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Research article

EFFECTS OF ROSE-HIP AND GRAPESEED DIETARY SUPPLEMENTATION ON SERUM OXIDATIVE STRESS PARAMETERS IN DOGS BEFORE AND AFTER PHYSICAL EXERCISE

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The aim of this study was to evaluate the effects of 60 days of rose-hip and grapeseed dietary supplementation of a balanced home-cooked diet on serum oxidative stress parameters: ROMs, MDA and FRAP in army service dogs before and after regular physical exercise. The dogs were fed a balanced cooked diet as instructed by army standards until the initial blood sampling in June. Thereon the dogs were randomly allotted to 4 groups according to the dietary regime: dogs maintained on a balanced cooked diet according to army standards, branded dry dog food, cooked diet with added 500 mg rose-hip extract, and cooked diet with added 100 mg grapeseed extract for a 60 day period from June to September after which all 4 groups were fed the standard cooked meal diet. Sampling was performed at the beginning of the experiment (June), 60 days from the start of the treatment (September) and finally 60 days after the end of supplementation (November). Statistical analysis of the results included descriptive statistical parameters: mean (M), standard deviation (SD), and variation coefficient (CV%). In order to test the statistical significance of the differences between treatments a multifactor variance test (ANOVA) was performed for ROM, MDA and FRAP and the combined effects of diet, exercise and time period were observed. The initial (in June) increase in MDA and ROMs after exercise indicates the presence of oxidative stress 30 minutes after exercise. However, the antioxidative effects of rose-hip and grapeseed extracts are not conclusive, as multifactor ANOVA testing of time, diet, and exercise factors did not reveal for MDA statistically significant differences either at 60 days of supplementation nor 60 days after withdrawal of the supplements. Only one distinct exception was recorded for the prolonged antioxidative effects reflected in significantly decreased ($p<0.01$) ROMs before and after exercise in dogs fed the branded dry food 60 days after the end of such dietary regime (November). FRAP values tend to be higher ($p>0.05$) after exercise compared to before exercise in all experimental groups

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in November, indicating on a possible redistribution and upregulation of endogenous antioxidants during the experiment.

Key words: dogs, oxidative stress, rose-hip extract, grapeseed extract.

INTRODUCTION

Exercise has been shown to increase the production of reactive oxygen species (ROS) to a point that can exceed antioxidant defenses, thus leading to a state of oxidative stress. Exceeding ROS have been shown to induce damage in all cellular macromolecules such as lipids, proteins and DNA [1-4]. ROS react with practically every organic molecule they come into close contact with, producing reactive oxygen metabolites (ROMs). The ROMs are more stable than ROS and thus can be easily quantified [5]. The generated free oxygen radicals attack cellular components, especially those containing lipids. The generated chain reaction leads to the formation of more free radicals and ROS that can damage other cellular components. The formed end-product malondialdehyde (MDA) is frequently used as a marker of oxidative stress in response to exercise.

Physical exercise can induce peroxidation of lipids in cellular membranes and increased levels of thiobarbituric acid reactive substances (TBARS, i.e. mainly MDA) in the blood observed in post-exercise samples is a consequence of leakage of peroxides from tissues, especially from muscles into the blood plasma. The so formed oxidative modifications of plasma constituents are an expression of oxidative damage that occurred in the tissues during exercise [6]. Kanter et al. [7] reported that peroxidation products were observed in the blood of athletes after extreme exercise and further studies by Ilhan et al. [8] disclosed differences due to type of exercise to which the athletes were exposed. However, the results of studies that addressed the question whether exercise induced oxidative stress are still not consistent, probably due to the different levels of training, environmental conditions, dietary regimens, timing of sampling, etc. Basically, it can be concluded that the majority of studies found some increase in oxidative stress as a response to physical exercise.

Cells have developed a multi- leveled system of defense against the damage caused by free radicals released during oxidative stress. The reactive free-radical molecules are neutralized by an elaborate antioxidant defense system consisting of enzymes such as catalase, superoxide dismutase, glutathione peroxidase, and numerous non-enzymatic antioxidants, including vitamins C, E and A, glutathione, ubiquinone and flavonoids.

To date there are various antioxidant activity assays, but all of them having advantages and disadvantages. There is not a single method that can provide unequivocal results and the best solution is to use a combination of methods. FRAP is often referred to as the measure of "antioxidant power" and it embraces both exogenous and endogenous factors [9]. It is characterized by a reduction of Fe3+ to Fe 2+ depending on the available reducing species. Exercise has been shown to affect the value of FRAP in

humans and animals depending on the intensity and duration of the physical strain [10]. Parallel to the studies on the beneficial effects of exercise and its effects on oxidative stress investigations on the effects of dietary supplementation with antioxidants have been carried out by a number of authors. In the recent years special attention has been focused on the effects of ingested free radical scavenging polyphenols present in grapes [11,12], rose-hips [13,14], blueberries [15,16], and pomegranates [17-19] on oxidative stress markers in animals and humans. The major bioactive compounds within these fruits are besides polyphenols, ascorbic acid, tocopherol, lycopene, tannins, ß carotene, sugars, amino acids, and essential fatty acids [20]. Since plant phenolics (e.g. quercetin, gallic acid, resveratrol) possess well documented antioxidant properties, many new polyphenol enriched foods and dietary supplements are being introduced to the market. Such products are becoming more popular not only for human consumption, but their role in veterinary medicine and animal health is increasing. However, it still remains unclear whether the protective effects are attributable only to the phenolic composition or to their interaction with other present molecules such as vitamins.

The aim of this study was to evaluate the effects of 60 days of rose-hip and grapeseed extracts dietary supplementation of a balanced home-cooked diet on serum oxidative stress parameters in army service dogs before and after regular physical exercise.

MATERIALS AND METHODS

Experimental animals and design

The experiment was carried out on a total of 40 army service patrol dogs body weight 30 – 35 kg, both sexes, 2 -5 years of age. The animals were of the German shepherd (n= 16), Belgian shepherd (n=15) and Labrador retriever (n= 9) breeds. All dogs were exercised according to army protocols. Their exercise routine consisted of 60 minutes of running and obstacle jumping (anaerobic exercise), followed by 120 minutes of obedience training. The exercise routine was carried in the morning hours, thus avoiding environment temperature extremes.

The dogs were fed a balanced cooked diet until the initial blood sampling in June. Thereon the dogs were randomly allotted to 4 groups according to the dietary regime: dogs maintained on the balanced cooked diet (n=10), commercially available branded dry food for working dogs (Eucanuba Active Dog®) (n=10), cooked diet with added 500 mg rose-hip extract (Swanson®, rose-hip extract) (n=10), and cooked diet with added 100 mg grapeseed extract (Swanson®, Grapeseed extract standardized to 90% polyphenols) (n=10) for a 60 day period from June to September. Thereon all 4 groups were fed the standard cooked meal diet. The animals were fed once a day in the evening hours. The trial groups were balanced for age, sex and ability. The study was carried out with the support and approval of the Macedonian Army Veterinary Service in accordance to the Animal Welfare Regulations.

Blood samples were collected by venipuncture of the jugular vein in the morning hours prior to exercise and 30 minutes after exercise. Sampling was performed at the beginning of the experiment (June), 60 days from the start of the treatment (September) and finally 60 days after the end of supplementation (November).

Serum was obtained by centrifugation of blood samples at 3000 rev/min for 10 minutes, aliquotted and stored at -70 C, pending analysis.

Reactive oxygen metabolites (ROM) assay

The ROM assay is intended for the measurement of the concentration of total hydroperoxides in serum or plasma samples. The method as described by Alberti et al. [5] is based on the following principle: *in vitro,* in a buffered solution (pH = 4.8) the iron ions are released from serum proteins and catalyze the reaction of transformation of hydroperoxides into alkoxyl and peroxyl radicals, which further react with the chromogen N,N-diethyl-p-phenylenediamine. The concentration of the colored complex is directly proportional to the concentration of the hydroperoxides which are present in the sample. The absorbance is measured at 505 nm (ChemWell2910®), and the results are expressed in CARR U. One CARR U corresponds to 0.08 mg/100 mL H_2O_2.

Malondialdehyde (MDA)

Lipid peroxidation was determined as described by Slater [21] modified as follows. To a 15% trichloracetic acid (TCA) solution 0.375% thiobarbituric acid (TBA) was added. To 3mL of the so prepared solution 200 μL of serum was added. After thorough mixing the solution was heated in a boiling water bath for 15 min. After cooling the solution was centrifuged at 3000 rev/min for 10 minutes and the supernatant decanted. Absorbance was measured at 535 nm and the extinction coefficient measured 1.56 10^5 dm^3/mol.cm. MDA concentration was expressed in μmol/l.

Ferric Reducing Ability of Plasma (FRAP) assay

The measurement of FRAP was done according to the procedure described by Benzie and Strain [9], slightly modified. The method is based on the principle of reduction of the ferric-tripyridyltriazine complex to the ferrous form, upon which an intense blue color develops. The change of absorbance was measured on a Chemwell® analyzer at 593 nm in a microplate format. Briefly, 10 μL of sample and 40 μL of water were pipetted on a microplate in duplicate. After that, 200 μL of working reagent were added in each well (a: acetate buffer pH 3.6; b: $FeCl_3$ solution; c: 2,4,6,-tripyridyl-s-triazine solution; 10:1:1), and the reaction mixture was incubated for exactly 8 min at 37 °C. Standards of 500, 1,000 and 2,000 μmol/L $FeSO_4$ were used for calibration. The results are expressed as μmol/L $FeSO_4$. The intra-assay variation of the FRAP assay was 3.6%, as determined with two quality control samples.

Statistical analysis

Statistical analysis included the determination of descriptive statistical parameters: mean (M), standard deviation (SD, and variation coefficient (CV%). In order to test the statistical significance of the differences between treatments a multifactor variance test (ANOVA) was performed. Three factors were tested: diet (home-cooked, commercial dry food, home-cooked with added rose-hip extract, and home-cooked with added grapeseed extract), exercise (before and after), and time (June, September, and November). Individual comparisons were determined with the Tukey test. Significance level was set at 5% and 1%. Values were expressed as mean ± SD. Analysis was performed using the PASW Statistics18 and MS Excel software.

RESULTS

Reactive Oxygen Metabolites (ROMs)

The ROM value in June (Table 1) was significantly higher ($p<0.01$) after exercise (333.97 ± 44.48) compared to the value before exercise (245.26 ± 38.03).

Table 1. ROM, MDA and FRAP values *(expressed as mean* ± SD) before and after exercise in June before dietary rose-hip and grapeseed supplementation

Parameter	Exercise (n=40)	June (Mean ± SD)	CV%
ROM (CARR U)	Before	245.26 ± 38.03[a]	15.51
	After	333.97 ± 44.48[a]	13.32
MDA (µmol/l)	Before	2.42 ± 1.53[b]	63.01
	After	3.45 ± 1.79[b]	52.07
FRAP (µmol/l)	Before	1629.45 ± 145.43[c]	8.93
	After	1518.21 ± 175.10[c]	11.53

aa, bb – mean values differ significantly ($p<0.01$)
cc – mean values differ significantly $p<0.05$)

The combined effects of all three (diet, exercise, time) tested factors (Table 2) on ROM values in September have shown a significant ($p<0.01$) increase after exercise in dogs fed the home-cooked diet. Sixty days (September) after the start of supplementation dogs fed the commercial dry diet, home-cooked diet supplemented with rose-hip or grape extracts did not show significant differences between before and after exercise.

After 60 days from the withdrawal of supplementation (November) and reversal to the home-cooked diet a marked significant ($p<0.01$) decrease in ROM values was measured both before and after exercise in dogs fed the commercial dry diet. These values were not lower only when compared to the other groups at this time, but were

also significantly ($p<0.01$) lower when compared to the values measured in September. The rose-hip and grapeseed extract supplemented groups did not show a significant change in ROM concentrations 60 days after withdrawal of supplementation when compared to the values obtained at the end of the supplementation period (September).

The combined effects on ROM of all three (time, diet, exercise) tested factors are shown in Table 2.

Table 2. Serum ROM (CARR U) levels (expressed as mean ± SD) of dogs fed different dietary regimens from June to September, thereon all maintained on the home-cooked diet from September to November before and after exercise

Diet	Exercise	September (Mean ± SD)	November (Mean ± SD)	Sept:Nov (statistical significance)
Home-cooked	Before	297.69 ± 37.16 ^{xy}	304.96 ± 55.82 ^{xy}	NS.
	After	376.13 ± 43.96 ^{xz}	279.5 3±46.04 ^{zq}	p<0.01
Branded dry dog food	Before	328.22 ± 24.49	192.37 ±16.88 ^{xzmnor}	p<0.01
	After	323.96 ± 28.19	180.13 ±14.62 ^{yqcdef}	p<0.01
Home-cooked + rose-hip extract	Before	368.32 ± 27.77 ^{yq}	351.14 ±28.14 ^{mc}	NS.
	After	335.18 ± 27.98	317.20 ±19.19 nd	NS.
Home-cooked + grapeseed extract	Before	344.48 ± 29.78	334.64 ±55.26 ^{oe}	NS.
	After	285.99 ± 71.35 ^{zq}	305.52 ± 51.32 ^{rf}	NS.

Statistical significance within the same column is described by the same superscripts: x,y, z, q, c, d, e, f, m, n, o, r - $p<0.01$
Statistical significance between columns September and November (Sept:Nov) is described as not significant (NS.) at $p>0.05$

Malondialdehyde (MDA)

Tested MDA values in June showed a statistically significant ($p<0.01$) higher value after exercise (3.45 ± 1.79) compared to the value before exercise (2.42 ± 1.53). CV% were high (63.01% before and 52.07% after exercise), which indicate a high heterogeneity of the observed parameter (Table 1).

The combined effects on serum MDA concentration of all three tested factors (time, diet, exercise) are shown in Table 3.

MDA concentrations before and after exercise showed an almost parallel increasing trend from September to November (Table 3). No significant difference was recorded for MDA values before or after treatment, nor between different diet regimens after 60 days of supplementation (September). Equally, no significant differences between treatments nor between before and after exercise were recorded 60 days after the withdrawal of rose-hip or grape extract supplementation (November). Comparison between the results obtained in September and November showed no statistically significant differences.

Table 3. Serum MDA (μmol/L) levels (expressed as mean ± SD) of dogs fed different dietary regimens from June to September, thereon all maintained on the home-cooked diet from September to November before and after exercise

Diet	Exercise	September (Mean±SD)	November (Mean±SD)	Sept:Nov (statistical significance)
Home-cooked	Before	1.83 ± 1.04	2.12 ± 0.91	NS.
	After	2.58 ± 0.92	2.87 ± 1.46	NS.
Branded dry dog food	Before	2.48 ± 1.16	2.33 ± 1.15	NS.
	After	2.75 ± 1.1	3.03 ± 1.09	NS.
Home–cooked + rose-hip extract	Before	2.15 ± 0.64	1.86 ± 0.77	NS.
	After	2.81 ± 1.31	2.87 ± 1.6	NS.
Home–cooked + grapeseed extract	Before	1.52 ± 0.55	2.78 ± 1.06	NS.
	After	3.11 ± 0.84	3.37 ± 0.75	NS.

Statistical significance between columns September and November (Sept:Nov) is described as not significant (NS.) at $p>0.05$

Ferric Reducing Ability of Plasma (FRAP)

At the start of the experiment (June) serum FRAP values were significantly ($p<0.05$) lower after exercise (1518.21±175.10) compared to the values before exercise (1629.45±145.43) (Table 1).

FRAP values in September were uniform and no significant differences were recorded due to exercise or diet regimen (Table 4).

Table 4. Serum FRAP (μmol/L) levels (expressed as mean ± SD) of dogs fed different dietary regimens from June to September, thereon all maintained on the home-cooked diet from September to November before and after exercise

Diet	Exercise	September (Mean ± SD)	November (Mean ± SD)	Sept:Nov (statistical significance)
Home-cooked	Before	1982.38 ± 235.82	1356.94 ± 162.88 [a]	p<0.01
	After	2082.3 ± 319.72	2035.36 ± 342.34	NS.
Branded dry dog food	Before	1928.93 ± 278.72	1599.34 ± 560.38	NS.
	After	1946.85 ± 353.18	1980.58 ± 355.94	NS.
Home –cooked + rose-hip extract	Before	1967.73 ± 199.07	1605.56 ± 290.82	NS.
	After	1938.98 ± 128.73	1809.3 ± 148.55	NS.
Home –cooked + grapeseed extract	Before	1928.7 ± 524.38	1813.65 ± 156.19	NS.
	After	2125.43 ± 346.96	2145.38 ± 366.22 [a]	NS.

Statistical significance within the same column is described by the same superscript: a - $p<0,01$
Statistical significance between columns September and November (Sept:Nov) is described as not significant (NS.) at $p>0.05$

FRAP values in November tended to be lower (compared to September) in serum samples taken before exercise. However, this trend was statistically significant only in samples taken before exercise in the group of dogs fed the home-cooked diet.

The recorded FRAP values before and after exercise in September for all diet regimens showed no significant differences.

The combined effects on FRAP of all three (time, diet, exercise) tested factors are shown in Table 4.

DISCUSSION

In this study we evaluated the oxidative stress status in army service dogs before and after physical exercise by assessing serum ROMs, MDA and FRAP, with the aim to establish the effects and persistence of natural antioxidants present in rose-hip and grapeseed extracts, and compare them to the effects of a quality commercial dry diet and a not supplemented home-cooked diet. The dogs were exposed to a daily morning training routine and housed under standard husbandry conditions, thus avoiding temperature extremes throughout the study. No differences in the measured oxidative stress parameters were observed between males and females before and after exercise, which is in accordance to the data published by Ilhan et al. [8] for athletes of both sexes exposed to aerobic-anaerobic exercise.

Initial measurements carried out in June showed that ROMs and MDA concentrations significantly increased ($p<0.01$) after exercise. The results obtained in this study are in agreement with previously published data [10] which revealed a similar trend for ROMs in hunting dogs. An extensive study on the effects of intense and exhaustive exercise [22] revealed an increase in MDA concentration by 131% in rats exposed to strenuous swimming. Azizbeigi et al. [23] confirmed a significant increase in MDA concentration in athletes regardless of the type of intensive training. The assessment of lipid peroxidation during exercise was also carried out in race horses and reported on a significant increase in plasma MDA values as long as 18h after the ride [24].

Oxidative stress is often reflected in a number of pathological processes which result in disease, poor productive and reproductive features in humans and animals. Jović et al. [25,26] observed the influence of long lasting physical activity of racehorses on the blood biochemical profile and concluded that free radical production results in extensive damage of the myocardium, muscles and liver. Due to such conditions numerous studies on the possible protective effects of dietary antioxidants and natural plant extracts have been carried out in the last decades. The common goal of such antioxidant supplementation is the enhancement of protective antioxidative mechanisms (both enzymatic and non enzymatic) and subsequent decrease in the concentration of peroxidation end-products such as MDA and ROMs. A correlation between the antioxidant activity of plant extracts, such as grape and rose-hip extracts and content of different groups of polyphenols has been repeatedly observed by

Katalinic et al. [27]. However, despite extensive studies the obtained results are still not consistent. Just as the studies on exercise-induced oxidative stress produced varied results, so did studies regarding dietary supplementation. The type of supplement, timing and the outcome measures differed among studies, thus making an overall interpretation difficult. A study carried out on cyclists by Morillas – Riuz et al. [28] described that on ingestion of a drink high on plyphenols the concentration of MDA did not significantly increase after exercise, compared to the placebo group where the increase was significant. At the same time polyphenolic antioxidants supplementation had no effect on plasma antioxidant capacity pre and post exercise. These results are not much different from ours, as the effect of diet and supplementation on MDA before and after exercise was not significant. Statistical analysis of our results revealed in November the lowest ROMs before (192.37 ± 16.88) and after (180.13± 14.62) exercise in dogs which prior reverting to the home-cooked diet were fed commercial dry food (Table 2). The significantly lower ($p<0.01$) values for ROMs obtained for this group of dogs in November indicate the persistence of the antioxidative effects of the commercial diet which were still present 60 days after reverting to the cooked diet. High quality commercial diets contain a wide selection of antioxidant ingredients. Such differences were not observed for MDA values, as there was no significant difference between groups when the diet and exercise factor were observed. Regardless the fact that the differences were not statistically significant it is evident that in September and November the MDA values were higher after exercise. It is noteworthy to emphasize that plasma MDA concentrations change over time after exercise. A study carried out on horses showed that the MDA concentration peak was reached 48 hours after a race [29], these changes were not concurrent with the changes measured for ROM. In our study blood sampling was done 30 minutes after exercise, thus not revealing the potentially significant differences which could have been possible at a later stage. However, it must be taken into account that recent studies by Gomez – Cabarera et al. [30] defined moderate exercise as an antioxidant, as the mild burst of ROS generated by training acts as a signal responsible for the activation of signaling pathways that lead to the induction of antioxidant enzymes. Macedo et al. [31] described that resveratrol (active polyphenol present in grape extract) supplementation of trained fireman did not promote additional plasma antioxidant capacity compared with the placebo group nor it was able to reduce the biomarkers of oxidative stress beyond the placebo levels. These results are in agreement with the results obtained in our study which showed no statistically significant difference for time, exercise and diet effect on ROMs, MDA levels for dogs supplemented with grape extract.

The FRAP assay is a test used to measure the biological antioxidant potential of serum/plasma samples. It allows the substantial measuring of the blood concentration of antioxidants as agents able to reduce the iron from its ferric (Fe3+) to its ferrous (Fe2+) form. The test provides a global measurement of many antioxidants including uric acid, ascorbic acid, α- tocopherol, glutathione, carotenoids and so on [32]. The results obtained in our study indicate a significant decrease in FRAP concentration

($p<0.05$) after exercise in June, before antioxidant supplementation was implemented (Table 1). Such difference was not present 60 days after supplementation (September) (Table 4) as the concentrations leveled up to an almost uniform value. Widen et al. [14] tested the effects of rose-hip extracts on erythrocyte antioxidant protection and reported a significant increase in the FRAP assay confirming that rose-hips contain many different antioxidant compounds among which vitamin C stands out due to its protective features. Serum FRAP values in November showed an interesting change, as 60 days after reversing to cooked meals all groups showed higher FRAP values after exercise. Nevertheless these differences were not statistically significant ($p>0.05$) (Table 4). This may result from redistribution of the tissue reserves of antioxidants to the oxidative site and/or from the upregulation of endogenous antioxidants as previously reported by Watson et al. [33]. Studies carried out by McAnulty et al. [34] revealed that the antioxidant potential of plasma generally increases after endurance or resistance exercise even in the absence of oxidative stress evidence Panza et al. [35] reported on the protective effects of polyphenols extracted from green tea in weight-trained man as FRAP values were significantly higher in the supplemented groups before and after exercise compared to the placebo group. Results obtained by Panza et al. [35] are different from ours as in our study there was no statistically significant FRAP change between groups, nor before and after exercise during the supplementation period (September). Such differences can be attributed to different experimental designs (humans vs. dogs, time of sampling, type of exercise, supplements used, and so on).

It can be concluded that in the present study the changes of biochemical parameters reflect a physiological response to standard regular exercise of army patrol service dogs. The increase in MDA and ROMs after exercise indicates the presence of oxidative stress 30 minutes after exercise. However, the antioxidative effects of rose-hip and grapeseed extracts are not conclusive, as multifactor ANOVA testing of the time, diet, and exercise factors did not reveal statistically significant differences for the measured parameters (ROM, MDA, FRAP) between September and November (Tables 2-4). Only one distinct exemption was recorded for the prolonged antioxidative effects reflected in significantly decreased ($p<0.01$) ROMs in dogs fed branded dry food 60 days after the end of such dietary regime (November). FRAP values tended to be higher ($p>0.05$) after exercise in all experimental groups in November, indicating on a possible redistribution and upregulation of numerous endogenous antioxidants during the experiment.

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EFEKTI DIJETARNE SUPLEMENTACIJE ŠIPURKOM I SEMENKAMA GROŽĐA NA PARAMETRE OKSIDATIVNOG STRESA U SERUMU PASA PRE I POSLE TRENINGA

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Cilj ovog rada bila je procena efekata šezdesetodnevne suplementacije balansiranog kuvanog obroka radnih pasa ekstraktima šipurka i semenki grožđa na serumske parametre oksidativnog stresa: ROM, MDA i FRAP pre i posle treninga. Do prvog uzorkovanja (juni) psi su hranjeni kuvanim izbalansiranim obrocima, nakon čega su raspoređeni u 4 ogledne grupe u skaldu sa režimom ishrane. Prvu grupu čine psi i dalje hranjeni kuvanim obrocima, druga grupa je hranjena visokokvalitetnom komercijalnom suvom hranom za radne pse, treću i četvrtu grupa čine psi kojima je u kuvane obroke dodato 500 mg ekstrakta šipurka ili 100 mg ekstrakta semenki grožđa. Nakon 60 dana suplementacije vršeno je uzorkovanje krvi, narednih 60 dana svi psi su hranjeni standardnom nesuplementiranom kuvanom hranom. Porast vrednosti MDA i ROMa 30 minuta nakon treninga ukazuje na oksidativni stres. Međutim antioksidativni efekti šipurka i semenki grožđa nisu ubedljivi obzirom da multifaktorijalna analiza varijanse ANOVA efekta vremena, dijete i treninga nije ukazala na statistički signifikantne razlike kako 60 dana nakon suplementacije tako 60 dana nakon obustave. Izdvaja se samo jedan jasan izuzetak za produžen antioksidativni efekat koji se ogleda u signifikantnom ($p<0,01$) smanjenju ROMa hranjenih komercijalnom hranom 60 dana nakon prestanka njenog korišćenja (novembar). Vrednosti FRAPa su bile više ($p>0,05$) nakon treninga u svim eksperimentalnim grupama u novembru, što ukazuje na moguću redistribuciju i regulaciju endogenih antioksidansa tokom ogleda.