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# Evaluation of hematological parameters, oxidative stress, Iron and unsaturated Iron binding capacity levels in Kangal shepherd dogs, before and after orchiectomy

## Evaluación de parámetros hematológicos, estrés oxidativo, niveles de capacidad de unión de hierro y hierro insaturado en perros pastores Kangal, antes y después de la orquiectomía

***Ibrahım Yurdakul[1], Barıs Atalay Uslu[2]****

*[1]Sivas Cumhuriyet University, Faculty of Veterinary Medicine, Department of Surgery. Sivas, Türkiye.*
*[2]Sivas Cumhuriyet University, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination. Sivas, Türkiye.*
**Corresponding Author: atalayuslu@hotmail.com*

**ABSTRACT**

Gonadectomy is a widely used method in reproductive health management, and behavior disorder therapies of domestic animals. Anemia and its development, one of the complications of surgical castration, which has been done so much recently in shelter dogs and dogs kept at home, has been investigated in male Kangal shepherd dogs. In castrated male Kangal shepherd dogs was to investigate the relationship between gonadectomy and oxidant and antioxidant levels, Iron (Fe) concentrations, unsaturated iron binding capacity (UIBC) and some blood parameters (RBC, HGB, HCT, MCV, RDW, MCHC). Blood samples were taken from 20 adult Kangal dogs brought to a clinic before castration (Day 0) and on the 1st, 3rd and 7th days. Some biochemical analyzes and blood parameters were evaluated in the blood samples taken. For this purpose, serum total oxidant–antioxidant capacity (TAC–TOC), Iron (Fe) concentrations, unsaturated iron binding capacity (UIBC) and some blood parameters (RBC, HGB, HCT, MCV, RDW, MCHC) were examined. In this study, it was evaluated the relationship between gonadectomy and oxidant–antioxidant capacity and Fe metabolism at some serum parameters in male dogs. After the orchidectomy operation, a remarkable decrease in clinical and statistical blood parameters was observed. As a result of this study, in the analysis of blood parameters, a severe picture of anemia was observed. It was determined an important role in erythropoiesis, with orchidectomy. The statistical difference in blood parameters ($P<0.05$) was indicative of this.

**Key words:** Antioxidant; Kangal shepherd dog; iron; oxidative stress; hematology

**RESUMEN**

La gonadectomía es un método ampliamente utilizado en el manejo de la salud reproductiva y en las terapias de trastornos del comportamiento de los animales domésticos. La anemia y su desarrollo corporal, son una de las complicaciones de la castración quirúrgica, que tanto se ha hecho recientemente en perros de refugio y en perros mantenidos en casa, se ha investigado en perros pastores Kangal machos. En perros pastores Kangal machos castrados se investigó la relación entre la gonadectomía y los niveles de oxidantes y antioxidantes, las concentraciones de hierro (Fe), la capacidad de unión al hierro insaturado (UIBC) y algunos parámetros sanguíneos (RBC, HGB, HCT, MCV, RDW, MCHC). Se tomaron muestras de sangre de 20 perros Kangal adultos traídos a una clínica antes de la castración (día 0) y en los días 1, 3 y 7. En las muestras de sangre tomadas se evaluaron algunos análisis bioquímicos y parámetros sanguíneos. Para ello, se examinaron la capacidad oxidante–antioxidante total en suero (TAC–TOC), las concentraciones de hierro (Fe), la capacidad de unión al hierro insaturado (UIBC) y algunos parámetros sanguíneos (RBC, HGB, HCT, MCV, RDW, MCHC). En este estudio, se evaluó la relación entre la gonadectomía y la capacidad oxidante–antioxidante y el metabolismo del Fe en algunos parámetros séricos en perros machos. Tras la operación de orquidectomía se observó una notable disminución de los parámetros sanguíneos clínicos y estadísticos. Como resultado de este estudio, en el análisis de parámetros sanguíneos se observó un cuadro severo de anemia. Se determinó un papel importante en la eritropoyesis, con orquidectomía. La diferencia estadística en los parámetros sanguíneos ($P<0,05$) fue indicativa de esto.

**Palabras clave:** Antioxidante; perro pastor Kangal; hierro; estrés oxidative; hematología

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## INTRODUCTION

Gonadectomy is a widely used method in reproductive health management, and behavior disorder therapies of domestic animals. It is referred to as castration in male mammalians that is the ending of most functions of external and internal reproductive organs by either extirpation or removing of testes from the body, or the closing of the canal system. Some of its positive effects can be count such as being more resistant to cancer and the immune system diseases compared to non-sterilized dogs [1]. However, negative major effects (obesity, infection, trauma, vascular diseases, and anemia) that are linked to castration should not be missed [2]. Anemia and decrease of hemoglobin levels have been shown by numerous studies as triggering effects for the majority of these negative effects [3].

Anemia is when the erythrocytes that carry the oxygen needed by the body are less than normal or the oxygen carrying capacity of the erythrocytes is low [4]. The low erythrocyte production in the organism is under the influence of many factors. One of them, Testosterone has a stimulating role in erythrocyte production [5]. It has been reported that a decrease in Testosterone production associated with bilateral orchidectomy may result in normocytic anemia in men [6]. Post–orchiectomy anemia is thought to be related to the hypothesis of this study and to the hypo–androgenic state.

Another key player in erythrocyte production, Fe is an essential element because of its necessity for the normal function of erythrocyte production, oxidative metabolism, and immune system [7]. Its deficiency affects erythrocyte status as a prominent effect by increasing oxidative stress [8]. As a result of this, increased oxidative stress causes lipid peroxidation in the cell membrane of erythrocytes and anemia occurs with broken erythrocytes imperiously [9]. On the parallel perspective, there is a close relationship between Fe and Testosterone. The Testosterone works with a negative feedback mechanism that mediates the production of erythrocytes. It enhances not only Fe absorption but also inhibits the production of Testosterone to protect Fe homeostasis when excessive amounts of iron are involved in the body [10]. There is a great balance between Testosterone, OS and Fe parameters in male dogs. An imbalance in any of these parameters is thought to occur as a result of sterilization.

The aim of this study was to investigate the relationship between blood parameters and Fe, UIBC, TAC and TOC in some male dogs after castration.

## MATERIAL AND METHODS

### Animal material

A total of 20 male dogs of same species and weights (20–30 kg, PNR, İstanbul, Turkey) were used in the study. Dogs were at the age of 2–5 years and brought to the clinics of Sivas Cumhuriyet University Veterinary Faculty Animal Hospital, Sivas, Turkey, by their owners. Dogs (*Canis lupus familiaris*) that undergoing castration operation were kept in the clinic for 7 days for post–operative care with the permission of the animal owners. During the study, daily care was performed according to the asepsis rules of the operation area to avoid postoperative infection formation. At the end of the day 7, the animals were delivered to the animal owners in good health.

The permission of the Sivas Cumhuriyet University Animal Experiments Local Ethics Committee was taken for this study design (Permission date and number: April 9, 2018, and 155).

### Operation

Dogs had been fasted and not allowed to access water pre–operation overnight. Afterward, they were sedated with Xylazine HCl (1.0 mg·kg$^{-1}$, Alfazyne 2%, Ege Vet, Turkey) and Ketamine (1.0 mg·kg$^{-1}$, Alfamine, Ege Vet, Turkey) intramuscularly. Dogs were placed on a surgical table in lateral recumbency, fixed, and surgical sites were shaved. Lidocaine HCl (6.0 mg·kg$^{-1}$, Jetokain, Adeka, Turkey) was administered to the testicles and incision site.

Operations were performed with an open castration method by using a scrotal incision. In the scrotal approach to castration, the scrotum was covered with a sterile fenestrated drape so that the testicles were visible. A single thick incision was made on the ventral surface of the testicle through the skin and subcutaneous tissue, just lateral to the median raphe, approximately one–third of the length of the testicle. After tying knots 4 times with absorbable 2/0 suture material to create complete hemostasis around both the ductus deferens and the vascular cord, the cord was cut. The same procedure was applied to the remaining testicle. After the testicles were removed, the scrotum was closed with a non–absorbable 3/0 synthetic monofilament suture so that it did not close completely. After surgery, the area was cleaned and an e–collar was applied to prevent the dog from licking or tackling the wound. Postoperative analgesia was provided with 0.3 mg·kg$^{-1}$ Meloxicam (Anaflex®, Hektaş, Turkey) administered SC for 3 days. Additionally, all dogs were administered Procaine Penicillin G (20,000 IU·kg$^{-1}$ IM) for 7 days after surgery.

### Collection of blood samples

Blood samples were collected from all the dogs five times during the experiment. Blood samples were taken on the day 0 (as control), 1st, 3rd and 7th days before surgery. Blood samples were taken into 5 mL sterile tubes without anticoagulants from the dogs' Vena cephalica antebrachii. Samples were kept at room temperature for 30 min, then centrifuged (Nüve NF 800, Nüve Laboratory & Sterilization Technology,Turkey) at 3000 G·15 min$^{-1}$. The sera obtained were stored at -20 °C (Haier, DW–86L828S, China) until analysis.

### Hematology

After all clinical examinations, 5 mL of blood samples were taken from the Vena cephalica antebrachi of the dogs to the tubes with anticoagulant and without anticoagulant once before castration. In the blood samples with Ethylenediaminetetraacetic acid (EDTA) the levels of erythrocyte (RBC), hemoglobin (HGB), hematocrits (HCT), mean corpuscular volume (MCV), red cell distribution width (RDW) and mean corpuscular hemoglobin concentration (MCHC) were determined by a hematological analyzer (BC2800 Vet hematology analyzer, Mindray Bio–Medical Electronics Co. Ltd., Nanshan, Shenzhen, Chine).

### Serum biochemical analysis

Serum total antioxidant capacity (TAC, Rel Assay Diagnostics kit; Mega Tıp, Gaziantep, Turkey) and total oxidant (TOC, Rel Assay Diagnostics kit; Mega Tıp, Gaziantep, Turkey) capacity levels were determined according to the procedure of manufacturers' directories. The optical density was determined using a microplate reader (Multiskan GO, Thermo Scientific, USA). Serum Fe and unsaturated iron–binding capacity (UIBC) levels were determined using an auto–analyzer (Abbot C8000–USA) using commercial kits.

### Statistical analysis

The sample size was determined as 20 dogs as a result of the power analysis before starting the study. In the power analysis, type 1 error probability ($\alpha$), power (1-$\beta$) and effect size (f) were taken 0.05, 0.80 and 0.40, respectively. G * Power Version 3.1.9.2 statistical program was used for power analysis.

Descriptive statistics for each variable were calculated and presented as "Mean ± Standard Error of Mean". Before performing the statistical analysis, data were examined with the Shapiro–Wilk test for normality assumptions. To test the differences in each parameter between time sampling, the Friedman test was used for repeated measures. The relationship between parameters was analyzed with the Spearman correlation coefficient. For all analyses, SPSS® 22.0 (IBM, New York, USA) for Windows was used and $P$<0.05 was considered as significant.

## RESULTS AND DISCUSSION

Descriptive statistics (Mean ± Standard Error of Mean) of serum and blood parameters in relation to castration in male dogs are presented in TABLES I and II, and FIG. 1. Serum TAC ($P$=0.494), TOC ($P$=0.668), Fe ($P$=0.187) and UIBC ($P$=0.229) values showed a non-significant ($P$>0.05) difference over time and non -significant correlations between each other except Day 0; TAC and TOC levels (r=0.0842, $P$=0.002) [TABLE II, FIG. 1].

***TABLE I***
**Descriptive statistics of serum TAC (mmol Trolox equivalent·$L^{-1}$), TOC (µmol $H_2O_2$ Eq·L $unit^{-1}$), Fe (ng·$mL^{-1}$) and UIBC (µg·$dL^{-1}$) levels in the pre and post castrated dogs (Mean±SEM)**

| | Preoperation | Postoperation | | | *P*-value |
|---|---|---|---|---|---|
| | Day 0 | Day 1 | Day 3 | Day 7 | |
| TAC | 1.446±0.084 | 1.403±0.063 | 1.502±0.032 | 1.520±0.059 | 0.494 |
| TOC | 8.590±2.708 | 8.850±2.257 | 5.622±1.182 | 8.115±2.365 | 0.668 |
| Fe | 130.680±12.990 | 115.520±7.240 | 101.990±5.900 | 109.860±10.350 | 0.187 |
| UIBC | 151.320±17.580 | 168.080±17.490 | 181.240±18.850 | 172.400±16.320 | 0.229 |

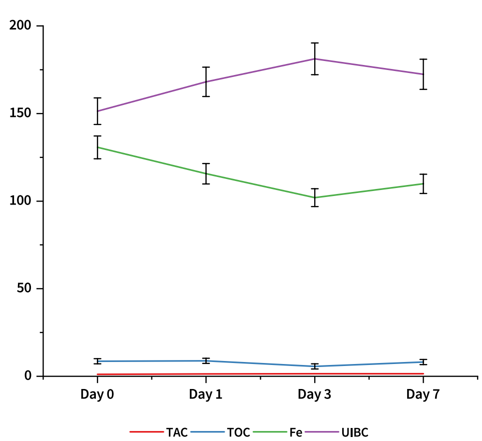


**FIGURE 1. Serum TAC (mmol Trolox equivalent·$L^{-1}$), TOC (µmol $H_2O_2$ Eq·L $unit^{-1}$), Fe (ng·$mL^{-1}$) and UIBC (µg·$dL^{-1}$) levels in the pre and post castrated dogs (Mean±SEM)**

It is well known that Testosterone has anabolic and androgenic effects on the organism. While anabolic activity plays an important role in the development of muscles, androgenic effect responses development of primary (growth and development of reproductive organs, the continuation of spermatogenesis) and secondary (sex characteristics, thickening of the voice, rate of gain, feed conversation, and the development of somebody regions, among others) [11]. Additionally, Testosterone has been reported to reduce development and growth, increase stress levels, suppress the immune system, and increase the risk of death [12]. For this purpose, it is important that analyze the effects of the absence of Testosterone on various body parameters to evaluate the pros and cons of gonadectomy. In this study, it was evaluated the relationship between gonadectomy and oxidant-antioxidant capacity and Fe metabolism at some serum parameters in male dogs.

After the orchidectomy operation, a remarkable decrease in clinical and statistical blood parameters was observed [TABLE III]. It is thought that a sudden androgen decline occurs with orchiectomy and blood production is disrupted as a result. Among the main factors reported by researchers for erythropoiesis, androgens are of great importance [13]. Hematological parameters [14], which have been determined to be decreased with many disease states, have been associated with the use of androgenic steroids in athletes [15]. The hypothesis of the current study is that a decrease in hemoglobin concentration follows the apparent androgen deprivation that occurs after castration. It is clear from measurements that blood parameters tend to drop suddenly after Day 1.

Surgical procedures performed on any part of the body cause undesirable changes in normal body homeostasis, and even surgical traumas increase the TOC level alone [16]. In dogs, after an ovariectomy operation [17], an increase was determined in plasma TOC levels on the first post-operation day, which indicated that the oxidative stress because of anesthesia and surgical trauma. In another study, lipid peroxidation levels were found high on the first day after the operation and lipid peroxidation levels were low on Days 3, 5, and 7 [18]. Contrary to previous studies performed by Mahalingam *et al.* [18] and Lee and Kim [17], the obtained results showed relative stability in levels of TOC. TOC and TAC levels on post-operation 1. Day was similar to pre-operation levels. Except Day 0, a significant difference could not be found between serum TAC and TOC levels was found ($P$>0.05), [TABLE II]. Besides, when the serum TAC levels were examined in terms of the time change [TABLE I, FIG. 1], it was determined that there was not a significant difference ($P$>0.05). Similarly, it was determined that the changes in serum TOC levels over time are not significant ($P$>0,643) [TABLE I, FIG. 1]. These unexpected oxidative and anti-oxidative status stability could be highly related to the difficult determination of post-operative conditions on intra and extracellular inflammatory response mechanisms from blood samples [3, 16, 17].

Environmental factors, various diseases, and stress can alter the results of the anti-oxidative status of the organism [19]. Stress consists of three stages, alarm, reaction, and exhaustion stages. Oxidative stress could affect by this complexity of stress [20, 21]. The results of the present study showed no difference over time and they followed a stable line. This phenomenon could be explained by stress caused by operation, injection, or collection of blood samples.

In studies conducted in rats (*Rattus norvegicus*) [22] and rabbits (*Oryctolagus cuniculus*) [23] reported that high levels of Testosterone

***TABLE II***

**Spearman's correlation coefficients (rs), describing the relation between TAC (mmol Trolox equivalent·$L^{-1}$), TOC (µmol $H_2O_2$ Eq·L unit-1), Fe (ng·$mL^{-1}$) and UIBC (µg·$dL^{-1}$) levels in the pre and post castrated dogs**

| | **Day 0** | | **TAC** | **TOC** | **Fe** | **UIBC** |
|---|---|---|---|---|---|---|
| | | Correlation Coefficient | 1.000 | | | |
| | **TAC** | Sig. (2–tailed) | | | | |
| | | n | 10 | | | |
| | | Correlation Coefficient | 0.842** | 1.000 | | |
| | **TOC** | Sig. (2–tailed) | 0.002 | | | |
| | | n | 10 | 10 | | |
| Spearman's rho | | Correlation Coefficient | 0.006 | 0.455 | 1.000 | |
| | **Fe** | Sig. (2–tailed) | 0.987 | 0.187 | | |
| | | n | 10 | 10 | 10 | |
| | | Correlation Coefficient | -0.115 | -0.430 | -0.600 | 1.000 |
| | **UIBC** | Sig. (2–tailed) | 0.751 | 0.214 | 0.067 | |
| | | n | 10 | 10 | 10 | 10 |
| | **Day 1** | | **TAC** | **TOC** | **Fe** | **UIBC** |
| | | Correlation Coefficient | 1.000 | | | |
| | **TAC** | Sig. (2–tailed) | | | | |
| | | n | 10 | | | |
| | | Correlation Coefficient | 0.539 | 1.000 | | |
| | **TOC** | Sig. (2–tailed) | 0.108 | | | |
| | | n | 10 | 10 | | |
| Spearman's rho | | Correlation Coefficient | 0.236 | 0.370 | 1.000 | |
| | **Fe** | Sig. (2–tailed) | 0.511 | 0.293 | | |
| | | n | 10 | 10 | 10 | |
| | | Correlation Coefficient | -0.212 | -0.018 | -0.297 | 1.000 |
| | **UIBC** | Sig. (2–tailed) | 0.556 | 0.960 | 0.803 | |
| | | n | 10 | 10 | 10 | 10 |
| | **Day 3** | | **TAC** | **TOC** | **Fe** | **UIBC** |
| | | Correlation Coefficient | 1.000 | | | |
| | **TAC** | Sig. (2–tailed) | | | | |
| | | n | 10 | | | |
| | | Correlation Coefficient | 0.006 | 1.000 | | |
| | **TOC** | Sig. (2–tailed) | 0.987 | | | |
| | | n | 10 | 10 | | |
| Spearman's rho | | Correlation Coefficient | 0.164 | -0.430 | 1.000 | |
| | **Fe** | Sig. (2–tailed) | 0.651 | 0.214 | | |
| | | n | 10 | 10 | 10 | |
| | | Correlation Coefficient | -0.212 | -0.018 | 0.091 | 1.000 |
| | **UIBC** | Sig. (2–tailed) | 0.556 | 0.960 | 0.803 | |
| | | n | 10 | 10 | 10 | 10 |
| | **Day 7** | | **TAC** | **TOC** | **Fe** | **UIBC** |
| | | Correlation Coefficient | 1.000 | | | |
| | **TAC** | Sig. (2–tailed) | | | | |
| | | n | 10 | | | |
| | | Correlation Coefficient | -0.018 | 1.000 | | |
| | **TOC** | Sig. (2–tailed) | 0.960 | | | |
| | | n | 10 | 10 | | |
| Spearman's rho | | Correlation Coefficient | -0.261 | 0.006 | 1.000 | |
| | **Fe** | Sig. (2–tailed) | 0.467 | 0.987 | | |
| | | n | 10 | 10 | 10 | |
| | | Correlation Coefficient | 0.564 | 0.200 | -0.661* | 1.000 |
| | **UIBC** | Sig. (2–tailed | 0.090 | 0.580 | 0.038 | |
| | | n | 10 | 10 | 10 | 10 |

*: Correlation is significant at the 0.05 level (2–tailed). **: Correlation is significant at the 0.01 level (2–tailed)

*TABLE III*
**Hematological parameters before and after surgery in castrated dogs**

| | Preoperation | Postoperation | | |
|---|---|---|---|---|
| | **Day 0** | **Day 1** | **Day 3** | **Day 7** |
| RBC ($\times 10^{12} \cdot L^{-1}$) | 5.6±0.96[a] | 4.7±0.82 | 3.0±0.81 | 3.6±1.73[a] |
| HGB ($g \cdot dL^{-1}$) | 14.5±0.97 | 12.5±1.78[a] | 9.0±0.94 | 10.1±1.96[a] |
| HCT(%) | 44.9±2.46 | 40.7±2.45 | 31.5±4.4 | 30.4±4.32 |
| MCV(fL) | 73.1±3.72[a] | 59.9±3.6[b] | 43.0±3.29[ac] | 51.2±9.44[bc] |
| RDW (%) | 14.9±0.73 | 11.4±1.26 | 9.4±1.50 | 9.7±3.02 |
| MCHC ($g \cdot dL^{-1}$) | 33.0±1.56[a] | 22.7±4.57 | 20.3±2.75 | 22.1±3.54[a] |

[a,b,c]: There is a statistically significant difference (*P*<0.05) for lines bearing the same letter, RBC: Erythrocyte, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean Corpuscular Volume, RDW: Red Cell Distribution Width, MCHC: Mean Corpuscular Hemoglobin Concentration

increased oxidation levels in testicular tissues and decreased antioxidant levels such as SOD, CAT, GSH-Px and GSH. In another study [21]. that supports this information; It has been reported that antioxidant levels such as SOD and GSH-Px decreased compared to the control group, and the reason for the decrease in these antioxidant levels was that testosterone deficiency disrupted antioxidant defense mechanisms. In this study, similar serum TOC and TAC levels were assessed at 0th, 1st, 3rd and 7th days. On the post-operation 1st, 3rd and 7th days, decreased serum TOC levels could be interpreted as a decrease in the risk of OS for the elimination of Testosterone hormone secretion from testicles, mainly because of the elimination of the inducing effect of Testosterone on ROS. However, this study is in a contrast with the results of some studies [11, 12, 24]. The balance in serum TAC levels (FIG. 1) can be interpreted as a decrease in oxidative stress because of a decrease in Testosterone concentration and an increase in the antioxidant defense activities of the cells in response to harmful effects such as lipid peroxidation/oxidation in the membranes caused by ROS.

In this study, serum Fe and UIBC levels were examined also. It was not found a significant correlation except Day 7 (r= -0.661, *P*<0.05) between serum Fe and UIBC levels [TABLE II]. Additionally, changes of serum Fe and UIBC levels during the time were analyzed and the relation was similar to other parameters and there was not a significant difference (*P*>0.05) [TABLE II, FIG. 1] also determined. Fe is a particularly important element because of its necessity for the normal function of erythrocyte production. There is a close relationship between Fe and Testosterone. It is working with Testosterone during the production of erythrocytes by a negative feedback mechanism [10]. Fe deficiency is reduced in Testosterone deficiency and is one of the most important causes of anemia [25]. It has been shown that there is a significant decrease in erythrocyte and hemoglobin concentration in the elderly or the littered male animals, parallel to the decrease in Testosterone level [26]. It has also been shown that in castrated male animals, blood parameters can be normalized by the administration of androgens in the presence of anemia [25, 27]. Because of all these stimuli, serum Fe concentration is also expected to increase due to the need for absorption and consumption. Contrary to the work of Shahidi [25], a significant decrease in serum levels of Fe was not detected in parallel with the decrease of Testosterone hormone secreted from testes in this study.

## CONCLUSION

As a result, it was determined by measurements that UIBC increased and Fe ratio decreased due to the sudden decrease in androgens after orchiectomy. In conditions such as inflammation and anemia, serum Fe level decreases and UIBC increases. The statistical difference in blood parameters (*P*<0.05) is an indication of this. Although these values try to return to normal as the inflammation heals in the future, they will not be able to return due to long-term androgen deficiency and anemia will occur. Orchiectomy-induced anemia is a process that can be explained by androgens that support the hematopoiesis mechanism. Although the data obtained in this study prove that orchiectomy is slightly associated with anemia, the relationship between orchiectomy and anemia will be revealed more clearly in future studies with more animals, more parameters and longer periods of time. In addition, it is thought that different results may occur due to difficulties in determining serum TAC, TOC and Fe levels in dogs, and the relationships between these parameters can be clarified with clinical observations and longer studies.


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### Ethical statement

This study was approved by the Sivas Cumhuriyet University Animal Experiments Local Ethics Board.

### Conflict of ınterest

The authors declare that they have no conflict of interest. Statement of Animal Rights all applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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