

Does the frequency of hunting dogs' activity influence their oxidative stress status subsequent to endurance?

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ABSTRACT

Oxidative stress is implied in various pathological conditions in both humans and animals, and recent studies have suggested its role in performance, recovery, immunity, and health. This study's objective was to investigate whether the higher frequency of activity by hunting dogs influences the oxidative stress indicators subsequent to endurance. The research involved forty-one hunting dogs, with equal representations of Alpine Brack Dachshund, Istrian Coarse-Haired Hound, and Posavina Hound. The dogs were categorized into higher frequency activity (n=22) and lower frequency activity (n=19) groups. Endurance was defined as a wild-boar hunt lasting for eight hours at an outside air temperature amounting to 10°C. Endurance had an impact on several parameters: an increased white blood cell count, neutrophils, and mean corpuscular hemoglobin concentration values, but decreased values of eosinophils and lymphocytes. Non-enzymatic biochemical indicators revealed higher energy expenditure (decreased glucose, total protein, and albumin concentration), hemoconcentration (decreased iron concentration), and elevated enzyme activity (AST, ALT, ALP, and CK-MB) but lower catalase activity. In terms of activity frequency, enzyme biomarker activity showed significant differences. ALT activity in both low- and high-frequency activity canines was notably higher ($P<0.05$) post-endurance compared to pre-endurance levels. ALP activity was significantly higher ($P<0.05$) post intense physical activity in higher frequency activity canines, yet lower ($P<0.05$) in lower activity frequency canines compared to their pre-endurance status. In conclusion, both tested canine groups demonstrated notable signs of oxidative stress subsequent to endurance, without differences between activity frequencies.

Key words: hunting dogs; oxidative stress; activity frequency; endurance

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Introduction

Oxidative stress, characterized by an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize or eliminate them, can lead to potential damage inflicted on biological molecules, such as proteins, lipids, and DNA (LUSHCHAK, 2014; SIES et al., 2017). It has been implied in various pathological conditions in both humans and animals, whereby recent studies suggest its role in performance and recovery (SHARIFI-RAD et al., 2020). Despite extensive research on oxidative stress in humans (ELOSUA et al., 2003; LISI et al., 2023), there is limited knowledge about its impact on working dogs, particularly in the context of hunting.

Hunting dogs, regardless of their activity frequency, face intense physical demands during hunting activities. These demands involve prolonged exercise, often characterized by bursts of high-intensity activity, and environmental stressors, such as temperature fluctuations and dehydration. These factors may potentially contribute to increased oxidative stress in hunting dogs. However, the extent to which oxidative stress is influenced by training and these dogs' work intensity remains poorly understood (ZANNONI et al., 2020).

Understanding the impact of oxidative stress on hunting dogs is both scientifically and practically significant. Hunting dogs play a globally crucial role in hunting and conservation efforts. Their performance is not only vital for the success of hunting, but it also affects the welfare of the animals involved. A hunting dog's ability to endure strenuous activities is influenced by factors such as fitness level, training, and overall health. Thus, oxidative stress may play a critical role in determining how hunting dogs cope with these physical challenges (BURR et al., 1997).

Determining the biochemical and hematological parameters in the blood of hunting dogs after strenuous activity is crucial for monitoring oxidative stress. These parameters provide insights into the dog's antioxidant defense system and overall health status. By assessing markers such as enzyme activity and blood cell counts, we can

identify potential oxidative damage and tailor interventions to support the dog's recovery and performance (ROVIRA et al., 2008).

This study's objective is to investigate whether a higher frequency of activity in hunting dogs has any effect on the oxidative stress indicators subsequent to endurance. Various oxidative stress markers and blood parameters were determined prior and subsequent to a standardized hunting exercise to shed light on how activity frequency (training) and exercise intensity (endurance) influence the oxidative stress responses of hunting dogs, providing valuable insights concerning their well-being and performance.

Materials and Methods

Animals. The research was conducted on forty-one hunting dogs, with equal breed representation: Alpine Brak Dachshund and the autochthonous hunting dog breeds, Istrian Coarse-Hair Hound and Posavina Hound. "Endurance" refers to a wild-boar hunt lasting for eight hours, at an outside air temperature amounting to 10 °C. The dogs were divided into two groups: dogs with a higher activity frequency (n=22) and dogs with a lower activity frequency (n=19). The dogs with a higher activity frequency included those that went hunting three times a week, and the dogs with a lower activity frequency included those involved in this kind of activity once a month, having followed that regimen for three months.

Hematological and Biochemical Parameters. Canine blood samples were taken by venipuncture of the cephalic vein twenty-four hours prior to intensive physical activity (whereby the blood samples were taken from the dogs in their owners' houses) and five minutes subsequent to intensive physical activity—that is, subsequent to participation in an eight-hour wild-boar hunt. The blood was taken in a Vacutainer system, in lithium heparin anticoagulant tubes (Becton Dickinson, Plymouth, England, UK). The samples were centrifuged (1.500 g/10 mins. At 4°C), and the plasma was separated and frozen at -80°C until analysis. The samples for hematological analysis were taken in

Ca-EDTA tubes (Becton Dickinson, Plymouth, England, UK) and analyzed within two hours on a Poch 100Veff analyzer (Sysmex, Japan), whereas differential blood count (DBC) was determined using a light microscope (Olympus BX40, Germany) on Pappenheim-stained samples. The plasma was analyzed using a Beckman Coulter AU680 clinical chemistry analyzer (Beckman Coulter, Germany). The blood plasma analysis implied determination of non-enzymatic parameters (glucose, urea, bilirubin, total protein, globulins, HDL-cholesterol, LDL-cholesterol, and iron, Fe) and enzymatic parameters (catalase, CAT, superoxide dismutase (SOD), glutathione peroxidase (GPx), aspartate aminotransferase (AST), alanine aminotransferase, (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), and creatine kinase, MB iso-enzyme (CK-MB). The activities of SOD, GPx, and CK-MB were determined by Randox (Randox, Crumlin, UK) kits RANSOD®, RANSeI® and Randox CK-MB®, respectively, and all other parameters were determined by FRG-produced Beckman Coulter standardized kits.

Statistical Analysis. Descriptive statistics concerning the plasma-related biochemical parameters were performed using STATISTICA software (TIBCO Software Inc. 2018). The assumption of normality was checked by administering the Shapiro–Wilk test. The variables not normally distributed were subjected to log₁₀ transformation to obtain normal distribution of values prior to statistical analysis. For evaluation of the endurance and activity-frequency effect in the experiment, pertaining to the variability of the biochemical and hematological parameters of individual animals, the following fixed statistical model was applied:

$$y_{ijk} = \mu + T_i + D_j + T_i D_j + e_{ijk}$$

where

y_{ijk} = estimated trait (complete blood count, enzyme biomarkers or biomarkers of the non-enzymatic part of the natural antioxidant defense in the canine blood);

μ = intercept;

T_i = fixed effect of activity level i (groups = prior and subsequent to an endurance activity);

D_j = fixed effect of training level j (j = the dogs with a higher-frequency activity and the dogs with a lower-frequency activity);

e_{ijk} = residual.

The significance of differences between the analyzed traits due to the fixed endurance effect and activity frequency, as well as any interactions, were tested by the post hoc Tuckey's test at the level of $P < 0.05$.

Results

Subsequent to endurance-based activity, both groups of canines, categorized by high or low activity frequency, exhibited a significantly higher ($P < 0.05$) average hemoglobin concentration in erythrocytes (MCHC) compared to their resting state. Subsequent to endurance-based activity, there was a decrease ($P < 0.05$) in the proportion of lymphocytes and eosinophils in the canine blood compared to the pre-endurance levels. Conversely, the proportion of segmented and non-segmented neutrophils in the canine blood increased ($P < 0.05$) subsequent to endurance activity. No impact of activity frequency on these indicators was determined in our study.

In relation to activity frequency, the average values of enzyme biomarker activity, representing a natural antioxidant defense system in the canine post-endurance blood sample revealed significant differences. The ALT activity in the blood of both groups of canines engaged in low- and high-frequency activity was significantly higher ($P < 0.05$) subsequent to endurance compared to the pre-endurance levels. Regarding ALP activity, it was significantly higher ($P < 0.05$) in the canines engaged in higher-frequency activity subsequent to intense physical activity, but lower ($P < 0.05$) in the canines engaged in lower-frequency activity, both values being compared to their respective pre-endurance status (see Table 2). The CAT activity was lower in the canine blood subsequent to intense physical activity than prior to endurance (Table 2). Additionally, the AST and CK-MB activity in the canine blood subsequent to endurance surpassed the levels observed prior to endurance (Table 2).

Table 1. The average values of complete blood count in dogs prior to and subsequent to endurance-based activity according to the activity frequency

Parameters	Before endurance		After endurance		Statistical parameters			
	Higher frequency-activity (n=22) \bar{x}	Lower frequency-activity (n=19) \bar{x}	Higher frequency-activity (n=22) \bar{x}	Lower frequency-activity (n=19) \bar{x}	SEM	P -Endurance	P -Frequency-activity	*P - E x F-A
Leukocytes, x 10 ⁹ /L	16.46	15.14	21.13	19.25	0.84	0.01	0.36	0.49
Erythrocytes, x 10 ¹² /L	7.66	7.46	7.49	8.38	0.15	0.74	0.46	0.17
Hemoglobin, g/L	169.6	167.6	168.1	190.70	3.45	0.92	0.29	0.19
Hematocrit, L/L	0.49	0.49	0.47	0.53	0.01	0.48	0.24	0.13
MCV, fL	64.26	65.86	63.81	64.01	0.41	0.17	0.51	0.37
MCH, pg	22.18	22.46	22.46	22.74	0.16	0.08	0.47	0.55
MCHC, g/L	345.3 ^a	341.0 ^a	352.1 ^b	355.3 ^b	1.28	0.01	0.61	0.03
Thrombocytes x 10 ⁹ /L	304.9	327.1	332.2	258.7	14.3	0.43	0.64	0.17
Neutrophils, seg, %	52.8	49.3	68.6	70.3	1.60	0.01	0.53	0.63
Neutrophils, band, %	5.82	6.2	10.73	8.7	0.65	0.01	0.96	0.22
Lymphocytes, %	22.2	24.5	11.6	11.4	1.12	0.01	0.71	0.92
Monocytes, %	6.55	6.3	5.59	4.5	0.36	0.08	0.68	0.55
Eosinophils, %	12.1	13.2	3.5	4.6	0.90	0.01	0.61	0.70
Basophils, %	0.5	0.5	0.01	0.4	0.08	0.08	0.52	0.37

MCV – average volume of erythrocytes; MCH – average amount of hemoglobin in erythrocytes; MCHC – average concentration of hemoglobin in erythrocytes; Neutrophils, seg – segmented neutrophils; Neutrophils, band – unsegmented neutrophils

*P interaction ExF-A /endurance x Frequency-activity/

^{a, b}different letters signify the differences (P<0.05) prior and subsequent to endurance-based activity

Table 2. The average values of enzyme biomarkers of natural antioxidant defense in the canine blood prior to and subsequent to endurance-based activity according to the activity frequency

Parameters	Before endurance		After endurance		Statistical parameters			
	Higher frequency-activity (n=22) \bar{x}	Lower frequency-activity (n=19) \bar{x}	Higher frequency-activity (n=22) \bar{x}	Lower frequency-activity (n=19) \bar{x}	SEM	P-Endurance	P-Frequency-Activity	*P-E x F-A
CAT, U/gHb	1482.76	1514.75	1212.23	1129.60	53.75	0.01	0.43	0.95
SOD, U/gHb	0.0031	0.0032	0.0031	0.0028	22.6	0.77	0.97	0.18
GPx, U/gHb	675.28	681.44	637.32	571.10	22.9	0.38	0.73	0.28
AST, U/L	38.54	19.80	84.50	82.10	6.9	0.01	0.98	0.10
ALT, U/L	84.72 ^a	30.00 ^a	103.59 ^b	52.10 ^b	3.2	0.03	0.91	0.04
GGT, U/L	5.13	2.80	6.36	3.10	0.7	0.25	0.46	0.98
ALP, U/L	110.36 ^a	77.10 ^a	123.63 ^b	65.90 ^b	17.9	0.21	0.72	0.02
CK - MB isoenzyme, μ g/L	106.01	97.67	216.79	153.20	11.7	0.01	0.23	0.35

CAT - catalase; SOD - superoxide dismutase; GPx - glutathione peroxidase; AST - aspartate -aminotransferase; ALT – alanine-aminotransferase; GGT - gamma-glutamyltransferase; ALP - alkaline phosphatase; CK - MB isoenzyme-creatine-kinase-MB isoenzyme

*P interaction ExF-A /endurance x Frequency-activity/

^{a,b}different letters signify the differences (P<0.05) before and after endurance

The average values of non-enzymatic biomarkers related to the natural antioxidant defense system in the blood of dogs, both before and after endurance, exhibit distinct patterns in relation to activity frequency. Specifically, Fe levels in the blood of higher frequency-activity dogs after endurance were significantly lower (P<0.05) compared to the levels observed in the

same group at rest (refer to Table 3). Moreover, post-endurance, the concentrations of glucose, total proteins, globulin, and HDL cholesterol decreased in the blood of dogs compared to their levels at rest (Table 3). Conversely, after intense physical activity, the concentrations of urea, bilirubin, and LDL cholesterol increased compared to the levels observed in dogs before endurance (Table 3).

Table 3. The average values of biomarkers of the non-enzymatic part of natural antioxidant defense in the canine blood prior to and subsequent to endurance-based activity according to the activity frequency

Parameter	Before endurance		After endurance		Statistical parameters			
	Higher frequency-activity (n=22) \bar{X}	Lower frequency-activity (n=19) \bar{X}	Higher frequency-activity (n=22) \bar{X}	Lower frequency-activity (n=19) \bar{X}	SEM	P -Endurance	P -Frequency-activity	*P - E x F-A
Glucose, mmol/L	4.95	4.89	4.19	3.86	0.13	0.01	0.29	0.14
Urea, mmol/L	4.28	4.18	6.74	5.52	0.32	0.01	0.63	0.51
Bilirubin, μ mol/L	1.92	1.86	2.18	2.73	0.10	0.01	0.36	0.08
Total protein, g/L	72.89	72.88	65.29	66.45	1.01	0.01	0.48	0.27
Globulin, g/L	45.46	41.18	36.80	33.96	1.21	0.01	0.58	0.63
HDL cholesterol, mmol/L	3.27	3.28	2.71	2.87	0.08	0.03	0.52	0.99
LDL cholesterol, mmol/L	1.20	1.06	1.83	1.86	0.08	0.01	0.83	0.94
Iron, μ mol/L	35.66 ^a	29.68	28.76 ^b	37.76	1.56	0.77	0.58	0.01

HDL cholesterol - high-density lipoproteins; LDL cholesterol - low-density lipoproteins

*P interaction Ex-F-A /endurance x Frequency-activity/

^{a, b}different letters signify the differences (P<0.05) before and after endurance

Discussion

We measured the biomarkers of antioxidant defense to assess the ability of the animals' organisms to respond to intense physical activity. Additionally, we performed a complete blood count to verify the health status.

In our study, the dogs exhibited a higher number of leukocytes (WBC) and an increased proportion of segmented and non-segmented neutrophils in their blood subsequent to intense physical activity compared to a period when they were at rest. Intensive exercise is known to temporarily elevate the white blood-cell count in the bloodstream, representing a natural response to the inflammation and tissue damage that are a consequence of physical exertion (ILKIW et al., 1989; DAVIS et al., 2008; SAND et al., 2013). Thus, intense physical activity, such as a high-intensity interval training or a prolonged endurance exercise, can lead to a temporary

increase in the number of circulating neutrophils, indicating the partial recruitment of bone-marrow neutrophils. This phenomenon is part of the acute inflammatory response to exercise, aiding the body to manage stress and potential damage (SUZUKI et al., 1996). In our research, the proportion of lymphocytes and eosinophils in the blood decreased after intense physical activity when compared to the resting state. Intensive exercise, especially high-intensity or prolonged endurance training, induces significant stress, triggering the body's "fight or flight" response, with the release of stress hormones such as cortisol and epinephrine. This hormonal surge initiates "immunomodulation," a process involving the redistribution of immune cells, including lymphocytes (CAPLIN et al., 2021). The body redirects immune resources to the active muscles, perceiving the exercise as a

potential threat, leading to a decreased number of circulating lymphocytes. The eosinophils involved in combating the parasitic infections and associated with allergic reactions and certain inflammatory conditions, undergo a temporary increase during an intense exercise (ALI et al., 2003). However, due to their pro-inflammatory nature, eosinophil numbers drop by 33.33% below the baseline after thirty minutes of rest, a state referred to by immunologists as an "open window" of immunodepression during recovery from intense exercise (ALI et al., 2003; PEAKE et al., 2017). Our research supports these findings, figuring a lower percentage of eosinophils in the blood after intensive physical activity. For hunting dogs, monitoring these changes in leukocyte counts and differentials, commonly known as a "stress leukogram," is crucial for assessing their health status and ensuring their readiness for rigorous activities in the field.

In our investigation, the mean corpuscular hemoglobin concentration (MCHC) in the blood of both the high- and the low-frequency activity dogs significantly increased after intense physical activity when compared to the resting state. In contrast, BALTZER et al. (2012) found no change in the MCHC values in the plasma of dogs subsequent to their completion of an agility course, as well as four hours following the exercise.

The body possesses a sophisticated antioxidant defense system, comprised of various enzymes, such as superoxide dismutase (SOD), catalase (CAT), and GPx, alongside the antioxidant molecules such as glutathione, working together to maintain a delicate balance between the production and removal of ROS (MICHALEK et al., 2020). In instances where the ROS levels become excessively elevated, the body typically upregulates its antioxidant enzymes to counteract oxidative stress. During prolonged and intense exercise, contracting skeletal muscles generate free radicals, potentially leading to oxidative damage to cellular components.

Despite the prominent role of SOD in shielding cells from the detrimental effects of the superoxide radical ($O_2^{\cdot-}$), our experimental model demonstrated no discernible differences prior to and subsequent to endurance-based activity.

Concerning the CAT activity, there is no unanimous consensus in the literature regarding the relationship between exercise training and CAT activity (POWERS and JACKSON, 2008). In our study, the CAT activity was lower in the canine blood subsequent to intense physical activity compared to their resting state. Extended periods of intense physical activity may deplete the body's antioxidant reserves, potentially resulting in decreased catalase activity, especially if the duration of oxidative stress induced by exercise is sustained over a prolonged time. This aligns with the results obtained by RUSH et al. (2000) concerning unchanged CAT activity in porcine coronary arterioles after a treadmill exercise, possibly explained by the suppressive effect of the substrate on CAT activity. Although CAT and GPx share common substrates, CAT's lower affinity for H_2O_2 at low concentrations, when compared to that of the GPx, might contribute to this phenomenon. In our study, the reason for the lack of increase in GPx activity after a long training session remains unclear, but corresponds to the findings of ERJAVEC et al. (2022).

Creatine kinase - isoenzyme (CK-MB) and AST are commonly used markers for evaluating skeletal muscle damage during exercise (CHANOIT et al., 2001). Our study observed an increase in AST and CK-MB activity in the serum after endurance. Intensive exercise, such as high-intensity resistance training, can cause muscle damage, releasing AST and CK into the bloodstream and temporarily elevating blood AST and CK levels (HUNTINGFORD et al., 2014). Similar observations were made by other authors (ILKIW et al., 1989; HINCHCLIFF et al., 1993; BURR ET. al. 1997; MCKENZIE et al., 2007; FRYE et al., 2018) in dogs after a race. The positive correlation between CK and AST activities in canine plasma suggests that AST is a biomarker of muscle cell injury rather than liver damage (FRYE et al., 2018).

Alkaline phosphatase (ALP) is present in all tissues throughout the body, predominantly in the liver, bile duct, kidney, bones, and placenta (FRANK et al., 2015). HUNTINGFORD et al. (2014) reported an increase in ALP activity in the serum of untrained dogs after two consecutive days

of low-intensity endurance exercise, consistent with the findings of ILKIW et al. (1989) and FRANK et al. (2015). This aligns with higher ALP activity in the blood of dogs exposed to higher-frequency activity after endurance compared to the same group at rest. Alanine-aminotransferase (ALT) is the most abundant enzyme in liver cells, although it is not specific for them (FRANK et al., 2015). In our research, the ALT activity in the canine blood subsequent to intense physical activity was significantly higher when compared to that of the dogs at rest, which is consistent with similar results in other studies (McKENZIE et al., 2007; FRYE et al., 2018). Elevated ALP and ALT activity may be associated with liver stress or injury. During endurance, the liver may be under increased stress due to factors such as increased blood flow, metabolic demands, or oxidative stress.

Being engaged in moderate to high-intensity exercise often results in a reduction in blood glucose levels during the activity. This occurs because the working muscles utilize glucose as their primary energy source, and insulin sensitivity increases, facilitating more efficient glucose uptake by the cells during exercise. Following exercise, the blood-related glucose levels may remain lower than the baseline, especially after high-intensity and relatively prolonged activities. In our investigation, the blood glucose concentration in dogs after physical activity was significantly lower compared to that of the dogs at rest, aligning with the findings of ERJAVEC et al. (2022).

Urea, a final nitrogenous product of protein metabolism, exhibits increased concentration in the plasma of dogs due to protein catabolism and reduced blood flow through the kidneys during exercise (FRANK et al., 2015). In the canines involved in our study, the observed rise in urea may be attributed to exercise-induced hemoconcentration, an anticipated phenomenon in untrained dogs (BURR et al., 1997; McKENZIE et al., 2007; FRANK et al., 2015; FRYE et al. 2018). In our investigation, the urea values in the blood of dogs subjected to intense physical activity was higher compared to that of the same dogs at rest.

In our study, the concentration of bilirubin in the blood of dogs after intense physical activity was

found to be higher than in the same dogs at rest. These results are congruent with those obtained by CHEVION et al. (2003), who identified an increase in bilirubin concentration in their subjects' plasma after two strenuous exercises involving weight loading. This elevation may be attributed to increased erythrocyte hemolysis, being itself the consequence of strenuous physical activity. In contrast, our study observed lower concentrations of total proteins and globulin in the blood of dogs after intense physical activity compared to the same dogs at rest, which is consistent with the findings of HINCHCLIFF et al. (2004), who noted a decrease in total protein and globulin concentrations in the plasma of racing dogs within one hour after a race. By promoting protein synthesis and preventing excessive muscle breakdown, exercise can thus contribute to the preservation of lean muscle mass and overall protein balance. In dogs, physical exercise caused a significant reduction in total lipids, total cholesterol and low-density lipoprotein (LDL) cholesterol, whereas high-density lipoprotein (HDL) and very low-density lipoprotein (VLDL) cholesterol remained unaffected (CHOUDHARY and SHARMA, 1989). Our study revealed a lower concentration of HDL cholesterol in the blood of dogs after intense physical activity compared to the same dogs at rest, and a higher concentration of low-density lipoproteins (LDL cholesterol, Table 3). This is opposite to the findings of CHOUDHARY and SHARMA (1989), who reported that the HDL:LDL cholesterol ratio rose significantly, from 0.36 ± 0.01 to 0.58 ± 0.01 . Similarly, PASQUINI et al. (2010) reported an increase in the concentration of LDL cholesterol in the plasma of Labrador Retrievers. Endurance exercises, known for releasing fatty acids into the bloodstream to fuel the working muscles, may contribute to the elevated levels of circulating lipids, including LDL cholesterol. Additionally, microscopic damage to muscle fibers during exercise may temporarily raise the LDL cholesterol levels as a part of the body's repair and recovery process, aiding in lipid transport to the damaged tissues. The elevated cortisol levels, influenced by exercise, can also impact lipid metabolism and temporarily elevate the LDL cholesterol (USUI et al., 2015).

Iron (Fe), an essential element for life, is acquired through food intake, and any reduction, increased requirement, or heightened loss can lead to Fe deficiency (FRANK et al., 2015). HUNTINGFORD et al. (2014) noted a significant increase in Fe concentration in the serum of untrained dogs after low-intensity exercise, which is an expected outcome due to hemoconcentration. In our study, the concentration of Fe in the blood of dogs exposed to higher-frequency activity after endurance was significantly lower compared to the same group at rest, while the dogs exposed to lower-frequency activity showed an increase after physical activity. This aligns with the findings of KENYON et al. (2011), who observed a decrease in Fe concentration in the serum of sled dogs immediately after a race.

Conclusions

Our data indicate that training does not significantly influence their antioxidative capacity and immune system. Blood cell count was impacted by endurance, primarily resulting in increased WBC, neutrophils, and MHC values, but also in a decrease in eosinophils and lymphocytes. Non-enzymatic biochemical indicators suggested higher energy expenditure, hemoconcentration, and heightened transaminase activity, yet, all parameters remained under robust homeostatic control. Enzymatic indicators indicated muscle damage and liver oxidative stress after endurance.

Ethics approval

Approval from Bioethics committee for research on animals of University in Osijek, Faculty of Agrobiotechnical Sciences Osijek (CLASS: 602-02/24-07/02; UB: 2158-94-02-24-24)

Authors contribution

Author Contributions: Conceptualization and designing experiments, M. Š., M. Đ., I. P. K. Methodology, M. Š., N. P. M., Data collection: I. B., T. F., I. P. K., M. P. V., Laboratory analysis, M. Đ., A. M., Statistical analysis M. P. V., Draft manuscript preparation and results interpretation I. P. K., Review and editing, M. Đ., M. Š. N. P. M.

Declaration of competing interest

No potential conflict of interest was reported by the authors

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PARČETIĆ-KOSTELAC, I., I. BOŠKOVIĆ, M., ĐIDARA, T. FLORIJAČIĆ, N. POLJIČAK MILAS, M. PAVIĆ VULINOVIĆ, A. MATIJEVIĆ, M. ŠPERANDA: Utječe li učestalost aktivnosti lovačkih pasa na stanje oksidacijskog stresa nakon dugotrajnog fizičkog napora? *Vet. arhiv* 94, 487-498, 2024.

Oksidacijski stres javlja se u raznim patološkim stanjima kod ljudi i životinja, a nedavna istraživanja sugeriraju njegov znakovit utjecaj na proizvodne pokazatelje, oporavak, otpornost i zdravlje. Cilj istraživanja bio je utvrditi utječe li veća učestalost aktivnosti lovačkih pasa na pokazatelje oksidacijskog stresa nakon dugotrajne fizičke aktivnosti. U istraživanju je sudjelovao 41 lovački pas, s podjednako zastupljenošću pasmina alpski jazavčar, istarski kratkodlaki gonič i posavski gonič. Psi su kategorizirani u skupine koje treniraju (n=22) i skupine koje ne treniraju (n=19). Dugotrajna fizička aktivnost definirana je kao lov na divlje svinje u trajanju od osam sati, na vanjskoj temperaturi zraka od 10 °C. Dugotrajna fizička aktivnost utjecala je na nekoliko pokazatelja: broj leukocita, neutrofila i srednje vrijednosti koncentracije hemoglobina su povećani, a vrijednosti eozinofila i limfocita su snižene. Neenzimski biokemijski pokazatelji otkrivaju veću potrošnju energije (smanjena koncentracija glukoze, ukupnih proteina i albumina), hemokoncentraciju (smanjena koncentracija željeza) i povišenu aktivnost enzima (AST, ALT, ALP i CK), te nižu aktivnost katalaze. Što se tiče frekvencije aktivnosti, pojedini enzimi biomarkeri aktivnosti pokazuju znakovite razlike. ALT je i kod pasa niske i kod pasa visoke frekvencije aktivnosti bila veća (P<0,05) nakon dugotrajne fizičke aktivnosti. Usporedbom vrijednosti ALP-a nakon dugotrajne fizičke aktivnosti u odnosu na vrijeme prije dugotrajne fizičke aktivnosti, utvrđeno je da je ovaj enzim bio znakovito povišen (P<0,05) kod pasa veće frekvencije aktivnosti odnosno znakovito snižen (P<0,05) kod pasa manje frekvencije aktivnosti. Zaključno, obje testirane skupine pasa pokazale su izražene znakove oksidacijskog stresa nakon vježbe izdržljivosti, bez obzira na utreniranost.

Ključne riječi: lovački psi; oksidacijski stres, frekvencija aktivnosti (utreniranost); dugotrajni fizički napor
