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Research Article

Total antioxidant, total oxidant and oxidative stress levels in free-living birds

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ABSTRACT

Antioxidants provide protection against free radicals formed as a result of increased metabolism in living organisms and the damage these radicals cause to the cell. Determining the antioxidant levels can help eco-physiologists in the field in understanding the physiological state of the animal at that moment and in conservation biology. In this study, TOC (Total Oxidant Capacity), TAC (Total Antioxidant Capacity) and OSI (Oxidative Stress Index) values of 12 Long-legged Buzzards (Buteo rufinus), 7 Common Buzzards (Buteo buteo) and 6 Golden Eagles (Aquila chrysaetos), 15 Grey Herons (Ardea cinerea), 7 Eurasian Eagle Owls (Bubo bubo) brought to rehabilitation centre with injuries due to various reasons were examined. The birds brought to the center were first examined physically. Species-specific rooms were kept until each bird had recovered. After being rehabilitated, blood was taken from the birds 1-2 days before being released into the wild. As a result of blood analysis, TOC and OSI values were found to be high in Eurasian Eagle Owl, Golden Eagle, Long-legged Buzzard and Common Buzzards which were brought with a diagnosis of gunshot wounds, soft tissue trauma, femur or wing fractures. Although these birds had been rehabilitated, the reason for the high TOC and OSI values in individuals with these diagnoses may be the trauma experienced by these free-living birds in nature and then being held in captivity. After the birds were treated and rehabilitated, they were released back to nature in habitats specific to each species.

INTRODUCTION

The habitats of wild birds living freely in nature are open areas that can be a few kilometers or thousands of kilometers. Therefore, keeping these animals under captivity for various reasons may cause an increase in their metabolism and stress as a result of this (Coles, 2007; Cohen et al., 2008; Fischer & Romero, 2018). In addition, the sources of nutrient they feed in nature or the contamination of these sources for various reasons may change the levels of antioxidants and oxidants in their bodies. This can also be a source of extra stress to the animal (Costantini & Verhulst, 2009; Espín et al., 2014; Abbasi et al., 2017). In nature, wild birds, especially raptors are more sensitive to environmental influences than many birds. Human interventions to the habitats of wild free-living birds in nature make it difficult for these animals to survive and even put many species of birds to the category of endangered species (Coles, 2007). According to the data we have, it is stated that 65% of the birds are brought to rehabilitation centres due to traumatic injuries caused by human impact, while 85% are brought due to human-related deaths (Fix & Barrows, 1990; Desmarchelier et al., 2010). A significant number of wild birds are brought to rehabilitation centres due to reasons such as traffic accidents, pesticide intoxication, gunshot wounds, hitting electric wires or high voltage lines (Desmarchelier et al., 2010; Malik & Valentine, 2018). While these free-living animals already have stress caused by their natural enemies, human pressure also causes this stress to increase. As a result of this, the metabolism of these birds may increase and their oxidant and antioxidant states may change. The balance between oxidant production and antioxidant defense is considered an important indicator of an individual's health, and the individual oxidative stress level is the best indicator of this (Costantini et al., 2006). Antioxidant capacity of all living organisms is among the factors that affect their longevity and plays a very important role in their physiology. Agents that prevent the oxidation caused by free radicals and that have the ability to capture and stabilize free radicals are called 'antioxidant' (Şahin et al., 2015). Any negative effect that occurs in the organism can be prevented by antioxidants. The main task of antioxidants is to keep the oxygen in the existing environment and to prevent the initiation or further progress of oxidation reactions. When free radicals exceed the physiological levels in the cell, they cannot be destroyed by antioxidants. In this case, oxidative stress occurs. Disruption of the balance between oxidants and antioxidants at cellular level is defined as oxidative stress. In this process, as a result of lipid peroxidation caused by free radicals, phospholipids in the membrane are oxidized and as a result, membrane permeability increases. As a result, the ion balance in the cell is disrupted, functions of membrane-bound surface receptors are disrupted and many biochemical functions are not fulfilled (Mis et al., 2018).

Wild birds are very sensitive to environmental changes and

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any changes that may occur in their environment cause significant physiological responses due to the stress they experience. As a result of these reactions, the animal transfers its existing resources predominantly to survival, which puts an extra physiological burden on the animal (Costantini & Verhulst, 2009; Cram et al., 2015; Fischer & Romero, 2018). Reasons such as smell, sound, light conditions and daily diet in rehabilitation centres may cause wild birds to get extra stressed (Morgan & Tromborg, 2007; Fischer & Romero, 2018).

Therefore, understanding the responses of wild birds to these stressors, their adaptation mechanisms, which strategies are used and the differences between species is very important in terms of conservation biology (Fischer & Romero, 2018). Not only clinical examination, but also determination and evaluation of hematological, biochemical and physiological anomalies are very important in the success of such survival and conservation strategies (Black et al., 2011). In the rehabilitation of wild raptors, it is also very important to determine when the bird will be considered suitable and healthy to be released, that is, the release criteria.

There are a large number of studies in literature on TOC (Total Oxidant Capacity), TAC (Total Antioxidant Capacity) and OSI (Oxidative Stress Index) values showing the stress states of broilers and wild birds kept in captivity in cages for a long time (Mis et al., 2018; Wang et al., 2021). In recent years, studies on oxidative stress index, antioxidant levels, DNA damage of wild birds living free in nature have gained momentum. Based on previously conducted studies, the expected hypothesis of the present study is that oxidative stress index will increase especially in birds which are injured by gunshot fire, which have fractures and bone tissue trauma (Yaprakci et al., 2016). However, considering that the birds are rehabilitated, it is a question mark whether these values as a result of rehabilitation will reflect the results of normal and healthy individuals or whether they will be high due to the stress they increase, the presence of trauma and humans. For this reason, blood samples were taken from free-living wild birds brought to the centre due to various reasons and their TOC, TAC and OSI data were evaluated.

MATERIAL and METHODS

Kafkas University/Kafkas Wild Animal Protection, Rescue, Rehabilitation Application and Research Centre

Kafkas University/Kafkas Wild Animal Protection, Rescue, Rehabilitation Application and Research Centre located in Kars in Türkiye is a center where exhausted or injured wild animals animals in the Eastern Anatolia and Black Sea regions are brought to be rehabilitated. There are administrative, clinical and rehabilitation departments in the center in the Kafkas University campus, which serves under the 13 Regional Directorates of Nature Conservation and National Parks of the Ministry of Agriculture and Forestry. Many wild animals such as brown bear, lynx, fox, wolf, roe deer, mountain goat, golden eagle, red hawk and eagle are brought to the center, which are injured for various reasons. After these animals are treated, they are released back into nature. The center serves provinces of Erzurum, Gümüşhane, Erzincan, Bingöl, Bayburt, Artvin, Ardahan, Ağrı and Iğdır. Since 2020, 380 wild animals have been brought to the center, nearly 200 of them were cured and released into their natural environment.

Animal material

In this study, TOC, TAC and OSI values of 12 Long-legged buzzard (*Buteo rufinus*), 7 Common buzzard (*Buteo buteo*), 6 Golden eagle (*Aquila chrysaetos*), 15 Grey heron (*Ardea cinerea*) and 7 Eurasian eagle-owl (*Bubo bubo*) brought to Kafkas University/Kafkas Wild Animal Protection, Rescue, Rehabilitation Application and Research Centre were examined. All Grey herons were brought to the rehabilitation centre because the trees they nested in fell down during storm and they could not leave the nest. No problems were found in their examination and the diagnosis was fatigue due to dehydration. Other birds were injured in nature due to various reasons and they were randomly brought to rehabilitation centre both by citizens and by wild life protection teams. All birds brought to the rehabilitation center were adults.

Table 1 and 2 show the reasons why all of the birds were brought to the centre. All of the birds in the centre were kept for 1-3 months in specially arranged, isolated bird care rooms with an international size and standards and then in flight tunnels suitable for the species (5m/6m/30m). To avoid stress, they were not disturbed for any reason other than the routine visits of researchers. They were fed with red or white meat specific to the species for 6 days a week. Food and water were given *ad libitum*. Since the important thing in rehabilitation and conservation biology is to treat the animal and return it to wild life in a healthy way, no unnecessary medical intervention was performed during treatment process on birds which were brought injured, sick or destitute to the centre. In their first admission to the centre, no blood sample was taken from these birds for examination. Due to these medical reasons, we do not have any control group data that show the initial disease status of the birds in our study. 1-2 days before the birds to be released into the wild, blood samples were taken and all individuals were released back to wild life in suitable sites.

Obtaining blood samples and analyses

Blood was taken from the wing ulnar/basilic vena of the birds brought to the rehabilitation centre and transferred to EDTA (BD vacutainer, K_2 EDTA) tubes; after they were centrifuged for 5 minutes at 3000 r.p.m. (Electro-mag M815 M), the plasma samples were transferred to clean tubes. The plasma samples were kept in a deep freezer (Profilo 6600) at - 20 °C until they were analyzed.

TOC and TAC Analysis of blood samples

TOC and TAC measurements (Rel Assay Diagnostics) were carried out with colorimetric test according to the recommended procedure.

TOC measurement

Plasma total oxidant capacity is measured with the ferric ion forming a color intensity based on the ration of oxidants in the environment as a result of creating a colorful complex with acidic chromogenic and spectrophotometric determination of this. Its principle is based on the oxidation of oxidants present in the plasma to iron ion with iron on-chelate complex. Based on this principle, the measurement was made in accordance with the procedure in commercial test kits. A commercial kit was used for the determination of TOC (Rel Assay Diagnostics®) (Acke et. al., 2015). The initial solution containing reagent 1 (assay buffer) and the sample or standard was read at 530 nm for the first absorbance value. After that, prochromogen solution was added and incubated for 10 min at room temperature or 5 min at 37° C to produce colour complexing between the ferric ions and chromogen which can be measured spectrophotometrically and related to the total oxidant concentration. After incubation, the solution was read again at 530 nm (DAS Plate Reader) (Allam & Lemcke, 1975).

TAC measurement

Measurement of plasma total antioxidant capacity is determined on the basis that antioxidants in the sample reduce the dark blue-green procromogen 2,2'-azinobis (3-etilbenzotiyazolin-6-sülfonik acid) (ABTS) radical to colorless ABTS form. Based on this principle, the measurement was made in accordance with the procedure in commercial test kits. For the measurement of TAC (Rel Assay Diagnostics®) (Acke et. al., 2015) the initial solution contained reagent 1 (assay buffer) and the sample or standard absorbance was read at 660 nm for the first value. After that, ABTS radical solution was added and the mixture was incubated for 10 min at room temperature or 5 min at 37° C to allow the antioxidants to reduce the dark blue-green ABTS to form colourless, reduced ABTS before the absorbance was read again at 660 nm (DAS Plate Reader) (Allam & Lemcke, 1975).

The calculation of OSI

In order to calculate OSI, which is an indicator of oxidative stress degree, TOC value was divided by TAC value and multiplied with 100.

$$OSI = TOC / TAC \times 100$$

Analyses of data

The data were analysed with SPSS 22.0 (Statistical Package for Social Sciences). The normality test of the data was performed using the Shapiro-Wilk test, and it was found that the the all bird groups were not normally distributed separately ($p<0.05$). Statistical analysis of blood samples taken from rehabilitated birds was performed by Chi-Square test and $p \leq 0.05$ was considered as significant. Chi-Square tests of goodness of fit was used in single group comparisons. The purpose of this test; is to investigate whether a sample of *n* volumes drawn from the population is representative of the population. In this study, we tested the suitability of TOC, TAC and OSI values obtained from each bird species to the population (Table 1-5). For example, a comparison was made in the TAC values for the Eurasian eagle owls group of 7 individuals. Likewise, TOC and OSI values were also compared separately.

We used the Kruskal-Wallis and Mann-Whitney test to separately compare the TOC, TAC and OSI values in birds. For example, we compared the TAC of Eurasian eagle owls, Golden eagles and Grey herons (only 3 birds). Then we compared the TOC and OSI values for the same birds separately. We used Kruskal-Wallis test (non-parametric equivalent of One-way ANOVA) to make these comparisons. For the Long legged buzzard and Common buzzard (only 2 birds), we used the Mann-Whitney U test, which is a pairwise comparative test (Figures 1, 2 and 3).

Table 1. Plasma TAC, TOC and OSI values of rehabilitated Eurasion eagle-owl and the reasons why they were brougth to the clinic

	TAC (mmol Trolox Equv./L)	TOC (µmol H_2O_2 Equv./L)	OSI (Arbitrary Unit)	Reason
1	1.77	46.78	2.64	Weakness
2	1.55	262.00	16.90	Weakness
3	0.92	108.15	11.75	Left leg atrophy, neural
4	1.74	440.09	25.29	Weakness
5	0.17	44.75	26.32	Weakness
6	1.65	924.00	56.00	Shotgun Fire
7	0.46	836.36	18.18	Weakness
Mean±S.E.	**1.18±0.25**	**380.30±139.64**	**22.44±6.37**	

There is no statistical difference in TAC values ($p>0.05$). There are statistical difference in TOC and OSI values ($p<0.05$) (n=7, TAC: Chi Square= 0.444, df= 4, p= 0.979; TOC: Chi Square= 2152.312, df= 6, p= 0.000; OSI: Chi Square= 74.981, df= 6, p= 0.000). S.E.= Standart Error.

RESULTS

Table 1-5 show TOC, TAC and OSI values of all wild birds which were injured or tired for various reasons and the reasons why they were brought to rehabilitation centre, while Figure 1, 2 and 3 show the mean TOC, TAC and OSI values of birds.

As seen in Table 1, there was no statistical difference in plas-

Table 2. Plasma TAC, TOC and OSI values of rehabilitated Golden eagle and the reasons why they were brougth to the clinic

	TAC (mmol Trolox Equv./L)	TOC (μmol H_2O_2 Equv./L)	OSI (Arbitrary Unit)	Reason
1	0.49	375.75	76.68	Weakness
2	0.57	281.58	49.40	Weakness, cachexia
3	0.89	502.56	56.46	Weakness
4	1.96	46.52	2.37	Weakness
5	0.31	47.55	15.33	Shotgun fire, wing fracture
6	1.76	507.22	28.81	Shotgun fire, wing fracture
Mean±S.E.	**0.99±0.28**	**293.53±85.20**	**38.17±11.30**	

There is no statistical difference in TAC values ($p>0.05$). There are statistical difference in TOC and OSI values ($p<0.05$) (n= 6, TAC: Chi Square= 0.667, df= 3, p= 0.881; TOC: Chi Square= 737.968, df= 5, p= 0.000; OSI: Chi Square= 101.895, df= 5, p= 0.000). S.E.= Standart Error.

Table 3. Plasma TAC, TOC and OSI values of rehabilitated Grey heron and the reasons why they were brougth to the clinic

	TAC (mmol Trolox Equv./L)	TOC (μmol H_2O_2 Equv./L)	OSI (Arbitrary Unit)	Reason
1	0.69	105.36	15.26	Weakness
2	1.41	68.06	4.82	Weakness
3	1.41	18.39	1.30	Weakness
4	0.62	90.44	14.58	Weakness
5	0.48	151.98	31.66	Weakness
6	0.53	63.40	11.96	Weakness
7	0.70	32.63	4.66	Weakness
8	0.45	42.89	9.53	Weakness
9	0.23	54.07	1.24	Weakness
10	1.43	96.03	6.71	Weakness
11	1.47	15.85	1.07	Weakness
12	0.70	14.91	2.13	Weakness
13	1.58	55.94	3.54	Weakness
14	0.14	13.72	9.80	Weakness
15	0.59	41.02	6.95	Weakness
Mean±S.E.	**0.82±0.12**	**57.64±10.24**	**8.34±2.06**	

There is no statistical difference in TAC values ($p>0.05$). There are statistical difference in TOC and OSI values ($p<0.05$) (n= 15, TAC: Chi Square= 1.500, df= 8, p= 0.993; TOC: Chi Square= 380.965, df=14, p= 0.000; OSI= Chi Square: 107.921, df=14, p= 0.000). S.E.= Standart Error.

ma TAC values in Eurasian eagle-owls. On the other hand, there was a statistical difference in TOC and OSI values, and the highest TOC and OSI values were seen in Eurasian eagle-owls (No. 6) injured with gunshot fire. Similarly, there was no difference in the TAC values in the Table 2, while there are differences in the TOC and OSI values. In Golden eagles, the highest plasma TOC and OSI values (No: 1, 3 and 6) were seen in the birds that was brought with weakness, shotgun fire and wing fracture. Although OSI value was high in individual injured with gunshot fire (No. 6), they was not as high as those in the Golden eagles brought with weakness (No. 1 and 3). As a result of the statistical analysis, no differences were found in the plasma TAC values of Grey herons, but there were statistical differences in TOC and OSI Table 3.

While there was no statistical difference in TAC values in

Table 4. Plasma TAC, TOC and OSI values of rehabilitated Long-legged buzzard and the reasons why they were brougth to the clinic

	TAC (mmol Trolox Equv./L)	TOC (µmol H_2O_2 Equv./L)	OSI (Arbitrary Unit)	Reason
1	0.82	526.80	64.24	Bone tissue trauma without fractures
2	1.89	176.22	9.32	Soft tissue trauma
3	0.93	589.27	63.36	Soft tissue trauma
4	0.42	71.79	17.09	Soft tissue trauma
5	1.68	147.31	8.76	Soft tissue trauma
6	1.73	77.38	4.47	Soft tissue trauma
7	1.33	408.39	30.70	Femur fracture
8	1.02	151.04	14.80	Left claw paralysis
9	1.73	715.15	41.33	Closed wing fracture
10	1.08	841.02	77.87	Closed wing fracture
11	1.37	367.36	26.81	Closed wing fracture
12	1.19	680.65	57.19	Closed wing fracture

There is no statistical difference in TAC values ($p>0.05$). There are statistical difference in TOC and OSI values ($p<0.05$) (n= 12, TAC: Chi Square= 5.667, df= 9, p= 0.773; TOC: Chi Square= 2054.899, df= 11, p= 0.000; OSI: Chi Square= 205.559, df= 11, p= 0.000). S.E.= Standart Error.

Table 5. Plasma TAC, TOC and OSI values of rehabilitated Common buzzard and the reasons why they were brougth to the clinic

	TAC (mmol Trolox Equv./L)	TOC (µmol H_2O_2 Equv./L)	OSI (Arbitrary Unit)	Reason
1	0.88	11.18	1.27	Bone tissue trauma without fractures
2	0.70	139.86	19.98	Soft tissue trauma
3	0.82	470.86	57.42	Compound fracture
4	0.66	453.14	68.67	Femur fracture
5	1.79	390.67	21.82	Weakness
6	1.67	97.90	5.86	Weakness
7	1.75	411.18	23.49	Weakness

There is no statistical difference in TAC values ($p>0.05$). There are statistical difference in TOC and OSI values ($p<0.05$) (n= 7, TAC: Chi Square= 1.200, df= 6, p= 0.997; TOC: Chi Square= 783.096, df= 6, p= 0.000; OSI: Chi Square= 136.444, df= 6, p= 0.000). S.E.= Standart Error.

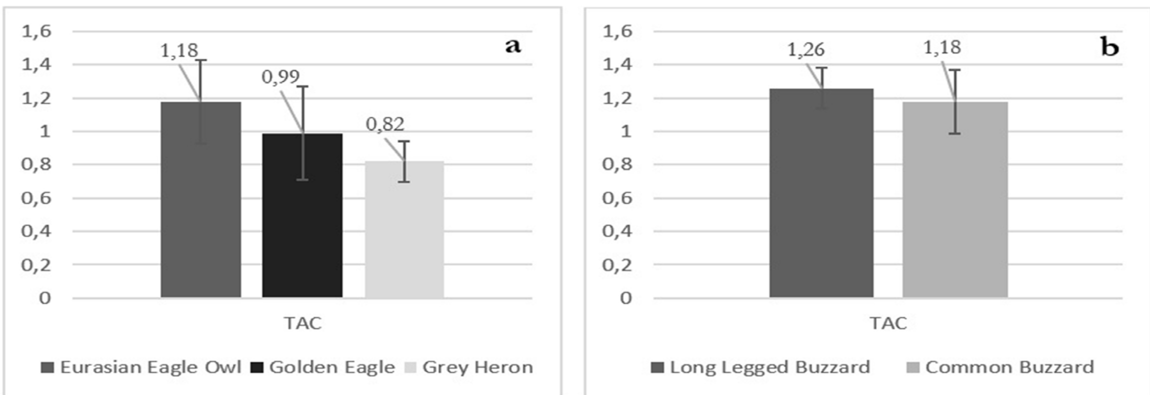

Figure 1. Figure 1.a. Mean plasma TAC (mmol Trolox Equv./L) data of Eurasion Eagle Owl, Golden Eagle and Grey Heron (Kruskal-Wallis H= 1.730, df= 2, p= 0.421). Figure 1.b. Mean plasma TAC (mmol Trolox Equv./L) data of Long Legged Buzzard, and Common Buzzard (Mann-Whitney U= 35.500, p= 0.582), $p>0.05$

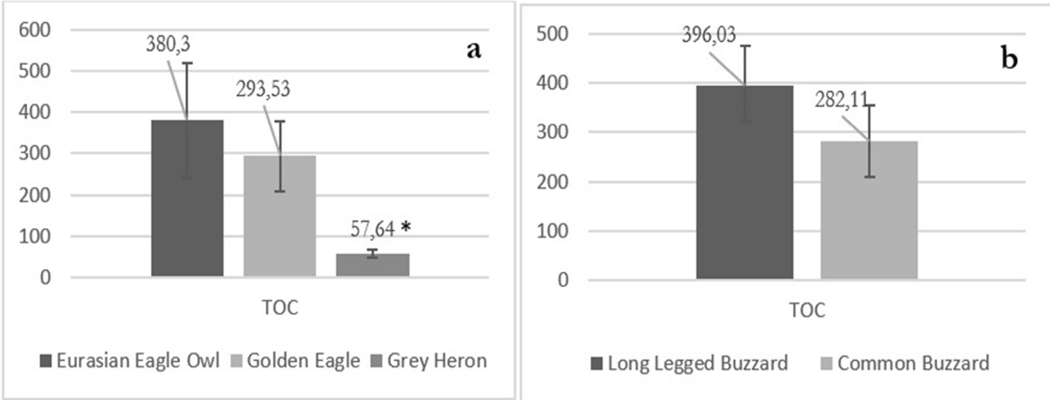

Figure 2. Mean plasma TOC (µmol H2O2 Equv./L) data of Eurasion Eagle Owl, Golden Eagle and Grey Heron (Kruskal-Wallis H= 8.831, df= 2, p= 0.012), * means statistically different, p<0.05. Figure 2.b. Mean plasma TOC (µmol H2O2 Equv./L) data of Long Legged Buzzard, and Common Buzzard (Mann-Whitney U= 31.000, p= 0.353)

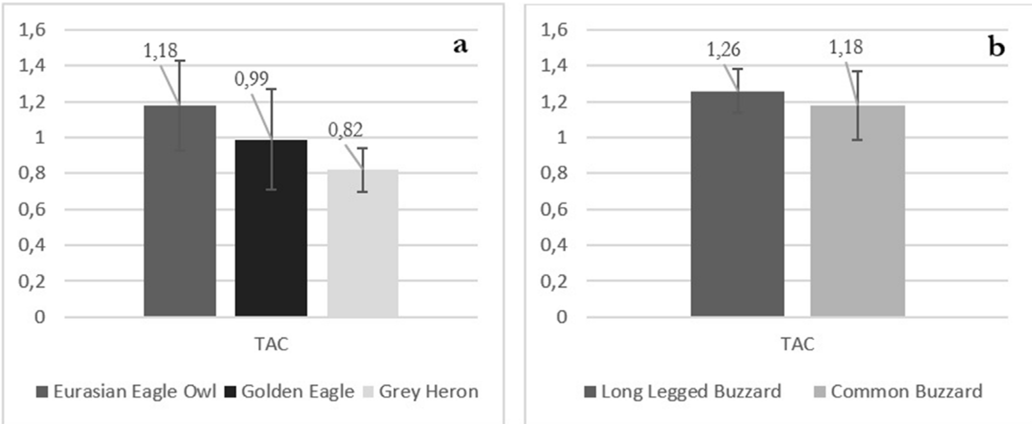

Figure 3. Mean plasma OSI (Arbitrary Unit) data of Eurasion Eagle Owl, Golden Eagle and Grey Heron (Kruskal-Wallis H= 9.45, df= 2, p= 0.09), * means statistically different, p<0.05. Figure 3.b. Mean plasma OSI (Arbitrary Unit) data of Long Legged Buzzard and Common Buzzard (Mann-Whitney U= 36.000, p= 0.612)

Long-legged buzzards and Common buzzards, there were differences in TOC and OSI Table 4 and 5. In terms of rehabilitated Long-legged buzzards, the highest plasma TOC and OSI values were found in the individual numbered 10 which was brought with closed fracture. In individuals which presented with closed fractures, OSI values are quite high. These values were found to be high in individual with the number 1 which was brought with unfractured bone trauma. OSI values were found to be high in all Long-legged buzzards except for those which were brought for soft tissue trauma (No. 2, 4, 5, 6). TOC and OSI values in all Common buzzards were high except for individuals numbered 1 and 6. Plasma TOC and OSI values were very high especially in individuals that were brought with compound fracture (No. 3) and femur fracture (No. 4).

DISCUSSION

Considering the plasma TOC, TAC and OSI values of all birds were examined, while TOC and OSI values were significantly high in all bird species except Grey heron, we believe it is necessary to draw attention to the fact that all of the Grey herons were brought with a diagnosis of weakness. Other birds were brought to the centre due to various and important traumatic reasons except for weakness.

The purpose of wild animal rehabilitation is treating the animals and releasing them to nature again. For this reason, care was taken to ensure that there is no different cause that may stress animals during the time they stayed at the centre, based on international practice standards (Redig, 1978; Chaplin et al., 1993). For example, according to international standards, birds in captivity should be kept in dark and quiet rooms so that they do not experience extra stress, they should not be disturbed, and the number of visitors should be kept to a minimum (Mander et al., 2003). The aim of rehabilitation of wild animals is to treat animals and release them back to natural life. Considering that all the birds evaluated in the study lived under relative captivity and the fact that the procedure of blood measurement may create stress, the birds were contacted each time by the same physicians and staff only when necessary for minimum stress conditions, no physical contact was experienced during the process and the same medical staff collected blood (Cooper, 1972; Campbell, 2012). In a study they conducted with *Falco tinnuculus,* Costantini and Dell'Omo (2006) suggested that especially OXY (total serum antioxidant level) was more affected by environmental components and the changes in OXY were due to antioxidants they took with nutrients. In a study they conducted with free-living *F. tinnunculus* during the breeding time, Costantini et al. (2006) found that stress levels increased due to decreased access to nutrient in larger hatch with more sibling rivalry. In the light of this information, starting from the moment they were accepted in the centre, the animals were fed sufficiently with natural food that is completely species-specific, similar to their hunt in natural life.

It is known that free radicals and oxidative stress in cells cause many disorders such as premature aging, reproductive capacity, arthritis, cardiovascular diseases and allergic reactions. Many things can also cause increased oxidative stress in wild life. Environmental variables (Costantini & Dell'Omo, 2006; Wang et al., 2021), predator and human pressure, poachers (Yaprakci et al., 2016), nutrition (Costantini et al., 2007; Mis et al., 2018), contamination of nutrients and water sources with chemicals, radiation (Fernie & Bird 2001; Fernie & Reynolds, 2005; Abbasi et al., 2017), whether the animals is in migration period (Arnold et al., 2010), size of hatch and nest (number of young birds) (Costantini et al., 2007) are among reasons that cause free radicals and oxidative stress index in animals to increase. Studies on oxidative stress, which has a direct effect on health and life capacity by being affected by many factors, have been increasing and becoming more important in recent years (Casagrande et al., 2011; Bize et al., 2014). Metabolic rate, diseases or injuries also cause the production of abundant reactive oxygen species. These reactive oxygen species may cause oxidative damage of biomolecules and accumulation of damage, resulting in disruption of homeostatic regulation mechanism and decrease in the longevity of the animal or death (Costantini & Verhulst, 2009; Yaprakci et al., 2016). At the same time, the assessment of oxidative stress levels may be a scale in evaluating immunity status (Cram et al., 2015). In this study, TOC and OSI values in Eurasian eagle-owls, Golden eagles, Long-legged buzzards and Common buzzards brought to the rehabilitation centre with diagnoses of gunshot wound, soft tissue trauma, femur and wing fractures were relatively higher than those of the other individuals. Although these raptors were rehabilitated, the reason why TOC and OSI values were high in individuals that were brought with trauma may probably be the fact that these birds were kept in captivity after the trauma/injury these individuals experienced. Similar to the results of this study, in a study conducted on common buzzards (*Buteo* species) with gunshot wound, TOC levels were found to be much higher than the control group (fatigue, thirst, weakness, simple bruise) (Yaprakci et al., 2016). An insignificant difference was found in TAC levels when compared with the control group. In this study, high OSI levels especially in individuals that were brought with gunshot wounds, tissue trauma and fractures shows the oxidative stress due to damage in all birds. Raptors may also be stressed due to poachers or human pressure in the environment (Finkel & Holbrook, 2000; Vágási et al., 2019). Birds in nests may also be exposed to an extra fracture or trauma when they are shot by hunters from a distance or due to falling from high. All these events may increase the metabolic rate and oxidative stress of animals.

CONCLUSION

In this study, the TOC, TAC and OSI levels of Eurasian eagle-owl, Golden eagle, Long-legged buzzard, Common buzzard and Grey herons that were brought to rehabilitation centre due to various reasons were evaluated. It was thought that the high oxidative stress index of especially birds that were brought to centre due to gunshot wounds, soft tissue trauma or fractures may be due to traumas experienced by these birds. Although blood collection and other procedures were performed by the same stuff in the rehabilitation centre so that the birds would not be stressed, the fact that they could be stressed and thus their metabolism increased due to presence of humans and the fact that they were kept under captivity was presented for the attention of readers by the authors. In addition, the data we reported may not completely represent the values expected for wild free-living birds due to individual effects of trauma, nutrition and captivity stress. Due to the small number of subjects obtained through completely natural means and those brought to a specific rehabilitation centre, it was thought that the data presented here may be evaluated as pre-data for studies to be conducted with higher number of subjects. It was concluded that conducting long-term studies with a higher number of subjects in the future will contribute to a stronger demonstration of these values.

DECLARATIONS

Ethics Approval

All procedures were approved by the Kafkas University Local Ethics Committee for Animal Experiments (KAÜ-HADYEK/2019-134) and the Republic of Turkey Ministry of Agriculture and Forestry, General Directorate of Nature Conservation and National Parks (14.11.2019/21264211-288.04-E.3469713).

Conflict of Interest

The authors declare no conflict of interest.

Consent for Publication

Does not need a publication consent

Author contribution

Idea, concept and design: ES, SÇ, MÇ, EU

Data collection and analysis: ES, AGE, SÇ, MÇ, MO, EU,

Drafting of the manuscript: ES, AGE, EU, SÇ, MO

Critical review: ES, SÇ, MO, EU,

Data Availability

The data used to prepare this manuscript are available from the corresponding author when requested. Acknowledgements

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