Effects of the consumption of polyunsaturated fatty acids on the oxidative status of adult dogs[1]

Gabriel F. E. Pacheco[*] Rafael C. Bortolin[†] Paloma R. Chaves[‡] José C. F. Moreira[‡] Alexandre M. Kessler[*] and Luciano Trevizan[2*]

[*] Department of Animal Science, Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, 91540-000, Brazil

[†] Department of Civil and Environmental Engineering, Universidad de La Costa, Calle 58 # 55-66, CP 080002, Barranquilla, Atlántico, Colombia

[‡] Department of Biochemistry - Institute of Basic Health Sciences of the Federal University of Rio Grande do Sul, 90035-003, Brazil

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[2] Corresponding author: ltrevizan@ufrgs.br

ABSTRACT: The present study evaluated the alterations of the oxidative stress markers in adult dogs fed with high levels of PUFA from the mixture of soybean oil enriched with docosahexaenoic acid (DHA) and supplemented with a natural algae-based antioxidant (AOX). Twelve healthy adult (2 years old) Beagle dogs (6 males and 6 females, 11.20 ± 1.92 kg BW), were distributed in 2 completely randomized blocks design and fed with 4 experimental diets coated with 2 lipid sources: saturated (13% bovine tallow) or unsaturated (13% soybean oil enriched with DHA), supplemented or not with 500 mg of AOX for 4 wk, intercalated with a 4 wk adaptation period. Blood samples were collected on days 0, 15, and 30 of each block. Glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), sulfhydryl group (SH), protein carbonylation, thiobarbituric acid reactive substances (TBARS), and total reactive antioxidant potential (TRAP) were evaluated in the serum, while GSH-Px, SOD, glutathione S-transferase (GST), catalase (CAT), SH, and TBARS were measured in erythrocytes. There was no significant difference in most of the oxidative markers evaluated. In contrast, GST activity in erythrocytes was greater in the animals that consumed the diets coated with bovine tallow compared to dogs that consumed diets coated with soybean oil enriched with DHA ($P < 0.05$). Serum from dogs fed on diets supplemented with AOX presented greater TRAP values ($P < 0.05$). These data demonstrate that the concentrations of unsaturated fatty acids used in the diets for dogs were not sufficient to cause large changes in the oxidative status. It was not possible to evaluate the efficiency of the natural antioxidant in maintaining the oxidative balance of the animals as it appears that the oxidative status of the dogs was not challenged by the unsaturated diets. Our findings also suggest that dogs, as descendants from carrion carnivores, may have some natural protection against oxidation.

Key words: algal meal, canines, free radicals, lipid oxidation, oxidative stress

INTRODUCTION

Ingredients rich in PUFA omega-3 (**n-3**) have been added to diets for pets due to their beneficial health potential for the animals (Hall et al., 2011; Hadley et al., 2017). However, experimental data that show the effect of such additions on the oxidative status in dogs, are scarce (Hall et al., 2003; LeBlanc et al., 2005). Algae meal is rich in n-3 fatty acids, especially eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**). When consumed, EPA and DHA accumulate in the cell membrane and play a key role in membrane structure and cellular functions (Calder, 2012). Because they have a high number of double bonds in their molecules, PUFA are highly susceptible to peroxidation. Thus, it is recommended to include adequate concentrations of antioxidants in the diets to reduce the vulnerability of the cell membrane to the action of free radicals and nitrogen reactive species and reactive oxygen species (Wander et al., 1997). The imbalance between the production of free radicals and the ability of the body's antioxidant systems to eliminate them is known as oxidative stress. The inclusion of natural ingredients with antioxidant properties has been investigated in companion animals (Salas et al., 2008; Ogoshi et al., 2016), mainly because supplementation with synthetic antioxidants, such as butylated hydroxytoluene (**BHT**) and butylated hydroxyanisole (**BHA**), has been perceived as unwanted by the owners. The present study evaluated the alterations of the oxidative stress markers in adult dogs fed with high levels of unsaturated fatty acids from the mixture of soybean oil enriched with DHA supplemented with natural algae-based antioxidant. Our hypothesis is that diets with greater concentrations of PUFA may require greater amount of tissue antioxidants to preserve the stability of the cell membranes.

MATERIALS AND METHODS

All procedures performed on the dogs were approved by the ethics committee of the Federal University of Rio Grande do Sul before the commencement of the experimental period, protocol number 29989, and were conducted according to ethical and animal welfare standards.

Experimental design and animals

Twelve, healthy, adult (aged 2 years old) Beagle dogs (6 males and 6 females, 11.20 ± 1.92 kg BW), with a BCS of 3.40 ± 0.23 based on a 5-point scale (Case et al., 2011), were used in a randomized block design with 4 treatments and 2 blocks during the 8 wk study period. Each block included a 4 wk adaptation phase in which all dogs received the same extruded commercial diet (Topi Dog, Nutribarrasul Alimentos Pet Ltda, Rio Grande do Sul, Brazil), free of long chain PUFA derived from fish oil. This period was used to standardize the oxidative status of the dogs. After the adaptation period, the dogs were fed the experimental diets. The dogs were individually allocated in metabolic cages (0.80 × 0.70 × 0.90 m) equipped with feeders and drinkers in an air-conditioned room with a temperature of 24°C and photoperiod control (14 h light: 10 h dark). The dogs were fed to maintain BW during the experiment. The BW was measured once a week and the amount of food was often adjusted when the BW decreased or increased by > 0.5 kg. The amounts supplied were initially calculated based on the ME content, which was estimated from the chemical composition of the diet, to meet the energy requirement recommended by NRC (2006) for the maintenance of dogs. The dogs were fed twice a day (0800 h and 1800 h) and had ad libitum access to fresh water throughout the experimental period.

Prior to the experiment, all dogs were vaccinated and evaluated with complete blood count and biochemical profiles, parasitological tests, and clinical examinations to ensure their health.

Experimental diets

The basal diet was produced by Nutribarrasul Alimentos Pet Ltda (Rio Grande do Sul, Brazil) (Table 1) and designed to be supplemented with one of two lipid sources: 13 g of bovine tallow or 13 g of soybean oil enriched with DHA (All-G-Rich, CCAP 4087/2; Alltech Inc., Nicholasville, KY)/100 g of basal diet. Four experimental diets were prepared using each of the lipid sources, supplemented or not supplemented with 500 mg of an algae-based natural antioxidant (**AOX** - EconomasE, Alltech Inc. Araucária, Brazil)/kg diet: 1) soybean oil enriched with DHA (**UNS**, unsaturated fatty acid), 2) soybean oil enriched with DHA and the natural algae-based antioxidant (**UNS AOX**, unsaturated fatty acid + EconomasE), 3) bovine tallow (**SAT**, SFA fat), or 4) SFA with AOX (**SAT AOX**; SFA + EconomasE) (Table 2). This level of AOX supplementation has been seen to have a beneficial effect on cats (Ogoshi et al., 2016). All diets were formulated to guarantee the nutritional and energetic needs of adult dogs (FEDIAF, 2016).

Blood collection

Blood samples were collected on days 0, 15, and 30 of each experimental block. After 12 h nocturnal fasting, blood samples were obtained by venous puncture of the cephalic vein using two vacutainer tubes: one containing EDTA for collection of erythrocytes and one without EDTA for serum collection (final concentration, 1.5 g/L for each tube). The erythrocytes and serum were extracted by centrifugation at 3000 × *g* for 10 min and stored at –80°C for subsequent analyzes. The erythrocytes were used to analyze the sulfhydryl group (**SH**), the enzymes catalase activity (**CAT**), glutathione peroxidase activity (**GSH-Px**), superoxide dismutase activity (**SOD**), glutathione S-transferase activity (**GST**) and thiobarbituric acid reactive substances (**TBAR**S), while the SH, protein carbonylation (**PC**), GSH-Px activity and SOD enzymes activity, TBARS, and the total reactive antioxidant potential (**TRAP**) were analyzed in the serum.

Quantification of antioxidant enzymatic activity

The activity of SOD (EC 1.15.1.1) in the erythrocytes was determined by the quantification of the adrenaline auto-oxidation inhibition in a spectrophotometer at 480 nm, as previously described by Misra and Fridovich (1972). The same protocol was used to determine the extracellular activity of CuZn-SOD (**EC-SOD**) in the serum samples. Results are reported as the unit of SOD/mg protein. The activity of blood CAT (EC 1.11.1.6) was determined by measuring the rate of H_2O_2 decomposition from the absorbance reading in a spectrophotometer at 240 nm (Aebi, 1984). The CAT activity is expressed as units of CAT/mg protein. The activity of GSH-Px (EC 1.11.1.9) was determined by measuring the oxidation rate of NADPH in a spectrophotometer at 340 nm, as previously described by Wendel (1981). GSH-Px activity was expressed as the unit: (nmol NADPH oxidized/min)/mg protein.

Measurement of non-enzymatic antioxidant defenses

The TRAP test was used as an indicator of plasma non-enzymatic antioxidant capacity based on the peroxyl radical [generated by AAPH solution, 2,20-azobis (2-amidino propane), with luminol] extracted from the components of the serum sample (Lissi et al., 1992). The reading was taken by chemiluminescence emission. Briefly, the AAPH solution was prepared and the luminol (system) was added. The first reading was only taken after a period of 2 h during which the system stabilized. Readings took 60 min to complete after the addition of each sample. The results were transformed into percentages and the area under the curve (**AUC**) was calculated by GraphPad software (San Diego, CA, USA) as described by Dresch et al. (2009). When the AUC of the sample was lower than that of the system, the sample had a greater antioxidant capacity.

Quantification of sulfhydryl group

The oxidative status of the thiol group was analyzed to quantify the total reduced SH in the sample (Ellman, 1959). Briefly, to measure total SH content, an aliquot of 100 µg of sample (erythrocyte or serum) was diluted in phosphate buffered saline and 10 mM 5,5'-dithiobis-2-nitrobenzoic acid and read in the spectrophotometer at 412 nm after 60 min of incubation at 25°C.

Protein carbonylation

Oxidative damage to serum protein was measured by quantification of the carbonyl group, based on the reaction with dinitrophenylhydrazine (**DNPH**) (Levine et al., 1990). The protein was precipitated by the addition of 10% trichloroacetic acid and was re-solubilized in DNPH. The absorbance was then read in a spectrophotometer at 370 nm. The results are presented as nmol of carbonyl/mg protein.

Lipid peroxidation

The TBARS test was used as an indicator of plasma lipid peroxidation, which is widely accepted as a method to measure the lipid redox status, as previously described by Draper and Hadley (1990). The TBARS consisted of the reaction of the final product of lipid peroxidation, malondialdehyde (**MDA**) with thiobarbituric acid (TBA, 4,6-dihydroxypyrimidine-2-thiol) under heating and low pH. The TBARS were determined at 532 nm and expressed as nmol/mg lipid.

Statistical analysis

All data were analyzed by analysis of variance (ANOVA) using the statistical package SAS University Edition. Contrasts were used to evaluate the effect of lipid sources and natural algae antioxidant supplementation (UNS vs. SAT and AOX vs. without AOX). The data were subjected to a normality test. Values that did not show normal distribution were transformed using log and square root. The statistical model used the degree of saturation, block, and treatment. The values of $P < 0.05$ were considered significant.

RESULTS

The dogs consumed diets enriched with 13% bovine tallow or with 13% soybean oil enriched with DHA without any evidence of refusal or any digestive disorder. All animals consumed 100% of the daily allocated feed and maintained BW and BCS throughout the experimental period. Throughout the experimental period, the dogs weighed (mean ± standard deviation) 12.0 ± 1.42, 12.0 ± 2.10, 11.0 ± 2.40, and 11.0 ± 1.43 kg for UNS, UNS AOX, SAT, and SAT AOX experiments, respectively. The BCS was also stable throughout the study, with mean values of 3.5, 3.7, 3.4, and 3.3 for UNS treatments; UNS AOX; SAT and SAT AOX, respectively, with score 3 being the ideal on the 5-point scale that was used (Case et al., 2011). Also, faecal score was similar among diets, close to the ideal for all diets.

Lipid oxidation

There was no evidence of lipid peroxidation in dogs in any of the treatments. The value of TBARS in serum and erythrocytes did not differ significantly between the four treatments, nor within each treatment over the experimental period (Table 4 and Table 5).

Enzymatic antioxidant activity in erythrocyte and serum

The antioxidant activity of GSH-Px and SOD enzymes showed no significant difference between serum and erythrocyte treatments regardless of the lipid source used in the diet and the presence or absence of AOX. No change in CAT activity was observed in erythrocytes of dogs fed any of the treatments. On the other hand, GST activity increased ($P < 0.05$) in the erythrocytes of the animals fed with diets coated bovine tallow compared to the animals fed diets coated with soybean oil enriched with DHA (Table 5).

Non-enzymatic antioxidant defense in red cells and serum

Substances such as polyphenols, vitamin E, vitamin C, glutathione, taurine, and reduced SH have chemical characteristics that directly affect the antioxidant balance of serum and tissues. Although the level of each of these components was not determined in the experimental diets, we observed a drop ($P < 0.05$) in the free radical scavenging potential, which was detected in sera from dogs supplemented with AOX, as determined by the TRAP test.

Protein oxidation

The level of protein oxidation, measured by the quantification of the carbonyl group in the serum and by the thiol content in the serum protein and erythrocytes, did not change during the experimental period. No effect of treatments was observed at any of the evaluated times.

DISCUSSION

Lipids are an indispensable source of nutrients for carnivores. In addition to providing essential fatty acids, they contribute to the greater energy value and improved palatability of a diet. Lipids can be sourced from the composition present in the ingredients or by the addition of animal or vegetable fat.

In general, trends for the development of high-quality diets are associated with high fat content as an energy source. This increases the final digestibility of the diets, since the utilization of this portion is extremely high in dogs (Marx et al., 2017). The lipid sources incorporated into the diets are protected against oxidation by the inclusion of antioxidants during the production process. Usually artificial antioxidants like BHA, BHT, propyl gallate, among others, are added to maintain the stability of the product and, apparently, to protect the components of the diet. However, our concern is associated with how this process affects the oxidation status of the animals that consume the products. According to several studies, lipid tissue concentration is modulated by food

concentration (Hall et al., 2006; Waldron et al., 2012). Because it increases the amount of PUFA in the diet, it must affect the fatty acids profile of the tissues.

With the advent of increased lipid composition of dietary energy, mainly in the form of PUFA, it is assumed that the tissue concentration is altered in a similar way to the diet concentration. This challenging factor or even deleterious to the oxidative balance of tissues because PUFA are recognized to be more susceptible to oxidation.

Previous studies have investigated numerous effects of n-3 fatty acid supplementation on the health of dogs and cats and the observed results are conflicting. Although some studies suggest that n-3 supplementation is beneficial to animal health (Ogilvie et al., 2000; Mueller et al., 2004; Mueller et al., 2005), its excess has been seen to cause adverse effects (Wander et al., 1997; Lenox and Bauer, 2013). This discrepancy between the results is justified, in part, by the scarcity of data on efficient levels of health maintenance and the maximum level of possible supplementation for pet animals.

Recently, Lenox and Bauer (2013) published a review on the potential adverse effects of n-3 supplementation for companion animals, highlighting the risks related to the excessive consumption of PUFA on the platelet functions, gastrointestinal, healing time, potential exposure to toxins and excess nutrients, weight gain, alterations in immunological functions, and lipid peroxidation, evidencing that supplementation may negatively affect the animal's health.

It is known that PUFA-rich diets such as n-6 and n-3 have the potential to increase the peroxidation of cells and tissues (Hall, 1996). Due to the number of double bonds, PUFA incorporated into cell membranes and organelles, such as mitochondria, endoplasmic reticulum, and peroxisomes, are particularly vulnerable to attacks by free radicals and reactive species. Evidence of increased lipid peroxidation has been found in studies with humans and animals consuming diets supplemented with n-3 (LeBlanc et al., 2005)

The generation of reactive species from oxygen occurs due to the successive addition of electrons in its molecule during the production of ATP in the respiratory chain. When in excess, reactive oxygen species and nitrogen reactive species suppress the body's antioxidant capacity and induce the formation of lipid peroxidation and protein oxidation products. When this imbalance occurs, oxidative stress is established, which can cause damage to DNA, RNA, lipids, and proteins.

The oxidation rate rises in response to increased consumption of oxidized lipids, the presence of pro-oxidant components in food, deterioration of PUFA, and the low consumption of exogenous antioxidants (Delles et al., 2014). According to NRC (2006) and FEDIAF (2016), supplementation of exogenous antioxidants is necessary to avoid vitamin E deficiency when PUFA consumption is high. However, establishing adequate levels of vitamin E intake to avoid animal deficiencies and preventing cell membrane peroxidation is very difficult given the variability of vitamin E and PUFA concentrations in the experimental diets used in each study.

LeBlanc et al. (2005), for example, did not observe changes in plasma concentrations of oxidated co-products or vitamin E deficiency after 12 wk of supplementation with fish oil for young dogs [n-6: n = 3 ratio = 3.4:1, EPA = 1.75 g/kg of diet and DHA = 2.2 g/kg of diet in DM, and did not find any evidence of oxidative damage in dogs in any of the treatments.

In contrast, Wander et al. (1997) found a difference in the plasma concentration of vitamin E when evaluating the effect of different n-6: n-3 (31:1, 5.4:1 and 1.4:1) relationships in elderly dogs. Dogs who consumed diets with n-6: n-3 ratio of 1.4: 1 had 20% lower plasma vitamin E concentrations than dogs that consumed diets with 31: 1 ratio, as well as a greater number of lipid peroxidation products in plasma and urine.

Although in our study we did not measure the serum concentration of vitamin E in dogs, we observed that the level of fat present in the treatments was not enough to cause changes in the oxidative stability of the Beagle specimen, or that these dogs are adaptable to the consumption of

levels of PUFA via diet. The diets contained approximately 20% of acid hydrolyzed fat (**AHF**), of which approximately 60% were in the form of PUFA in treatments with soybean oil enriched with DHA and 18% for treatments with bovine tallow (Table 3). This AHF concentration is much greater than the minimum recommendation in the FEDIAF (2016) for adult dogs (5% in DM). However, it is within the values recommended by NRC (2006) for dogs in maintenance (4–33% in DM).

Although Wander et al. (1997) reported increased lipid peroxidation in dogs supplemented with fish oil, we did not observe oxidative changes in serum and erythrocytes of dogs fed soybean oil enriched with DHA. TBARS are based on the thiobarbituric acid reaction of the hydroperoxide decomposition products and in the present study, no difference was observed between the treatments.

Likewise, serum and erythrocyte activity of SOD and GSH-Px and CAT in erythrocytes were not modified with the greatest amount of PUFA in UNS and UNS AOX treatments. This result was surprising, since the consumption of PUFA-rich foods was expected to raise antioxidant enzyme activities in response to increased oxidative stress.

The greater activity of GST observed in the erythrocytes of dogs that consumed saturated fat-coated foods may be an adaptive response to the production of toxic compounds derived from saturated fatty acids. The GST plays an important role in the defense against oxidative stress (Goto et al., 2009) and in the elimination of cellular xenobiotics and carcinogenic compounds (Aliya et al., 2003).

In a comparative study between elderly and young dogs, Kil et al. (2010) observed an increase in the expression of the glutathione S-transferase pi 1 gene (*GSTP1*), responsible for the synthesis of GST, in elderly dogs that consumed foods rich in saturated fatty acids. To our knowledge, however, no studies have been conducted to evaluate the effect of saturated fatty acid consumption on GST activity in dog erythrocytes. Hence, further studies are needed to explore the

mechanisms regulating the level of GST activity in response to the consumption of saturated fatty acids.

Another unexpected result was the greater antioxidant capacity present in the serum of the animals that consumed foods without supplementation of the natural antioxidant in relation to the animals that consumed diets with antioxidant supplementation (i.e., AOX treatments) ($P < 0.05$).

Evaluation of the antioxidant capacity of the ingredients is often hampered by the inability to induce oxidative stress in animals. This is due to the fact that it is necessary to promote large changes to the extent that it is sufficient to induce measurable changes in oxidative markers, while ensuring that the protocol is compatible with animal welfare (Ferreira et al., 2014).

In our study, dogs were subjected to PUFA-rich diets to modify oxidative stress markers, but it appears that the concentration used was insufficient to cause an imbalance between reactive species generation and elimination by the antioxidant defense systems of the animals. The reason for this observation is that the NRC (2006) cites diets with more than 50% of fatty acids in DM and therefore, perhaps, dogs are resistant to high consumption of PUFA.

Implications

The inclusion of high amounts of PUFA in dog diets has been questioned for the possibility of causing adverse effects such as lipid peroxidation. However, the present study showed that the consumption of foods that include 13% soybean oil enriched with DHA as a source of PUFA did not alter the oxidative status of dogs under maintenance. Likely, other factors and mechanisms that have not been well studied, such as alterations in gene expression, act on the oxidative equilibrium of the body when high amounts of PUFA are consumed via the diet, and hence, further studies are needed.

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Table 1. Basal diet composition [1]

Item	Amount, g/kg as fed basis
Poultry byproduct meal	295
Corn grain	269
Broken rice	158
Full-fat rice bran	95.0
Meat meal 45% CP	58.0
Wheat bran	52.8
Corn gluten 60% CP	52.8
Caramel dye	8.45
Salt	5.28
Premix mineral and vitamin[2]	4.22
Yucca extract	0.32

[1]The basal diet was reduced in total lipids and the formula was designed to be supplemented with beef tallow or soybean oil + microalgae meal (All-G Rich, CCAP 4087/2; Alltech Inc., Nicholasville, KY) at the level of 13 g/100 g diet. The diets were prepared by Nutribarrasul Alimentos Pet Ltda., Barra do Ribeiro, Rio Grande do Sul, Brazil.

[2]The mineral-vitamin premix added assured per kg of diet: vitamin A, 7,000 IU; vitamin B1, 2 mg; vitamin B12, 25 g; riboflavin, 4 mg; pyridoxine, 2 mg; vitamin D3, 600 IU; vitamin E, 50 IU; vitamin K3, 1 mg; niacin, 30 mg; folic acid, 0.2 mg; pantothenic acid, 10 mg; biotin, 0.03 mg; cobalt, 10 mg; copper, 7 mg; iron, 80 mg; iodine, 1.5 mg; manganese, 5 mg; selenium, 0.2 mg; zinc, 100 mg; butylated hydroxytoluene, 150 mg.

Table 2. Chemical composition of basal diet and experimental diets containing soybean oil + microalgae or bovine tallow, with or without algae-based natural antioxidant, DM basis[1]

Item	Diets[2]				
	Basal	UNS	UNS AOX	SAT	SAT AOX
DM, %	92.1	93.6	94.2	94.7	94.0
CP, %	23.7	26.2	26.2	26.2	26.0
Ash, %	6.77	7.22	7.24	7.09	7.10
Acid hydrolyzed fat, %	18.2	20.1	19.0	20.9	21.1
Crude fiber, %	2.87	3.63	3.47	2.34	2.35
GE, kcal/g	5,188	5,379	5,361	5,351	5,355

[1]The composition of the diet was determined from the analysis of subsamples collected throughout the experiment. Accuracy was assured by adequate replication with acceptance of the mean value of 5% between samples.
[2]UNS = diet with unsaturated fatty acid (soybean oil + microalgae flour); UNS AOX = diet with unsaturated fatty acid with algae-based antioxidant (EconomasE); SAT = diet with SFA (bovine tallow); SAT AOX = diet with SFA and antioxidant based on algae (EconomasE).

Table 3. Fatty acid composition of experimental diets containing soybean oil + microalgae (UNS) or bovine tallow (SAT)[1]

Fatty acids	g FA/100 g of dietary FA	
	UNS	SAT
C4:0	0.02	0.05
C6:0	0.05	0.11
C8:0	0.03	0.09
C10:0	0.04	0.11
C11:0	0.09	0.30
C12:0	0.04	0.13
C13:0	0.10	0.35
C14:0	0.79	3.92
C15:0	0.18	0.68
C16:0	23.3	37.9
C17:0	0.33	0.08
C18:0	8.99	25.2
C20:0	0.67	0.38
C22:0	0.11	0.11
C24:0	0.24	0.10
∑ SFA[2]	35.0	69.5
C14:1(n-5)	0.04	Nd
C16:1(n-7)	1.43	5.02
C17:1(n-7c)	0.12	0.81
C18:1(n-9t)	0.29	3.33
C18:1(n-9c)	2.06	1.96
C20:1(n-9c11)	0.36	0.39
C22:1(n-9)	0.05	0.11
C24:1(n-9)	0.03	Nd

∑ MUFA[3]	4.38	11.6
C18:2(n-6t9t12)	0.81	0.12
C18:2(n-6c)	58.2	17.0
C20:2(n-6c)	0.06	0.09
C22:2(n-6c)	0.07	0.09
C18:3(n-6)	0.35	0.20
C18:3(n-3)	0.06	0.80
C20:3(n-3c)	0.56	0.12
C20:4(n-6)	0.16	0.14
C20:5(n-3)	0.31	0.13
C22:6(n-3c)	0.05	Nd
∑ PUFA[4]	60.6	18.7
∑ (n-6) AG[5]	59.6	17.6
∑ (n-3) AG[6]	1.29	1.18
Ratio (n-6):(n-3)	46.2:1	14.9:1

FA = fatty acids; Nd = not detected (detection limit, 1 ng).
[1]Analysis performed by the Integrated Nucleus of Development in Laboratory Analyzes (Santa Maria, Rio Grande do Sul, Brazil).
[2] Sum of SFA: 4:0 + 6:0 + 8:0 + 10:0 + 11:0 + 12:0 + 13:0 + 14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0 + 22:0 + 24:0 + 25:0.
[3] Sum of MUFA: 14:1 (n-5) + 16:1 (n-7) + 17:1 (n-7c) + 18:1 (n-9t) + 18:1 (n-9c) + 20:1 (n-9c11) + 22:1 (n-9) + 24:1 (n-9).
[4] Sum of PUFA: 18:2 (n-6t9t12) + 18:2 (n-6c) + 20:2 (n-6c) + 22:2 (n-6c) + 18:3 (n-6) + 18:3 (n-3) + 20:3 (n-3c) + 20:4 (n-6) + 22:6 (n-3c).
[5] Sum of fatty acids (n-6): 18:2 (n-6t9t12) + 18:2 (n-6c) + 20:2 (n-6c) + 22:2 (n-6c) + 18:3 (n-6) + 20:4 (n-6).
[6] Sum of fatty acids (n-3): 18:3 (n-3) + 20:3 (n-3c) + 22:6 (n-3c).

Table 4. Oxidative markers in the serum of dogs fed with unsaturated diets (UNS), unsaturated with antioxidant (UNS AOX), saturated (SAT) and saturated with antioxidant (SAT AOX)

Item	Diets				SEM	*P*-value	
	Time, d	UNS	UNS AOX	SAT	SAT AOX	UNS × SAT	AOX × without AOX
Carbonila,	0	68.4	73.3	88.1	61.2	23.7	
nmol/mg protein	15	40.6	63.1	69.7	85.2	12.6	0.9231	0.7060
	30	77.2	64.9	76.3	27.7	23.7	
Sulfhydryl,	0	86.3	63.0	77.2	60.0	9.82	
µmol/mg protein	15	47.4	79.2	59.2	65.1	11.1	0.6817	0.3908
	30	77.2	61.6	76.1	61.1	11.7	
Glutathione peroxidase	0	271	327	308	316	45.9	
GSH-Px, IU/mg protein	15	300	336	311	248	38.5	0.5124	0.8585
	30	287	293	300	231	40.8	
Superoxide dismutase	0	131	131	141	179	13.7	
SOD, IU/mg protein	15	174	163	169	156	11.9	0.5755	0.4887
	30	148	158	139	145	6.54	
TBARS	0	1.37	1.43	1.74	1.76	0.24	0.4023	0.9134

nmol/mg protein	15	2.17	2.06	2.01	2.34	0.21		
Sulfhydryl	30	2.13	2.17	2.23	1.99	0.35		
TRAP	0	55238	73999	52572	78001	9284		
area under the curve	15	57437	92006	63266	74566	9008	0.5107	0.0069
	30	78732	90438	81670	75237	10135		

IU: international units; TBARS: thiobarbituric acid reactive substances; TRAP: Total non-enzymatic antioxidant potential

Table 5. Oxidative markers in the erythrocyte of dogs fed with unsaturated diets (UNS), unsaturated with antioxidant (UNS AOX), saturated (SAT) and saturated with antioxidant (SAT AOX)

Item	Diets				SEM	*P*-value	
	Time (d)	UNS	UNS AOX	SAT	SAT AOX		UNS × SAT	AOX × without AOX
Catalase	0	3.66	3.08	3.34	3.28	1.54		
CAT IU/mg protein	15	3.33	3.03	3.34	3.21	1.62	0.4973	0.6713
	30	3.51	3.60	2.79	2.97	1.26		
Sulfhydryl	0	20.0	20.6	20.5	22.6	0.52		
µmol/mg protein	15	22.4	22.0	23.1	19.5	0.55	0.9367	0.8037
	30	22.5	21.4	20.8	22.0	0.51		
Glutathione peroxidase	0	27.1	24.5	24.4	26.2	1.42		
GSH-Px, IU/mg protein	15	26.3	27.7	24.2	25.7	0.87	0.1292	0.0868
	30	27.3	27.9	24.5	29.3	1.16		
Superoxide dismutase	0	0.20	0.19	0.20	0.18	0.10		
SOD, IU/mg protein	15	0.15	0.16	0.15	0.15	0.10	0.7634	0.7307
	30	0.14	0.16	0.15	0.15	0.10		
TBARS	0	37.8	35.4	41.1	39.3	8.09	0.5641	0.8737

nmol/mg protein	15	38.9	41.1	46.6	43.9	8.44		
	30	35.3	43.1	35.2	35.3	7.33		
Glutathione S-transferase	0	1.89	2.00	2.80	2.44	0.44		
GST, IU/mg protein	15	1.92	2.27	2.40	2.34	0.42	0.0404	0.9577
	30	1.89	1.89	2.58	2.46	0.43		

IU: international units; TBARS: thiobarbituric acid reactive substances