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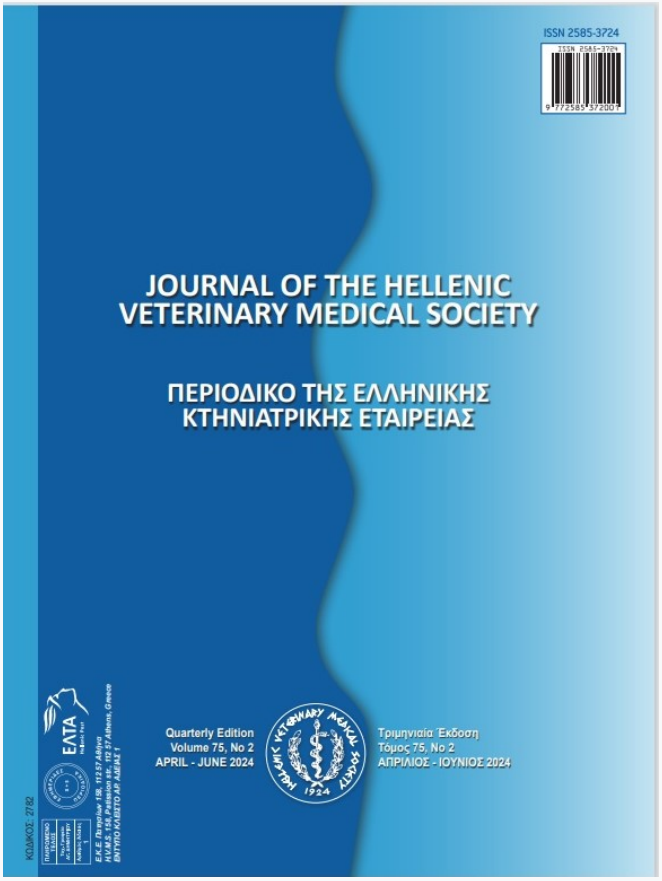

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Research article
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Redox Balance in Van Cats and It's Association with Age, Gender and Eye Color

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ABSTRACT: Objectives: The aim of this study was to determine the oxidative stress index and redox balance in healthy Van cats and to evaluate it's association with gender, age and eye color. **Methods:** 80 healthy Van cats from different eye color, gender and age were used in the study. All cats were grouped according to their age (A) as <12 months (A1, n=30), ≥1 and <3 years (A2, n= 22), ≥3 and <5 years (A3, n=12) ve ≥5 years (A4, n= 16); eye color (E) from left to right as blue-blue (E1, n=31), blue-amber (E2, n= 19), amber-blue (E3, n= 12), amber-amber (E4, n= 19) respectively and gender (G) as female (G1, n=43) and male (G2, n=37). Blood serum reactive oxygen species (ROM) and serum antioxidant power (PAO) analyzed and oxydative stress index (OSI) was measured in all groups. **Results:** There was no statistically significant difference between age, gender and eye color regarding ROM, PAO and OSI in healthy Van cats. Mean ROM (H2O2/dL), PAO (HClO/ml) and OSI CarrU/(µmol HClO/ml)) were 1.79, 434.8 and 0.05, respectively (P>0.05). **Conclusions:** Since oxidative stress plays a critical role as a biomarker of various diseases, determination of redox balance may provide a useful tool in healthy Van cats. It was observed that ROM, PAO and OSI were not affected by age, gender and eye color in healthy Van cats. Breed differences among cat population may lead to dramatic changes in redox balance.

Keywords: ROM; PAO; Van cats; Redox balance; OSI

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INTRODUCTION

Reactive oxygen metabolites (ROM) are chemically active free radicals that play an important role in numerous biological processes (Castro et al., 2021). ROM's overproduction causes various damages in organism such as protein and DNA oxidation, lipid peroxidation and cellular death (Lu et al., 2009; Krumova and Cosa, 2016; Purohit et al., 2019). Endogenous factors such as electron leakage from electron transport chains, cytochrome P450, peroxisomes, lipoxygenases, cyclooxygenases, xanthine oxidase, nicotinamide adenine dinucleotide phosphate oxidase (NADPH) and various inflammatory cells in the endoplasmic reticulum; exogenous factors such as UV light, drugs and cigarette smoke induce ROS formation (McMichael, 2007; Celi and Gabai, 2015). The increased production of ROM lead to activation of various antioxidant mechanisms in cells. These mechanisms also include antioxidant proteins such as albumin, haptoglobin, ferritin and ceruloplasmin; enzymatic antioxidants such as superoxide dismutase (SOD), catalase and glutathione peroxidase; water-soluble antioxidants such as ascorbic acid, uric acid, bilirubin, glutathione, zinc and selenium; and fat-soluble antioxidants such as tocopherols, β-carotene, coenzyme Q10 and lycopene (Halliwell, 1999). Neutralization capacity of those antioxidants is exceeded, the imbalance between oxidant and antioxidants causes oxidative stress (Celi et al., 2010).

It is known that the reactions mediated by ROM and free radicals cause oxidative damage to many biomolecules such as lipid, protein, DNA and eventually leading to many diseases (Huang et al., 2007). Due to the fact that oxidative stress plays a critical role in the pathogenesis of various diseases, considerable studies on the measurement of free radicals and their metabolites has been reported (Lefer and Granger, 2000; Iamele et al., 2002; Jenner, 2003; Madamanchi et al., 2005). Similarly, there are many studies in veterinary medicine investigating the relationship between various diseases and oxidative stress in cats and dogs (Sakallioglu et al., 2005; Brown, 2008; Webb and Twedt, 2008; Verk et al., 2017; Candellone et al., 2019). Furthermore, reference values of many oxidative stress parameters such as Malondialdehyde (MDA), SOD and Catalase (CAT) in healthy cats and dogs were determined (Todorova et al., 2005). Total antioxidant capacity (PAO) is defined as the sum of nonspecific antioxidant activities. It is used as a parameter measuring the resultant force counteracting undesired oxidation in a material (Bartosz, 2003). The oxidative stress index (OSI) has been used both as a biomarker in many diseases in humans and veterinary medicine and to determine the imbalance between PAO and ROM levels (Rabus et al., 2008; Izuta et al., 2010; Abuelo et al., 2013; Baltacıoğlu et al., 2014; Bottegaro et al., 2018)

Turkish Van cats are known to have lived in the Van Lake region in eastern Turkey for centuries. One of the characteristic features of Van cats, whose species are in danger of extinction, is eye color. According to eye color, they are divided into three groups as blue-blue, yellow-yellow, and blue-yellow (Cak, 2017). Besides the studies on Van cats are quite limited, there is no study evaluating the redox balance in Van cats. In this study, it was aimed to determine the oxidative stress index and redox balance in healthy Van cats and to evaluate it's association with gender, age and eye color.

MATERIAL-METHOD

Animals and Study Design

The study was carried out at Istanbul University-Cerrahpaşa, Faculty of Veterinary Medicine, Internal Medicine department and Van Yüzüncü Yıl University, Faculty of Veterinary Medicine, Van Cat Research Center and Perugia Zooprophylaxis Institute with the ethical approved obtained by Van Yüzüncü Yıl University, Local Ethics Committee. 80 Van cats from different eye color, gender and age were used in the study. All cats were grouped according to their age (A) as <12 months (A1, n=30), ≥1 and <3 years (A2, n= 22), ≥3 and <5 years (A3, n=12) ve ≥5 years (A4, n= 16); eye color (E) from left to right as blue-blue (E1, n=31), blue-amber (E2, n= 19), amber-blue (E3, n= 12), amber-amber (E4, n= 19) respectively and gender (G) as female (G1, n=43) and male (G2, n=37). Fasting blood samples were taken from jugular vein and centrifuged at 1500 rpm for 10 minutes. Serum samples were transferred to the eppendorfs and stored at -20 °C for about 3 months until ROM and PAO measurements were made.

In the study, all animals were fed with similar commercial dry food and hosted in Van Cat Research Center. They were routinely administered endoparasite and ectoparasite treatments and vaccines. Physical and laboratory examinations of all cats were assessed and only healthy cats were included in the study. Accordingly, serum biochemistry and hemogram analyzes were performed with the FUJI DRI-CHEM NX600V and VH5R Hemogram Device. Cats

whose blood values were between the reference values shown in Table 1 were included and using any medication or supplements were excluded from the study.

Informed consent has been obtained for client-owned animals included in this study.

ROM measurement

Free radicals (FRs) determination system (D-Roms test, Diacron; Grosseto, Italy) was used in this study. This test is based on the ability of transition metals to catalyse in the presence of peroxides with formation of FRs, which are trapped by an alchilamine. The alchilamine reacts forming a coloured radical detectable at 505 nm. ROM were assayed using the spectrophotometric d-ROM test (Diacron International, Oxidative Stress index Grosseto, Italy), which determines hydroperoxides (breakdown products of lipids as well as of other organic substrate, generated by the oxidative attack of ROS) through their reaction with the chromogen N, N-diethylparaphenylenediamine. The components of the ROM's kit are:

- R1 reagent that is a Chromogen mixture (alkyl-substituted aromatic amine)
- R2 reagent that is Acetate Buffer (pH 4,8) to preserve and stabilize
- Calibrator
- Lyophilized serum

Serum samples were used and calibrator was reconstituted with 1 mL of freshly distilled water. According to the end-point mode, the following procedure was performed (Table 2).

All the solutions were delicately mixed and after 90 min of incubation at 37 °C they underwent immediately photometric reading, by measuring their absorbance at 505 nm. The absorbance value of reagent blank was subtracted from that the calibrator and the samples. The intensity of color change in the sample solution is directly proportional with the concentration of ROMs in that sample, according to Lambert-Beer's law. The results were expressed as mg H2O2/dL.

PAO measurement

Serum antioxidant power was measured using the Oxi-adsorbent test (Diacron, Grosseto, Italy). Reaction with an alkyl-substituted aromatic amine produces a pink-colored product that can be detected spectrophotometrically. The concentration of the colored complex is directly proportional to the concentration of HClO and indirectly proportional to the antioxidant capacity. The results were expressed as mol of HClO consumed by 1 ml of serum sample (μmol HClO/ml).

Table 1. Reference values for hematology and serum biochemistry in laboratory analyzes performed in Van cats included in the study (FUJI DRI-CHEM NX600V, VH5R Hemogram Device)

Parameters	Reference Values
WBC (10^9/L)	5.50 - 19.50
RBC (10^{12}/L)	4.60 - 12.00
PLT (10^9/L)	100.00 - 518.00
ALP (U/l)	9.00 - 53.00
ALT (U/l)	22.00 - 84.00
GGT (U/l)	1.00 - 10.00
AMY (U/l)	200.00 - 1900.00
LIPAZ (U/l)	0.00 - 200.00
BUN (mg/dL)	17.60 - 32.80
CREA (mg/dL)	0.80 - 1.80
TP (g/dL)	5.70 - 7.80
TBIL (mg/dL)	0.10 - 0.40
GLU (mg/dL)	71.00 - 148.00

Table 2. The amount of solutions used in ROM test

	Reagent blank	Calibrator	Sample
R2 Reagent	1 ml	1 ml	1 ml
R1 Reagent	10μl	10μl	10μl
Distilled water	5μl	-	-
Calibrator	-	5μl	-
Sample	-	-	5μl

Values lower than 350 μmol HClO/ml suggest a reduced antioxidant capacity. Sample was mixed with HClO solution (one of the powerful reactive oxygen species emitted by leukocytes). HClO subjects the sample to massive oxidation; the antioxidant substances contained in the sample react with the acid and can be quantified by measuring the excess of HClO. Quantification of the unreacted acid was carried out by the spectrophotometric method (reading at λ = 490 nm), after addition of suitable buffered chromogenic agent, an aromatic alkyl diamine (N, N-diethyl-para-phenylendiamine). The color concentration was directly proportional to the concentration of HClO and was indirectly related to the antioxidant capacity (Table 3).

Solutions were kept at 37° C for 10 min. After adding 10μl of R2 reagent (chromogenic mixture) in each assay, all the solutions underwent immediatly photometric reading, by measuring their absorbance at 505 nm. The results were expressed as μmol of HCLO/ml of sample.

OSI calculation

The OSI was calculated as ROM/PAO and expressed as CarrU/(μmol HClO/ml). For this, the ROM values were converted to Carratelli Units (CARR U) according to the following formula:

CARR U = Abs sample/Abs calibrator X [calibrator]

1 CARR U = 0,08 mg H2O2/dL

where: Abs sample and Abs calibrator are the measured absorbance values (for the sample and the calibrator, respectively). The method is linear up to 500 CARR U (Table 4).

RESULTS

Results of ROM test were expressed as mg H2O2/dL and the results of PAO test are expressed as μmol HCLO/ml. OSI value was calculated by converting the ROM values to CarrU units and expressed as CarrU/(μmol HClO/ml). Accordingly, the mean ROM values in A1, A2, A3 ve A4 groups were measured as 1.76, 1.96, 1.53 and 1.78 respectively (mg H2O2/dL). In the same group, PAO values were 425.6, 430.2, 439.2, 438.4, respectively (μmol HClO/ml). Accordingly, there was no statistically significant difference in ROM and PAO values for all age groups (P>0.05). The mean OSI values were 0.07, 0.06, 0.03 and 0.05, respectively (CarrU/(μmol HClO/ml)) (Table 5).

Table 3. The amount of solutions used in PAO test

	Reagent blank	Calibrator*	Sample*
R1 Reagent	1 ml	1 ml	1 ml
Distilled water	10μl	-	-
Calibrator*	-	10μl	-
Sample*	-	-	10μl

*Diluite both solution 1:100 with distilled water

Table 4: ROM values conversion as CARR U and mg H2O2/dL

ROM (CARR U)	ROM (mg H2O2/dL)
300-320	24.08-25.60
321-340	25.68-27.20
341-400	27.28-32.00
401-500	32.08-40.00
>500	>40.00

Table 5. Mean ROM and PAO values in Van cats grouped by age

	ROM (mg H2O2/dL)		PAO (μmol HClO/ml)		OSI (CarrU/(μmol HClO/ml))	
Groups	M±SE	Min-Max	M±SE	Min-Max	M±SE	Min-Max
A1 (n=30)	1.76 ±0.12	1.51-2.01	425.6±8.33	409-442.2	0.07±0.01	0.003-0.61
A2 (n=22)	1.96 ±0.15	1.66-2.26	430.2±10.03	410.2-450.1	0.06±0.02	0.03-0.09
A3 (n=12)	1.53 ±0.19	1.15-1.92	439.2±12.87	413.6-464.9	0.03±0.02	0.02-0.07
A4 (n=16)	1.78 ±0.16	1.45-2.1	438.4±11.08	416.3-460.5	0.05±0.02	0.02-0.08

M ± SE: Mean ± standard error, OSI: Oxidative stress index, ROM: Reactive oxygen metabolites, PAO: Antioxidant capacity, y.o: years old

A1: <12 months, A2: ≥1 and <3 y.o, A3: ≥3 and <5 y.o, A4: ≥5 y.o

The mean ROM values for E1, E2, E3 and E4 were 1.71, 1.95, 1.68 and 1.69, respectively (mg H2O2/dL); mean PAO values were measured as 443.4, 438.5, 415.8 and 440.5, respectively (µmol HClO/ml). In these groups, it wasn't detected a significant difference (P>0.05) for both ROM and PAO. However, PAO was tended to be lower for the E3 compared to the other eye color groups. The mean OSI values were 0.04, 0.05, 0.07, and 0.04, respectively (CarrU/(µmol HClO/ml)) (Table 6).

The mean ROM values measured in the G1 and G2 groups were 1.77 and 1.72, respectively (mg H2O2/dL) and the mean PAO value was 426.8 and 439.9, respectively (µmol HClO/ml). There was no significant difference between genders for ROM and PAO (P>0.05). The mean OSI values for males and females were 0.06 and 0.04 respectively (CarrU/(µmol HClO/ml)) (Table 7).

The p values were given in Table 8. Accordingly, ROM and PAO didn't significiantly differ in each parameters. However, PAO was tended to be significant for group E (p=0.056) (Table 8).

In healthy Van cats, mean ROM (H2O2/dL), PAO (µmol HClO/ml) and OSI (CarrU/(µmol HClO/ml)) values were 1.79 (0.11-3.40), 434.8 (50.9-456.7) and 0.05 (0.003-0.61), respectively (Table 9).

Table 6. Mean ROM, PAO and OSI values in Van cats grouped by eye color

	ROM (mg H2O2/dL)		PAO (µmol HClO/ml)		OSI (CarrU/(µmol HClO/ml))	
Groups	**M±SE**	**Min-Max**	**M±SE**	**Min-Max**	**M±SE**	**Min-Max**
E1 (n=31)	1.71±0.12	1.47-1.95	443.4±10.50	422.4-464.5	0.04±0.01	0.003-0.09
E2 (n=19)	1.95±0.15	1.64-2.26	438.5±16.28	405.9-471.1	0.05±0.02	0.02-0.09
E3 (n=12)	1.68 ±0.19	1.30-2.06	415.8±15.04	385.7-446	0.07±0.02	0.02-0.61
E4 (n=18)	1.69±0.15	1.38-2.01	440.5±12.86	414.7-466.3	0.04±0.02	0.02-0.08

(M ± SE: Mean ± standard error, OSI: Oxidative stress index, ROM: Reactive oxygen metabolites, PAO: Antioxidant capacity, E1: blue-blue, E2: blue-amber, E3: amber-blue, E4: amber-amber)

Table 7. ROM, PAO and OSI values in Van cats grouped by gender

	ROM (mg H2O2/dL)		PAO (µmol HClO/ml)		OSI (CarrU/(µmol HClO/ml))	
Groups	**M±SE**	**Min-Max**	**M±SE**	**Min-Max**	**M±SE**	**Min-Max**
G1 (n=43)	1.77±0.13	1.49-2.04	426.8±7.10	412.6-441	0.065±0.014	0.003-0.61
G2 (n=37)	1.72±0.16	1.39-2.05	439.9±7.80	424.3-455.4	0.046±0.017	0.01-0.09

(M ± SE: Mean ± standard error, OSI: Oxidative stress index, ROM: Reactive oxygen metabolites, PAO: Antioxidant capacity, G1: female, G2: male)

Table 8. *P* values for gender, age group and eye color parameters

PARAMETERS	ROM (*p*)	PAO (*p*)
Gender	0.832	0.211
Age	0.672	0.717
Eye color	0.749	0.056

Table 9. ROM, PAO and OSI values in healthy Van cats

	Mean (n=80)	Minimum	Maksimum	Standart error
ROM (H2O2/dL)	1.79	0.11	3.40	0.64
PAO (HClO/ml)	434.8	50.9	456.7	44.3
OSI (CarrU/(µmol HClO/ml))	0.05	0.003	0.61	0.01

DISCUSSION

Van cats are originated from Van lake in Turkey (Lipinski et al., 2008). They have a long white body, semi-long hair and characteristically different eye colors as blue-blue, yellow-yellow, blue-yellow or yellow-blue. Considering gradually decrease in their numbers worldwide, they are a breed at risk of extinction. Furthermore, studies on Van cats are very limited (Eksen et al., 1992; Altunok et al., 2011; Erat et al., 2012; Hakkı Nur et al., 2020). This is the first study that evaluate the oxidative stress parameters and redox balance in healthy Van cats.

In order to evaluate the oxidative stress due to an imbalance between oxidant and antioxidant substanc-

es in the body, it is a useful method to analyze the relationship between oxidant and antioxidant substance concentrations, rather than evaluating them separately. This method has been used as a biomarker in various diseases in human medicine and many veterinary practices (Sharma et al., 1999; Celi, 2011; Abuelo et al., 2013; Yesilova et al., 2013; Baltacıoğlu et al., 2014). It was reported that many infectious and non-infectious diseases incats were associated with redox unbalance (Tecles et al., 2015; Candellone et al., 2019; Michałek et al., 2020). In a study conducted with the cats diagnosed with feline infectious peritonitis, it was reported that total antioxidant capacity decreased in affected cats compare to healthy group (Tecles et al., 2015). Similarly it was showed that hyperthyroidism in cats increases the free radical production and leads to compare to healthy cats (Candellone et al., 2019). OSI has been used to diagnose oxidative stress in many disease conditions (Kolesnikova, 2014). In a study in cattles with Brucellosis, it was reported that SAC value was lower, total oxidant capacity and OSI values were found to be significantly higher compare to healthy group (Merhan et al., 2017). In our study, reference OSI values were calculated by evaluating the mean oxidative load and antioxidant defense status in healthy Van cats. In a previous study evaluating the OSI ratio in healthy cats without any breed difference the mean ROM (CarrU) value was 155.9 (Castillo et al., 2013). This result is inconsistent with our mean ROM (CarrU) measurement (22.375) which may suggests that the ROM values may be affected by various factors such as cat breeds. However in the same study, mean serum antioxidant capacity (SAC) was 384.2 ± 25.1 which is consistent with our results (434.8 ± 44.3). Consequently, antioxidant status seems similar in healthy Van cats and healthy mixed breeds. In the study, it was also reported that the male cats are at oxidative risk comparing with the females (Castillo et al., 2013).

In veterinary medicine redox balance were also evaluated in different animal species such as cattles, horses and dogs (Lykkesfeldt and Svendsen, 2007; Pasquini et al., 2008; Panda et al., 2009; Abuelo et al., 2013; Candellone et al., 2022; Pugliese et al., 2022). In a study investigating the redox status in cows, mean ROM value (135.6 CarrU) was greater than our results while SAC (480 mmol HClO/ml) was measured as similar values (Abuelo et al., 2013). In a study investigating OSI in race horses, ROM values related to race distance and difficulty were greater than our results (Bottegaro et al., 2018). It is also stated that the d-ROM concentrations in horses were significantly higher ($p<0.001$) than in the other animals (Shono et al., 2020). All of these studies suggest that ROM values and redox status may differ between animals.

There are many human studies (Mezzetti et al., 1996; Taddei et al., 2001; Baumann et al., 2016; Luo et al., 2020;) evaluating the relationship between oxidative stress and age. It was reported that ageing is related to decrease in antioxidants and increase in oxidative stress components in human medicine (Junqueira et al., 2004) and suggested that oxidative stress has an important role in the pathogenesis of many age-related diseases (Baumann et al., 2016; Liguori et al., 2018; Luo et al., 2020). In a study investigating the relationship between ageing and oxidative stress markers in human, it was reported that total antioxidant capacity tented to decrease with age (Mendoza-Núñez et al., 2007). In veterinary medicine, it was reported that the older dogs had greater oxidative stress parameters such as MDA, SOD and glutathione peroxidase (GSH-Px) (Vajdovich et al., 1997). In the study using thoroughbred racehorses (Kusano et al., 2016), higher serum antioxidant potential and lower OSI were reported in low age of female horses. A similar study suggested that there is a significant negative correlation between age and serum antioxidant potential of older adult human (Pesce et al., 2018). In the study of Castillo et al. (2013), it was reported that ROM values were higher in young cats than in adult cats. In our study, it was observed that there was no significant age-related difference in oxidative load and antioxidant defense in Van cats.

In various studies in human medicine, it has been reported that the gender has a significant effect on the OSI level (Vassalle et al., 2008; Kaya et al., 2010; Vassalle et al., 2011). In a study of veterinary medicine, MDA levels measured greater in male cats and dogs and suggested that old and male dogs are subjected to more harmful effects of free radicals and lipid peroxidation (Todorova et al., 2005). In the study of Panda et al. (2009), it was stated that oxidative stress indices aren't affected by gender, breed or age in dogs. In a study in Thoroughbred foals, it was indicated that systemic and local OS biomarkers did not differ between gender of the horses (Po et al., 2013). In the study of Castillo et al. (2013), OSI levels were higher in male cats than females and it was stated that the male cats had a higher oxidative load than females. In a human study, it was reported that men are more exposed to oxidative stress (Pansarasa et al., 2000). Ide

at al. (2002) showed that oxidative stress is greater in healthy young males than in premenopausal females due to greater generation of ROS and reduced antioxidant activity. Unlike these studies, we observed that the gender factor did not show significant changes in oxidative stress and antioxidant defense in healthy Van cats.

The eye is an organ that is metabolically active, constantly in contact with light, therefore exposed to oxidative stress including photo-oxidative processes. In human studies, the relationship between age-related changes in the eye and oxidative stress has been investigated and age-related ocular pathologies and oxidative stress has been suggested (Beatty et al., 2000; Kruk et al., 2016; Goodman and Ness, 2023). Also in veterinary medicine, relationship between oxidative stress and ocular patholgies were evaluated (Chen et al., 2015; Simeonova et al., 2018). However, although the influence of eye colour on oxidative stress parameters has not been investigated and not clear yet, the use of eye as a biomarker of oxidative stress is promising in the future (Choodet et al., 2019).

In our study, two important points should be emphasized. Firstly, it was previously determined that there are different redox balances in different animal species (McMichael, 2007; Aktas et al., 2017; Bottegaro et al., 2018; Barbato et al., 2019) and the ROM and PAO values were determined in healthy cats without any breed consideration. However our study underlines that breed differences among cats may also lead to dramatic changes in redox balance. Secondly, this is the first study to evaluate redox balance in healthy Van cats. Since oxidative stress plays a critical role as a biomarker of various diseases, determination of redox balance may provide a useful tool in healthy Van cats.

CONCLUSION

Reactive oxygen species, total antioxidant capacity and oxidative stress index values in healthy Van cats were determined. It was observed that reactive oxygen species, total antioxidant capacity and oxidative stress index were not affected by age, gender and eye color in healthy Van cats.

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