppm) containing 16 elements was used in ICP-MS to perform elemental analysis. For each element, an intermediate stock solution with a concentration of 1 ppm and six standard solutions with densities of 10, 20, 50, 100, 200, and 400 ppb were prepared to be used in ICP-MS. The device was calibrated before detecting the heavy metal content of the dissolved samples. The Bruker 820-MS device was utilized to determine the heavy metal content in muscle and liver tissue samples from *Cyprinus carpio* species. The samples were analyzed for the presence of arsenic, beryllium, boron, cadmium, chromium, cobalt, copper, lithium, lead, manganese, nickel, rubidium, strontium, uranium, vanadium and zinc.

The statistical analysis of the results was carried out with the IBM SPSS Statistics 22 package program (IBM SPSS Statistics®, Chicago, IL, USA). The statistical values such as mean and standard error were determined from the muscle and liver samples. In the research, the normality of variables was examined through the implementation of Shapiro-Wilk and Kolmogorov-Smirnov tests. Anova analysis was utilized to assess variables that followed a normal distribution, while the Kruskal-Wallis analysis was employed for variables that did not exhibit a normal distribution. The posthoc test Duncan was used during the Anova analysis to detect any differences, while the Mann-Whitney U test was employed for the Kruskal-Wallis analysis. Results were deemed significant if their statistical significance level was below 0.05.

Results

The heavy metal concentrations in the muscle and liver tissue of *Cyprinus carpio* specimens that were captured are illustrated in Table 1 and Table 2. The results indicate that the third region had the highest levels of heavy metals. Additionally, it was inferred that the second region was the cause of heavy metal pollution in the third region.

Table 1. Muscle tissue heavy metal levels (µg/g).

Metals	Region 1 $\bar{X}\pm S_{\bar{x}}$ (n=10)	Region 2 $\bar{X}\pm S_{\bar{x}}$ (n=10)	Region 3 $\bar{X}\pm S_{\bar{x}}$ (n=10)	P*
Li	0.25±0.01^{a}	0.28±0.02^{a}	0.23±0.01^{a}	0.084
Be	1.62±0.09^{a}	1.62±0.08^{a}	1.46±0.07^{a}	0.450
B	1.56±0.08^{b}	2.07±0.17^{a}	1.48±0.07^{c}	0.028
Cr	1.44±0.24^{a}	1.30±0.10^{a}	1.24±0.10^{a}	0.650
Mn	0.24±0.07^{c}	0.36±0.04^{a}	0.36±0.04^{b}	0.012
Fe	17.46±1.81^{b}	26.96±2.46^{a}	25.84±2.81^{a}	0.018
Co	0.06±0.01^{a}	0.16±0.08^{a}	0.07±0.01^{a}	0.061
Ni	0.40±0.071^{b}	0.66±0.09^{ab}	0.93±0.12^{a}	0.004
Cu	0.83±0.02^{a}	1.89±0.61^{a}	1.57±0.36^{a}	0.118
Zn	9.74±1.16^{a}	13.35±1.88^{a}	14.43±2.62^{a}	0.229
Rb	3.10±0.19^{a}	0.61±0.04^{c}	0.71±0.08^{b}	0.000
Pb	0.43±0.03^{a}	0.49±0.05^{a}	0.42±0.04^{a}	0.338
Sr	0.78±0.27^{a}	0.95±0.14^{a}	1.82±0.74^{a}	0.256
Cd	0.08±0.01^{a}	0.09±0.01^{a}	0.11±0.04^{a}	0.588
As	0**	0.06±0.03^{a}	0.02±0.02^{b}	0.039
V	25.39±1.78^{a}	27.98±2.12^{a}	24.73±1.52^{a}	0.424
U	0.24±0.01^{a}	0.23±0.01^{a}	0.20±0.01^{a}	0.123

^{a,b,c} The difference between groups with different letters in each parameter line is significant.
* P<0.05 was accepted as statistically significant.
** :Below the limit of analysis.

Discussion

Heavy metals, which constitute a considerable part of water pollution, are among inseparable causes of environmental pollution. Heavy metals in fish tissues, though in small amounts, may accumulate and gradually reach levels causing toxic effect. According to previous studies, the amount of heavy metal accumulation in fish is influenced by the specific type of metal, as well as the

Table 2. Liver tissue heavy metal levels (µg/g).

Metals	Region 1 $\bar{X}\pm S_{\bar{x}}$ (n=10)	Region 2 $\bar{X}\pm S_{\bar{x}}$ (n=10)	Region 3 $\bar{X}\pm S_{\bar{x}}$ (n=10)	P*
Li	0.21±0.02[a]	0,17±0.01[a]	0.16±0.01[a]	0.118
Be	1.25±0.04[a]	1.19±0.07[a]	1.27±0.05[a]	0.557
B	1.66±0.023[a]	1.01±0.08[b]	1.06±0.15[c]	0.002
Cr	1.94±0.10[a]	1.71±0.14[a]	1.73±0.06[a]	0.272
Mn	0.51±0.08[c]	0.77±0.18[b]	1.03±0.21[a]	0.030
Fe	275.00±55.26[a]	407.00±92.21[a]	340.00±71.05[a]	0.123
Co	0.03±0.01[b]	0.05±0.01[a]	0.02±0.05[c]	0.037
Ni	6.60±1.12[a]	9.34±2.01[a]	7.82±1.47[a]	0.338
Cu	1.50±0.10[a]	2.83±0.62[a]	4.34±1.40[a]	0.065
Zn	40.47±3.79[c]	128.00±13.72[b]	167.00±65.03[a]	0.000
Rb	2.42±0.16[a]	0.50±0.03[b]	0.52±0.06[b]	0.000
Pb	0.70±0.13[a]	0.49±0.04[a]	0.69±0.10[a]	0.229
Sr	0.50±0.09[a]	0.59±0.06[a]	0.63±0.12[a]	0.442
Cd	0.11±0.04[b]	0.11±0.01[b]	0.30±0.09[a]	0.002
As	0[**]	0[**]	0.01±0.01[a]	0.368
V	38.78±1.37[a]	37.12±2.85[a]	39.96±1.58[a]	0.618
U	0.20±0.01[a]	0.19±0.01[a]	0.20±0.01[a]	0.751

[a,b,c] The difference between groups with different letters in each parameter line is significant.
* P<0.05 was accepted as statistically significant.
** Below the limit of analysis.

type of fish and tissue involved (Korkmaz et al., 2012; Petrovic et al., 2013). Uysal et al. (1986) conducted a study on the coasts of the Aegean Sea and discovered that fish species capable of adjusting to pollution displayed significant levels of heavy metal buildup. The degree of adaptation, however, varied among different species. Linde et al. (1998), reported that heavy metal accumulations in fish may vary depending on the age, size and length of the fish. Melgar et al. (1997) examined weekly cadmium toxicity and accumulation in tissues. In the first weeks, it was observed that heavy metal accumulation in other tissues was prevented as a result of detoxification in the liver, gills and kidneys. However, it has been reported that heavy metals accumulate significantly in the muscle, brain, bone, ovary and testicles by increasing the accumulation with long-term exposure, making the detoxification insufficient.

According to Cicik et al. (2004), there exists a correlation between the accumulation of heavy metals and various tissues and organs. The order of accumulation, from highest to lowest, was found to be kidney, spleen, gill, liver, and muscle. Furthermore, the study reported that the accumulation of heavy metals increased as the exposure time increased. De Conto et al. (1997) observed a simulated cadmium deposition exposure process in fish of the *Cyprinus carpio* species, heavy metal deposition in muscle and other tissues and organs. Accordingly, cadmium concentrations increased rapidly in the kidney and liver, while the level of pollutants in muscle increased significantly after 106 days. In the kidney and liver, they observed that as the concentration of pollutants in the water increased, their toxic concentrations increased and there was immediate loss of cadmium in the muscle during the initial 43 depuration days.

The study determined that the muscle tissue tends to accumulate higher levels of heavy metals such as Be, B, Co, Rb, Sr, and As than the liver tissue. Although this situation contradicts with other studies in the literature, it is thought that long-term exposure of fish to these heavy metals is caused. The concentration of metals such as Li, Cr, Mn, Fe, Ni, Cu, Zn, Pb, Cd, V, and U is higher in the liver tissue compared to the muscle tissue. Specifically, the accumulation of Fe, Ni, Cu, Cd, and V metals in the liver tissue is significantly greater than in the muscle tissue.

Akbulut and Akbulut (2010) investigated the levels of heavy metals, namely Br, Hg, Co, Cr, Cu, Pb, and Zn, in the muscle and gill tissues of three fish species, namely *Capoeta tinca*, *Capoeta capoeta*, and *Leuciscus cephalus*, caught in Kızılırmak River. Based on the study findings, the levels of heavy metals in the muscle tissue exhibit a relationship in the following order: Zn> Cu> Pb> Br> Cr> Hg> Co. Similarly, the heavy metal levels in the muscle tissue show the following relationship: V> Fe> Zn> B> Be> Rb> Cu> Cr> Sr> Ni> Pb> Mn> Li> U> Co> Cd> As. In terms of heavy metal levels in the liver tissue, the relationship is as follows: Fe> Zn> V> Ni> Cu> Cr> B> Be> Rb> Mn> Pb> Sr> U> Li> Cd> Co> As.

Table 3. Comparison of average heavy metal levels in muscle tissue with national studies, national limit and certified reference values DORM-4 (µg/g).

Metals	Measured Values	Türkmen et al. (2009)	Topçuoğlu et al. (2002)	National Standard Limit Values (TKB, 2002)	DORM-4 (NRC, 2014)
Li	0.25	-	-	-	0.10
Be	1.57	-	-	-	-
B	1.70	-	-	1.00	0.03-1.00
Cr	1.33	0.05-1.87	0.06-0.84	-	1.00
Mn	0.32	0.14-1.33	0.69-3.56	-	3.17
Fe	23.42	9.99-43.30	30.00-60.00	-	343.00
Co	0.09	0.01-0.53	0.05-0.40	-	0.25
Ni	0.67	0.06-4.70	0.01-2.04	-	1.34
Cu	1.43	0.21-5.89	1.01-4.54	20.00	15.70
Zn	12.51	3.85-15.90	25.70-44.20	50.00	51.60
Rb	1.47	-	-	-	0.05
Pb	0.45	0.09-0.81	0.05-0.06	0.20	0.40
Sr	1.18	-	-	-	10.10
Cd	0.09	0.01-0.38	0.02-0.24	0.10	0.10
As	0.03	-	-	1.00	0.10
V	26.04	-	-	-	1.57
U	0.22	-	-	-	0.05

The information presented in Table 3 compares the average levels of heavy metals detected in the muscle tissue of fish in present study to those found in other national studies and various national and international standards. In a research conducted by Turkmen et al. (2009) involving 20 fish species from the Aegean Sea and the Mediterranean, it was observed that the average levels of Cu, Zn, and Cd were similar to the findings of this study. However, the average level of Pb was significantly elevated in their research. Topcuoğlu et al. (2002) conducted an independent study examining a variety of marine organisms discovered along the Turkish Black Sea coastline. The findings of present study indicated that the mean levels of Fe, Ni, Cu, and Cd observed in this research were in agreement with the concentrations documented in previous studies concerning the muscle tissues of acorn, whiting, horse mackerel, and red mullet. However, the average levels of Mn, Co, and Zn were low in this research, while the average levels of Cr and Pb were high. Upon evaluation of this study based on national standards (Kalafatoglu et al., 1997; TKB 2002), it was discovered that the mean levels of Cu, Zn, and As were below the limit values, whereas the mean level of Cd was very close to the limit values. As for B and Pb, their mean levels were high based on national standards. On the other hand, when this study was evaluated using DORM-4: Fish protein certified reference material for trace metals (NRC, 2015), it was observed that the mean levels of Mn, Fe, Co, Ni, Cu, Zn, and Sr were below the references values. However, Li, B, Cr, Rb, Pb, V, and U had high average levels. Even though the average level of Cd was low compared to international standards, it was discovered that the interregional assessment exceeded the limit value in Region 3 (also see Table 2).

The mean levels of heavy metals in liver tissue obtained from various regions were compared with national and international studies in Table 4. Turkmen et al. (2008) conducted a study on *Engraulis encrasicolus* and *Spicara* sp. in the Aegean Sea, the Sea of Marmara, and the Black Sea coast of Turkey. The comparison of the minimum and maximum liver tissue levels measured in their study with those in this study showed that the average levels of Mn, Cu, Zn, Rb, Sr, As, Cd, Pb, Fe, and Ni were low. The liver tissue levels obtained in this study were also compared with the liver heavy metal levels measured in Usero et al. (2003) study, revealing that the mean levels of Mn and Cu were low, while the mean levels of Cr, Ni, Zn, and Pb were high. Additionally, the liver tissue levels in this study were compared with the DOLT-4: Dogfish liver certified reference material for trace metals values generated from shark liver measurements (NRC, 2014). The mean levels of Fe, Cu, Zn, Cd, and As were low, while the mean levels of Ni and Pb were high.

Table 4. Comparison of mean heavy metal levels in liver tissue with national studies, international studies and certificate reference values (DOLT-4) (µg/g)

Metals	Measured Values	Türkmen et al. (2008)	Usero et al. (2003)	DOLT-4 (NRC, 2014)
Cr	1.79	0.28–2.97	0,01-0.06	-
Mn	0.77	0.72–9.67	1.23-4.61	-
Fe	340.97	55.20–316.00	185.00-560.00	1833.00
Ni	7.92	0.47–11.60	0,08-0,39	0.97
Cu	2.89	0.99–30.70	13.70-164.0	31.20
Zn	111.69	12.50–145.00	15.00-81.80	116.00
Pb	0.63	0.26–3.38	0.20-0.60	0.16
Cd	0.17	0.06–0.69	0.08-0.51	24.30
As	0.01	-	-	9.66

The results obtained in present study align with the findings from previous studies conducted by Çolak Esetlili (2010) and Arslan (2010) in the Alangüllü region, as well as the study conducted by Beyhan and Algül (2018) in the Bafa Lake region. It should be noted that Alangüllü and Bafa Lake form a part of the Büyük Menderes Basin.

Conclusion

The main objective of this study was to evaluate the concentrations of toxic metals in the muscle and liver tissues of *Cyprinus carpio* fish that were collected from three different regions of the Büyük Menderes River. The purpose was to compare the measured levels with the acceptable limits established by both national and international standards, as well as other research studies previously conducted on this topic. Results indicated that levels of Li, Be, B, Cr, Co, Rb, Cd, Pb, V, and U metals in the muscle tissue were above acceptable limits. Additionally, Ni and Pb levels in the liver tissue exceeded those reported in previous national and international studies. These findings confirm that the Büyük Menderes River is contaminated with chemical wastes, industrial and domestic wastes, and agricultural practices including fertilization and pesticides used in mining activities. The build-up of Ni and B in the tissues of fish is believed to be the result of the waste produced by geothermal power plants. Other metals such as Be, Co, Rb, Sr, and As) were found to accumulate primarily in the muscle tissue of the fish, as opposed to the liver tissue. This suggests that exposure to heavy metals over an extended period of time has led to the continuous presence of pollutants. Without prompt intervention, this pollution is likely to worsen and have more severe consequences. Therefore, it is imperative that official institutions and organizations increase their audits of the Büyük Menderes River and Basin to eliminate environmental risk factors. The discharge of geothermal plant waste to rivers should be halted, or facilities that do not comply should face closure sanctions. Treatment plants to manage domestic and industrial wastes should be established immediately, and organic agricultural practices should be improved. Consumption of *Cyprinus carpio* fish caught from the Büyük Menderes River poses a risk to public health due to toxic metals such as Co, Cd, B, Pb, Li, Be, B, Cr, Rb, V, and U, which are especially high in muscle tissue and can cause various diseases in the long term. As such, consumption of these fish should be banned if necessary. It should be noted that pollution in the Büyük Menderes River is a threat to the entire ecosystem, and measures should be taken to reduce the release of ions into the environment.

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Conflict of Interest

The authors declare that they have no conflict of interest in this study.

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Review

Investigation of Organic Chicken Breeding and Meat Quality

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A B S T R A C T

In recent years, with the increase in income in our country, it has been seen that most consumers are awareness of healthy eating and are interested in organic food. The emergence of various diseases, such as cancer and obesity, play a significant role in this preference. In addition, with the prolongation of life expectancy, the desire to increase the quality of life is very important. Due to the harm caused by the foods obtained by conventional methods to human health and the environment, consumers' demand for healthy and quality food has led to a market segment consisting of organic products. The availability of many conventional foods with unsafe additives, preservatives, sweeteners, and colorants can be seen as another factor in the increasing importance of natural, safe, and healthy foods. This review study it is aimed to correct some existing misperceptions by giving detailed information about organic chicken breeding and meat quality.

Keywords*: Organic chicken, conventional chicken, chicken meat quality, fatty acid composition.*

Organik Tavuk Yetiştiriciliği ve Et Kalitesinin İncelenmesi

Ö Z E T

Ülkemizde son yıllarda gelir artışı ile birlikte tüketicilerin çoğunun sağlıklı beslenme bilincine sahip olduğu ve organik gıdaya ilgi duyduğu görülmektedir. Bu tercihte kanser ve obezite gibi çeşitli hastalıkların ortaya çıkması büyük rol oynamaktadır. Ayrıca yaşam süresinin uzamasıyla birlikte yaşam kalitesini artırma isteği de büyük önem taşımaktadır. Konvansiyonel yöntemlerle elde edilen gıdaların insan sağlığına ve çevreye verdiği zararlar nedeniyle tüketicilerin sağlıklı ve kaliteli gıdaya olan talebi organik ürünlerden oluşan bir pazar segmentinin ortaya çıkmasına neden olmuştur. Güvenli olmayan katkı maddeleri, koruyucular, tatlandırıcılar ve renklendiriciler ile piyasada pek çok geleneksel gıdanın bulunması, doğal, güvenli ve sağlıklı gıdalara verilen önemin artmasında bir başka faktör olarak görülebilir. Bu derleme çalışmasında, organik tavuk yetiştiriciliği ve et kalitesi hakkında detaylı bilgi verilerek, var olan bazı yanlış algıların düzeltilmesi amaçlanmaktadır.

Anahtar kelimeler: *Organik tavuk, konvansiyonel tavuk, tavuk eti kalitesi, yağ asidi kompozisyonu.*

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Introduction

Organic poultry is defined as a production model in which animals are allowed to exhibit their natural behavior. It has been determined that organic agriculture and food product markets are concentrated in big cities such as Istanbul, Ankara, and Izmir. In contrast, purchasing organic agriculture and food products is not common because consumers throughout Turkey do not have enough information about organic production and find the price expensive (Eryılmaz et al., 2015).

It has been determined that the studies on this subject need to be revised. This review study it is aimed to eliminate the misinformation about organic chicken by giving information about organic chicken breeding and meat quality.

Conventional Livestock

Conventional livestock farming is known as a production method in which a large number of animals are compressed into a small number of large enterprises. Studies show that conventional production has harmed many environmental, biological and socio-economic structures, especially human and animal health, leading to the extinction of small-scale agriculture and animal husbandry (Hanoğlu, 2013).

Today, conventional animal husbandry is a production method aiming for the highest yield with more animals and inputs (Çukur and Saner, 2005). Intensive breeding methods and tight housing systems applied to animals cause important health problems by weakening the immune systems of animals (Öztürk and Türkoğlu, 2012). One of the main drawbacks of industrial animal husbandry is using genetically modified (GMO) corn and soybean products as feed because they are cheaper. Studies in the literature reveal GMO DNA fragments in the meat of animals fed with GMO feeds. In a study, GMO DNA was detected in the tissues of animals fed GMO corn (Hanoğlu, 2013).

Especially in European countries, the increase in diseases caused by animal feeding causes consumers to turn to alternative products (Hanoğlu, 2013). Today, the European Union countries are starting to abolish chicken farming in traditional cage systems, taking into account animal rights, technological developments, and the improvement of the economic structure have also caused a change in consumption habits (Türker et al., 2017).

Organic Livestock

Agricultural enterprises have a complementary relationship between plant and animal production. Fertilizer for livestock, crop production; vegetative production also provides fodder for livestock with both the production of fodder crops and by-products and residues. However, later on, this relationship was brought to a breaking point (Hanoğlu, 2013).

Organic livestock can be defined as a production system where the number of animals is low, at appropriate feeding and shelter conditions, with the appropriate production and marketing methods to obtain high quality products and reach a high price. In the Regulation on the Principles and Implementation of Organic Agriculture, organic animal production: “Producing animals using breeding animals or semen, producing human food and animal and plant nutrition products from animal products, supplying organic raw materials to industries and scientific studies that obtain their raw materials from agriculture, every stage of which is authorized according to this Regulation. It is defined as “production activities controlled and certified by the organization” (Çukur and Saner, 2005; Anonymous, 2010).

The main differences distinguishing organic livestock from conventional livestock; are animal welfare, a natural environment, healthy products, and sustainable resources (Çukur and Saner, 2005). Considering these differences, organic livestock farming has come to occupy an important place as an alternative livestock production model, especially in the United States and the European Union (Çiçek and Tandoğan, 2009).

The countries with a say in organic animal production are listed as the USA, Canada, Austria, Denmark, Germany, England, France, and Argentina. Among them, the most important countries in organic livestock farming are the USA and Canada. In these countries, the trend towards organic meat was realized after some hormones in meat were examined under laboratory conditions and it was determined that these hormones increased the risk of some types of cancer. Thus, organic meat, milk, yoghurt, cheese, and eggs began to take their place in the market (Yunus, 2003; Çiçek and Tandoğan, 2009; Öztürk and Türkoğlu, 2012; Hanoğlu, 2013; Yenilmez and Uruk, 2014).

There is a general perception that chicken meat obtained through organic production methods is more delicious. This perception is also confirmed by the results of many studies. The studies stated that the meat obtained with organic production is superior in terms of crispness, texture, and juiciness while being darker in color and having a low cooking loss. The difference in chicken meat flavor is mainly due to genotype, age, feeding, and rearing style (Ceylan, 2014).

Basic Rules for Organic Poultry

According to the Regulation on the Principles and Implementation of Organic Agriculture (2010).

There are some rules regarding organic animal production. These rules cover the following basic elements such as genotype, nutrition, reproduction and shelter (Anonymous, 2010):

•In organic livestock breeding for breeding or production, breeds with high adaptability to environmental conditions and resistance to diseases are selected. For this reason, priority is given to native breeds and hybrids adapted to that region.

• Animals brought from organic farms and fed completely organic feeds, whose genetic structure has not changed.

• Natural methods are used in reproduction in organic

animal breeding.

• Animals must have access to pastures, outdoor promenades, or open spaces. The number of animals per unit area in pastures and open areas should be limited to provide sufficient animal manure for the crop production in the production unit.

Conventionally bred animals may be found in the same holding, provided that the barns and land on which they are reared are clearly separate from the organically bred units and that separate species are present.

• If organic animal products cannot be distinguished from conventional products, these products cannot be considered organic.

• The transition period in animal production is 10 weeks for meat production poultry, provided that they are not older than 3 days. Some breeders or breeds used in intensive production, which do not have special diseases or health problems, are used as breeders.

• If the number of organically raised animals insufficient in a flock created for the first time, conventionally raised broiler chickens can be used in organic livestock farming, provided that they are not older than 3 days of age when they leave the farm they came from.

• Organic animal breeding and animal production enterprises keep regular records regarding the entry and exit of animals and all treatment practices.

Animal Health and Veterinary Intervention in Organic Animal Breeding

According to the Regulation on the Principles and Implementation of Organic Agriculture (2010), it is important to take preventive measures rather than treatment in organic animal breeding. The necessary measures for the protection of animals against diseases are as follows:

• Disease-resistant, suitable breeding breeds are selected.

• Walking for regular exercise that boosts the natural immunity of animals access to fields or pastures and the use of quality feed are provided.

• To prevent health problems in animals due to overcrowding Appropriate placement frequency is provided.

The most appropriate vaccines or drugs should be used to minimize or prevent the risk of transmission of pathogens to animals (Ak, 2002).

Feed Supply and Animal Nutrition in Organic Animal Production

Vegetable-based feeds for animal nutrition should preferably be produced in the enterprise. Chemical pesticides and fertilizers should not be thrown on pastures. Animal fat and animal by-products cannot be added to the rations. Regulation rules should be followed in the use of vitamins and minerals (Ak, 2002).

Shelter

Another important point of organic animal husbandry is to provide good shelter conditions. All hygienic measures should be taken to establish and maintain shelters in ecological livestock farming. The shelter should receive adequate fresh air and daylight and should give freedom of movement to all species and races (Ak, 2002).

According to the Regulation on Implementation (2010), necessary shelter areas for poultry are given.

Table 1. Necessary shelter area for poultry

Shelter type	Internal area (Net area reserved for animals) Number of animals/m^2	Promenade (Rotatable area m^2/head)
Fattened poultry house animals (in fixed shelters)	10 up to 21 kg live weight/m^2	4
Fattened poultry (in portable barns)	16[(1)] up to 30 kg live weight/m^2 mobile poultry houses	2.5

[(1)]Only in the case of portable shelters not exceeding 150 m^2 of floor area

Transport

According to the Regulation on the Principles and Implementation of Organic Agriculture (2010), there are rules that must be followed especially for animal welfare in organic animal transportation. These rules:

• Animals are transported stress-free

• Loading and unloading operations are carried out carefully and without the use of an electrical stimulant device to force the animals. It is forbidden to use sedatives before and during transportation.

• In land transportation, a break is made every 8 hours for feeding, watering, and resting.

Slaughter

Appropriate measures should be taken so as not to create stress during slaughter. Slaughter should be done separately from the slaughterhouse of conventional animals, separate slaughterhouses should be used and synthetic additives cannot be used to protect products after slaughter (Ak, 2002).

Table 2. Organic broiler breeding and meat production in Türkiye (Ministry of Agriculture and Forestry, 2021)

Years	Total number of farmers	Number of chickens (pieces)	Meat production (tonnes)
2007	2	1.400	-
2008	1	500	1
2009	1	69.150	34.5
2010	2	273.910	550
2011	5	325.436	713.06
2012	5	102.082	210.31
2013	18	716.024	1030.06
2014	17	834.167	1823
2015	23	589.804	2130
2016	26	608.862	1485.9
2017	12	604.900	1266
2018	26	606.790	134.128
2019	6	94.583	45
2020	20	469.345	49.78

Organic chicken farming in Türkiye

Organic chicken farming showed an increasing trend. According to the 2021 data from the Ministry of Agriculture and Forestery (2021), there are 20 organic chicken breeding farmers in Turkey. Table 2 shows the number of farmers, the number of organic chickens, and the amount of organic chicken meat obtained between 2007 and 2020.

Türkiye also has an important place in organic chicken production. Organic poultry has developed in some of our provinces. Accordingly, a significant increase has been achieved in the production of organic chicken and chicken products. This shows that the demand for organic chicken products is also increasing (Aykutoğlu and Cakir, 2021).

According to 2020 data from the Ministry of Food, Agriculture and Livestock, organic chicken production was carried out by 20 producers in Turkey, and a total of 469.345 organic chickens and 49.78 tons of organic chicken meat were produced.

Table 3 shows the number of farmers in various provinces in 2021, the number of organic chickens grown and the amount of organic chicken meat. According to this table, a producer is in first place in Izmir, producing 171.559 organic chickens. This is followed by a producer in Sakarya with 132.500 organic chickens. A producer in Istanbul, which is the last place, raised 350 chickens in 2021 (Ministry of Agriculture and Forestry, 2021).

As a result of the study conducted by Husak (2007), it was determined that organic chicken meat is superior to conventional chicken meat in terms of protein amount, omega-3 and omega-6 fatty acids, mineral substance content and crispness, and also has a lower fat level. As a result of the sensory evaluations, it was stated that the chewiness of organic chicken meat was higher.

Średnicka-Tober et al. (2016) examined the fatty acid profile of organic and conventional chicken meats. It has been reported that saturated fatty acids and monounsaturated fatty acids are lower and omega-3 fatty acids and polyunsaturated fatty acids are higher in organic chicken meat.

Meluzzi et al. (2009) investigated the effects of different genotypes (slow, medium, and fast-growing) and feeding methods on the chemical composition of organic chicken meat. As a result of this study, the highest levels of polyunsaturated fatty acids, omega-3, and omega-6 fatty acids were detected in slow-growing organic chicken meat. Monounsaturated fatty acids were found to be lowest in slow-growing organic chicken meat. In addition, it has been determined that the amount of fat in slow-growing chicken meat is lower than in medium and fast-growing chicken meat.

Castellini (2005) and Castellini et al. (2002) investigated the effects of organic production systems on chicken meat. As a result of the studies, it has been stated that the water-holding capacity, pH, and fat content of organic chicken meat are lower than conventional chicken meat. At the same time, cooking loss and omega-3 fatty acids are higher. It has also been observed that organic breast meat's sensory quality is better than conventional breast meat's.

As a result of the study conducted by Grashorn and Serini (2006), it was reported that organic chicken meat and skin are more yellow in color than conventional chicken meat, cooking losses are lower, and texture values are higher. It has been concluded that the dry matter content, ash and protein amounts, omega-3 fatty acids of organic chicken meat are higher than conventional chicken meat. As a result of the sensory evaluation, it was stated that the panelists found organic chicken meat to be tougher and tastier than conventional chicken meat.

Table 3. Organic poultry breeding (Ministry of Agriculture and Forestry, 2021).

Provinces	Number of farmers	Number of poultry (pieces)
ADANA	1	13.300
BALIKESİR	1	12.000
BOLU	3	42.000
BURDUR	1	1.800
BURSA	1	2.617
ELAZIĞ	4	55.284
İSTANBUL	1	350
İZMİR	8	171.559
KIRKLARELİ	6	49.790
KOCAELİ	2	4.652
MANİSA	4	55.472
MERSİN	1	4.000
ORDU	35	109.000
SAKARYA	4	132.500
SAMSUN	1	83.838
TRABZON	2	1.500
UŞAK	9	48.590

Castromán et al. (2013) in a study conducted in Uruguay, observed that higher levels of polyunsaturated fatty acids were detected in conventional chicken meat. In addition, it was stated that the omega-3 and omega-6 fatty acids of conventional chicken meat had statistically higher values. Contrary to other studies, they reported that monounsaturated fatty acids were higher in organic chicken meat. Researchers emphasized that these differences are not due to the carcass region taken from the chicken, but to the production method.

Saleh et al. (2015) investigated the quality of organic chicken meat. As a result of the study, they reported that the amount of fat in organic chicken meat is lower and the cooking loss is higher compared to conventional chicken meat.

Sirri et al. (2010) investigated the effects of different genotypes (slow, medium, and fast- growing) on the fatty acid profile of organic chicken meat. As a result of the study, it was reported that slow and medium-growing chickens had lower fat contents than fast-growing chickens. It was observed that the breast meat of slow-growing chickens contained a statistically higher amount of protein than medium and fast-growing chickens. The highest amounts of arachidonic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) or docosapentaenoic acid (DPA) were detected in the meat of slow-growing chickens. On the other hand, a-linolenic acid was found in the lowest amount in the meat of slow-growing chickens. The total amount of polyunsaturated fatty acids is statistically different in each, with the highest in slow- growing chickens (413 g/kg fat), followed by medium fast-growing chickens (358 g/kg fat), and the lowest in fast- growing chickens (324 g/kg fat).

Total omega-3 fatty acid values are highest in slow-growing chickens (77.1 g/kg fat), followed by medium fast-growing chicken (54.6 g/kg fat) and lowest in fast-growing chicken (43.5 g/kg fat) was detected. Total omega-6 fatty acid values were highest in slow- growing chicken (336 g/kg fat), followed by medium fast-growing chicken (301 g/kg fat) and lowest in fast- growing chicken meat.

Napolitano et al. (2013), the sensory properties of fast-growing conventional chicken meats (CC), fast-growing organic chicken meats (OFG), and slow-growing organic chicken meats (OSG) were investigated. In the study, the chicken breast meat was used for sensory evaluation. As a result of sensory evaluation, CC was more brittle than OFG, and OSG was more fibrous than both OFG and OSG, OFG and OSG had a stronger aftertaste. In contrast, OSG was rated less watery than OFG and CC before swallowing and less fibrous than OFG.

Fanatico et al. (2007) investigated how fast and slow-growing chickens affect meat quality with and without open space access. It has been reported that the flesh and skin colors are more yellow when slow-growing chickens have open field access, but the same effect is not seen when fast-growing chickens have open field access. It was stated that breast meat obtained from slow-growing chickens contained more protein and a-tocopherol and half the amount of fat detected in fast-growing chicken meat. In addition, it was reported that chickens with open field access had more protein in

their meat. It has been concluded that the meat of slow-growing chickens has a lower water holding capacity and is more crispy than fast-growing chicken meat.

Capan and Bagdatli (2021) investigated the physicochemical, microbiological, and sensorial properties of organic and conventional retail chicken meat. The organic chicken breasts had a higher fat content than conventional chicken breasts. The protein content of organic thighs was higher than that of conventional thighs. Organic chicken meat contains more mineral substances than conventional chicken meat and has a higher pH value, cooking loss, and water holding capacity. Alpha-linoleic and docosahexaenoic acids were found to be higher in organic chicken meat. *Salmonella* spp. was detected in all conventional chicken and 66.66% of organic chicken.

Conclusion

As a result, omega-3 and omega-6 fatty acids are found in higher amounts in organic chicken meat compared to conventional chicken meat. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are omega-3 fatty acids, are known to have positive effects on health. These fatty acids lead to important biochemical and physiological changes in the body. Omega-3 fatty acids are recommended for their beneficial effects in preventing and treating diseases such as heart disease, cancer, diabetes, and high blood pressure that affect human health. As a result of research other than fatty acids, it has been determined that organic chicken meat contains higher protein and lower fat than conventional chicken meat. As a result of the studies, it is seen that organic chicken meat is superior to conventional chicken meat not only in terms of fatty acid composition and protein content but also in many quality characteristics. Today, it is seen that consumers are turning to foods rich in low-fat, high-protein, and unsaturated fatty acids in their diets. For this reason, it is thought that the demand for organic chicken meat is increasing day by day.

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Conflict of Interest

The authors declare that they have no conflict of interest in this study.

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