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Article *in* Animal Science Journal · November 2019

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Received: 30 March 2019 | Revised: 7 August 2019 | Accepted: 2 September 2019

DOI: 10.1111/asj.13294

ORIGINAL ARTICLE

Animal Science Journal WILEY

Microalgae *Schizochytrium* sp. as a source of docosahexaenoic acid (DHA): Effects on diet digestibility, oxidation and palatability and on immunity and inflammatory indices in dogs

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Abstract

The objective of this study was to evaluate the effects of the microalgae Schizochytrium sp., as a dietary source of docosahexaenoic acid (DHA), on diet palatability, coefficients of total tract apparent digestibility (CTTAD) of nutrients and metabolizable energy (ME), blood variables and indicators of immunity in dogs. We also evaluated oxidative stability. Two diets containing 0 and 0.4% of microalgae *Schizochytrium* sp. were evaluated in three experiments. On Experiment I the palatability of diets containing 0% versus 0.4% microalgae was compared. In Experiment II test diets were offered for 30 days to determine digestibility, fecal characteristics, and blood parameters. In Experiment III, the oxidative stability of diets containing microalgae versus anchovy oil was evaluated. There was a higher intake ratio of the diet containing microalgae ($p < .05$). The ME and CTTAD of nutrients increased ($p < .05$), except for ether extract after acid hydrolysis, with the inclusion of the microalgae in diet. The amount of monocytes and phagocytic granulocytes was higher ($p < .05$) in dogs fed 0.4% microalgae. There was greater oxidative stability for the sample containing microalgae. The addition of 0.4% microalgae presented high palatability, increased phagocytic cell numbers, and demonstrated oxidative stability superior to anchovy oil.

KEYWORDS
algae, immune system, lipids, omega 3

1 | INTRODUCTION

Studies on companion animal nutrition have been seeking to increase the longevity and quality of life of pets. In order to optimize nutritional status and to meet nutritional requirements, varieties of ingredients are utilized. In this context, polyuunsaturated fatty acids (PUFA) are important nutrients, being essential to growth and survival of dogs as well as providing an energy source.

Inclusion of omega 3 (n-3) PUFA in diets can improve nutritional quality profiles and are able to modulate inflammatory response. The eicosapentaenoic (EPA, 20:5, n-6) and docosahexaenoic (DHA, 22:6, n-3) fatty acids are of special importance. These compounds are precursors of eicosanoids (prostaglandins, leukotrienes, thromboxanes, and prostacyclins) with less inflammatory action, resolvins, and protectins, which reduce inflammation (Calder, 2012).

Studies in dogs and other species have shown DHA to modulate the inflammatory response, neurodevelopment, improvement in cognitive ability and in the immune system (Heinemann, Waldron, Bigley, Lees, & Bauer, 2005; Kelley, Siegel, Fedor, Adkins, & Mackey, 2009).

Considering that the conversion of alpha-linolenic acid to DHA is inefficient in dogs (Bauer, 2007), dietary sources are necessary. The main DHA sources are marine and cold-water fish oil (Kus & Mancini-Filho, 2010). However, the availability of these traditional sources is becoming limited, due to species seasonality, contamination of natural fish environments, and higher levels of vegetable-based diets fed to farmed fish (Lenihan-Geels, Bishop, & Ferguson, 2013). Therefore, alternative sources should be investigated.

Microalgae such as the genus *Schizochytrium* are potential alternatives to conventional omega 3-PUFA sources, given their nutritional value and high DHA concentration (approximately 20%). In addition, microalgae do not have seasonal variation, do not generate marine pollution, present high potential of production, and may have lower processing costs compared to fish (Junwei et al., 2015). However, further studies are needed regarding the stability of microalgae DHA for commercial use, in addition to effects on the gastrointestinal system, blood parameters, and immunity processes of dogs.

The objective of this study was to evaluate the diet palatability, the coefficients of total tract apparent digestibility (CTTAD) of nutrients, metabolizable energy (ME), fecal characteristics, and blood and immunity variables in dogs fed microalgae *Schizochytrium* sp. as a source of DHA. Furthermore, the oxidative stability of diets containing microalgae or anchovy oil was compared.

2 | MATERIAL AND METHODS

Three experiments were approved by the Committee of Ethics on Animal Use of the Sector of Agrarian Science of the Federal University of Paraná, Curitiba-PR, Brazil, under protocol number 027/2017. Experiments I and II presented the same diet, facilities and animal health conditions.

2.1 | Diets

Two test diets based on a commercial dry extruded diet for adult dogs were compared: control (no microalgae) and test (0.4% *Schizochytrium* sp. microalgae, Alltech Inc). The diet contained the following ingredients: poultry byproduct meal, meat meal, ground whole corn, soybean meal, poultry fat, swine liver hydrolyzate, sodium chloride, citric acid, antioxidants (BHA, BHT), propionic acid, vitamin A, vitamin D3, vitamin E, vitamin B1, vitamin B6, vitamin B12, vitamin K3, nicotinic acid, folic acid, biotin, calcium pantothenate, zinc sulfate, calcium iodate, sodium selenite, sulfate copper, iron sulfate, manganese monoxide, manganese sulfate, and zinc oxide. The analysed chemical composition of diets is described in Table 1.

Prior to each feeding, 0.4% microalgae were weighed with a digital scale (MH-Series, PocketScale) and subsequently added and manually homogenized in the diets, supplementing 814 mg of DHA/kg. The microalgae physical characteristics were a finely ground powder. Its chemical composition is shown in Table 2.

2.2 | Experiment I: Palatability assay

2.2.1 | Animals and facilities

In total 16 adult Beagle dogs (1.5 years old), eight males and eight females, with average body weight of 10.5 ± 1.8 kg were used in the palatability trial. The animals were individually housed in concrete kennels (5 m long × 2 m wide) with shelter and solarium for two days.

2.2.2 | Palatability protocol

Palatability was measured using a 2-plate test using diets containing 0 or 0.4% microalgae. The test was performed for two consecutive days. The two foods were offered simultaneously to the dogs once a day (08:00 hr). The amount supplied was 30% higher than the recommendations of the Nutrient Research Council (National Research Council Committee on Dog & Cat Nutrition [NRC], 2006) for the maintenance of adult dogs. Once one of the diets was completely consumed, both bowls were withdrawn and the amount remaining was quantified. The relative position of bowls was alternated on the second day of the experiment so that the animal was not conditioned to the feeding site.

Palatability test was determined using the intake ratio and the first choice between the diets offered to the dogs. The first choice was defined by noting the first bowl that the animal approached. To determine the intake ratio, the quantities supplied and remaining

TABLE 1 Analysed chemical composition (%, dry matter basis) of experimental diets

	Microalgae (%)	
Items (%)	**0**	**0.4**
Dry matter	92.13	91.93
Crude protein	21.98	21.35
Ether extract in acid hydrolysis	8.02	8.40
Ash	6.02	6.38
Crude fiber	3.67	3.72
Calcium	0.89	0.89
Phosphorus	0.51	0.55
Gross energy (kcal/g)	4.37	4.47
Fatty acid composition		
C18:2n6 (Linoleic acid)	2.0	1.98
C20:4n6 (Arachidonic acid)	0.03	0.02
C18:3n3 (Alpha-Linolenic acid)	0.10	0.10
C22:6n3 (Docosahexaenoic acid, DHA)	—	0.08
C20:5n3 (Eicosapentaenoic acid, EPA)	—	0.006

Note: Enrichment per kg of product: vitamin A (retinol) = 20,000 IU; vitamin D3 = 2,000 IU; vitamin E (alpha-tocopherol) = 48 mg; vitamin K3 = 48 mg; vitamin B1 = 4 mg; vitamin B2 = 32 mg; pantothenic acid = 16 mg; niacin = 56 mg; choline = 800 mg; Zn = 150 mg; Fe = 100 mg; Cu = 15 mg; I = 1.5 mg; Mn = 30 mg; selenium = 0.2 mg.

TABLE 2 Chemical composition of the microalgae *Schizochytrium* sp. (%, dry matter)

Items (%)	Dry matter (%)
Moisture	3.70
Crude protein	9.53
Ether extract	57.20
Crude fiber	9.00
Ash	3.60
Calcium	0.34
Phosphorus	0.47
Fatty acid composition	
C18:2n6 (Linoleic acid)	0.01
C20:4n6 (Arachidonic acid)	0.06
C18:3n3 (Alpha-Linolenic acid)	0.03
C22:6n3 (Docosahexaenoic acid, DHA)	20.48
C20:5n3 (Eicosapentaenoic acid, EPA)	0.27

were quantified using the following equation: Intake ratio = g of diet A or B intake/g of total food consumed (A + B) ×100.

2.2.3 | Statistical analyses

Data were analysed according to a completely randomized design. Data obtained in each day of palatability test were first submitted to the Kruskal–Wallis test, which did not reveal an influence ($p > .05$) of the sex (male and female), or test day (day one and day two) on results. Intake ratio results were compared by the Student's *t* test at 5% significance and the first choice by the 5% chi-square test, totaling 32 replicates per test (16 dogs, 2 days of evaluation).

2.3 | Experiment II: Digestibility assay and blood parameters

2.3.1 | Animals and facilities

Twelve healthy adult Beagle dogs (6 males and 6 females) with average body weight of 10.3 ± 1.7 kg were used in this experiment. The animals were individually housed in concrete kennels (5 m long × 2 m wide) with shelter and solarium for a period of 31 days.

2.3.2 | Digestibility protocol

Dogs were fed the experimental diets twice daily (08:00 and 15:30 hr) in sufficient amounts to meet ME needs as recommended by the National Research Council Committee on Dog and Cat Nutrition [NRC] (2006). Water was offered ad libitum.

Between the 25th and 31st experimental days all feces were collected twice a day. Stool collection followed the recommendations of (Association of American Feed Control Officials (AAFCO), 2004). Feces were weighed and frozen (−14°C) in individual containers, identified, and pooled by animal.

2.3.3 | Chemical analysis

After the collection period, the feces were thawed, homogenized, and dried in a forced air ventilation oven (320-S; Fanem) at 65°C for 48 hr. After drying, feces and diets were ground to 1-mm sieve in a Willey hammer mill (Arthur H. Thomas Co.) and analysed for dry matter (DM) at 105°C, crude protein (CP, method 954.01), crude fiber (CF, method 962.10), ether extract in acid hydrolysis (EEAH, method 954.02), and ash (method 942.05), according to Association of the Official Analytical Chemists (Association of the Official Analytical Chemists [AOAC], 1995). Gross energy (GE) was determined in a bomb calorimeter (Model 1261; Parr Instrument Co). The main fatty acids composition of the microalgae, anchovy oil, and diets were analyzed after one-step extraction/methylation (Ulbert & Henninger, 1992).

2.3.4 | Fecal characteristics

Fecal characteristics were evaluated by total fecal dry matter (DMf), feces production (g feces/g DM intake/5 days), fecal score, ammonia concentration, pH, and sialic acid. Fecal pH and ammonia concentration were measured in feces collected at a maximum of 15 min after defecation. The fecal score was always evaluated by the same researcher, using the following 1 to 5 scale: 1 = watery feces; 2 = soft and unshaped stools; 3 = soft, shaped, and moist stools; 4 = well-shaped and uniform stools; 5 = well-shaped, hard, and dry stools.

Fecal pH was measured using a digital pHmeter (331; Politeste Instrumentos de Teste Ltda) in 3.0 g of fresh feces diluted in 30 ml of distilled water. The concentration of ammonia in feces was determined according to Félix et al. (2013). For analysis of sialic acid, feces were lyophilized (Alpha 1-4 LO plus; Christ, Osterode Am Hans) and the analysis was done according to Jourdian, Dean, and Roseman (1971).

2.3.5 | Blood collection and analysis

Blood collection was performed on the 31st experimental day. Prior to the procedure, dogs fasted for food during 12 hr and for water 8 hr. After a physical containment of the dogs, a local antisepsis with iodized alcohol was performed. Four mL of blood was collected from jugular vein and transferred into tube containing anticoagulant (EDTA) for evaluation of the complete blood count (erythrogram and leukogram) and flow cytometry. For analysis of serum 2 mL of blood was collected and added into tubes without anticoagulant.

The biochemical profile was analyzed using a commercial chemical kit (Dialab®), and for analyze complete blood count was utilized B5-200 equipament (Mindray Chemistry). The blood parameters analyzed were: erythrocytes, hematocrit, hemoglobin, mean corpuscular volume, mean globular hemoglobin concentration, leukocytes, eosinophils, lymphocytes, platelets, total plasma protein, cholesterol, alkaline phosphatase, triglycerides, urea, and alanine transaminase. For cell counts, the slides were stained by the rapid panotype method.

For analysis of immunosuppressive and phagocytic activity, 2 ml of blood were treated as modified by Beirão et al. (2012). Basically, 1 ml

of whole blood was purified on histopaque for leukocyte isolation. Cells were permeabilized to allow intracellular labeling with FoxP3. One million cells were incubated for 30 min (37°C) with the specific antibodies and fixed with paraformaldehyde for 30 min at 4°C, then packed into cytometry tubes in final volume of 2 ml PBS.

The phagocytosis analysis after cell purification involved incubation of leukocytes with the pHrod reagent for 30 min at 37°C before being fixed. The samples were analysed on a FACS Calibur (FACS Calibur; Becton Dickinson and Co) equipped with argon laser. Suppressor lymphocytes were identified by fluorescence in green (CD4 cells) and yellow (FoxP3 cells). The phagocytes were separated for cellular granularity (CSS) in monocytes or granulocytes. The percentage of phagocytic cells of green color was calculated. The intensity of phagocytosis was measured as the mean fluorescence intensity.

2.3.6 | Calculations and statistical analyses

The organic matter (OM) was calculated as: 100 - Ash and the original fecal dry matter were obtained by: (DM55 × DM105)/100. The ME was estimated according to Association of American Feed Control Officials [AAFCO] (2004):

$$\begin{aligned}\text{ME (kcal/g)} = \Big\{ &\text{kcal/g GE intake} - \text{kcal/g GE faecal excretion} \\ &- \Big[(\text{g CP intake} - \text{g CP faecal excretion}) \\ &\times 1.25\ \text{kcal/g}\Big]\Big\}/\text{g of feed intake.}\end{aligned}$$

Based on the laboratory results obtained, the CTTAD of the diets were determined according to the following equation:

$$\text{CTTAD} = (\text{g of nutrient intake} - \text{g of nutrient excretion})/\text{g of nutrient intake.}$$

The experiment was in a completely randomized design, with two treatments of six replicate dogs each (three males and three females per treatment). Data were previously analysed for their normality by the Shapiro–Wilk test at 5% probability. Data with normal distribution were evaluated by the Student *t* test ($p < .05$) and the nonparametric data were analysed by the Mann–Whitney test at 5% probability.

The formulation of immuno-suppressive phagocytic and CD4 activity figures were performed by GraphPad Prism 6 software.

2.4 | Experiment III: Oxidative stability

Two 200 g samples of the control diet were collected (Table 1). In each sample, the amount of microalgae or anchovy oil (DHA = 19.71%, EPA = 11.32%) was added so that the samples had the same EEAH content at the end.

The oxidative stability of the samples was evaluated according to the MA - 0.80 method (ML Oxipres, Instruction Manual, version 2009.02.01, Mikrolab Aarhus A/ S) using the OXIPRESTM equipment (Microlab Aarhus, Denmark), consisting of a control unit, one heater block and two pressure vessels. The conditions established by the equipment were of temperature 30-150°C and maximum pressure of 1 Mpa. The estimated period of induction of dietary oxidation in days was evaluated.

3 | RESULTS

3.1 | Experiment I: Palatability assay

Dogs consumed as a first choice the diet containing 0.4% microalgae ($p < .05$). The same occurred in relation to the intake ratio ($p < .05$), in which a greater amount of diet was consumed with addition of the microalgae (Table 3).

3.2 | Experiment II: Digestibility assay and blood parameters

3.2.1 | Digestibility and fecal characteristics

Dogs totally consumed the diets. There were no episodes of vomiting or diarrhea. The diet with 0.4% microalgae *Schizochytrium* sp. presented higher CTTAD of DM, CP, GE, OM and ME, when compared to the control diet ($p < .05$). However, there was no difference ($p > .05$, Table 2) in relation to the CTTAD of the EEAH.

Microalgae supplementation did not change fecal characteristics of the dogs ($p > .05$). However, there was a tendency ($p = .062$) for lower fecal production in dogs fed the diet containing microalgae (Table 4).

3.2.2 | Blood parameters

The circulating percentage of T-helper suppressor lymphocytes (CD4) did not differ ($p > .05$) between treatments (Figure 1).

The numbers of phagocytic monocytes and granulocytes were higher ($p < .05$) in animals fed 0.4% microalgae *Schizochytrium* sp. (Figure 2a,c). In addition to being present in greater amounts in the blood, monocytes from DHA-fed dogs also phagocytosed more particles ($p < .05$; Figure 2b). However, phagocytic granulocytes in the blood were less active ($p < .05$) in dogs fed microalgae (Figure 2d).

There were no differences in the biochemical profile, erythrogram and leukogram in dogs given 0.4% microalgae and controls ($p > .05$). All blood variables remained within the normal range for dogs (Data not shown).

3.3 | Experiment III: oxidative stability

There was greater oxidative stability for the diet containing microalgae *Schizochytrium* sp. in comparison to the sample with anchovy oil. Time to oxidation was 214 and 178 days, respectively, as the pressure and temperature increased.

4 | DISCUSSION

4.1 | Experiment I: Palatability assay

The inclusion of 0.4% microalgae *Schizochytrium* sp. in the diet, positively influenced the intake ratio and the first choice by the dogs, indicating that it is a palatable ingredient. It is possible that

the characteristic flavor of the microalgae (fish smell) was responsible for manifesting interest of the dogs and influenced in the food preference.

Hadley, Bauer, and Milgram (2017) evaluating the dietary supplementation of *Schizochytrium* sp. in dogs verified that the animals accepted very well the diet, without signs of food refusal. Dogs may be attracted to foods that emit odor and taste, and if the taste matches the odor, the reference for that food is maintained, being important to intake behavior (Houpt & Smith, 1981). Also, Folador et al. (2006) reported that ingredients with high total concentrations of fatty acids can add palatability to the diet, which was consistent with the present study.

4.2 | Experiment II: Digestibility assay and blood parameters

4.2.1 | Digestibility and fecal characteristics

There are few published papers that studied diets for dogs containing the microalgae *Schizochytrium* sp. The increase in ME found in the present study with inclusion of the microalgae may be related to the high energy level of this algae (7.5 kcal/g of GE), as well as to the higher nutrient digestibility values presented. In addition, an in vitro digestibility study showed a great availability of microalgae oil (Junwei et al., 2015).

Most of the microalgae generally contains complex cell wall with cellulose, gums, and pectins, presenting lower digestibility (Anderson, Jackson, Matty, & Capper, 1984). The cell wall of *Schizochytrium* sp. is simpler, mainly consisting of proteins and galactose (Jain, Raghukumar, Tharanathan, & Bhosle, 2005). This may favor the higher digestibility of diet containing this microalgae.

Considering that most of the immune system is associated with the gastrointestinal system via the lymphatic tissue, it is possible that dietary supplementation of DHA directly regulates inflammatory processes in the gastrointestinal tract (Campos et al., 2002; Patterson, Wall, Fitzgerald, Ross, & Stanton, 2012). According to these same authors, the treatment with n-3 PUFA has had favorable results in histological improvement in the colon contributing to intestinal health, consequently favoring digestion and absorption of nutrients. Despite these evidences, it is important to highlight that the dogs used in the present study were clinically healthy and although we did not evaluate specific inflammatory markers on gut, the results of phagocytic activity obtained may be consistent with the control of inflammation in dogs supplemented with DHA.

In this study, the fecal DM, score, sialic acid, ammonia, and fecal pH were within the normal range for dogs (Félix et al., 2013). The tendency toward lower fecal production in dogs fed with the diet containing microalgae may be explained by the higher digestibility presented by this diet.

4.2.2 | Blood parameters

The amount and activity of phagocytic cells were more sensitive in dogs fed the diet with supplementation of the microalgae *Schizochytrium* sp., source of DHA. There are two types of phagocytes in peripheral blood, monocytes and granulocytes. The amount of both types was increased in the circulation of the animals supplemented with the microalgae (DHA). This result may seem to contradict the immunosuppressive function that is expected from DHA. However, phagocytosis is crucial in the resolution phases of inflammation, for the removal of dead cell debris, for example. In this period of the immune response, phagocytosis is performed by cells that are activated to a "suppressor" profile (M2 macrophages/monocytes), which assists in the completion of inflammation and

TABLE 3 First choice and intake ratio of dogs fed diets with 0% or 0.4% microalgae *Schizochytrium* sp

	Microalgae (%)		
Item	**0**	**0.4**	***p*-value**
First choice[a]	7	25	<.001
Intake ratio[b]	0.34	0.66	.003

Note: First choice by chi-square test ($p < .05$) and intake ratio by Student *t* test ($p < .05$).
[a]Number of visits of dogs to the bowl with food B is given by: 32–n.
[b]IR: g diet A or B intake/g total food consumed (A + B) ×100.

TABLE 4 Means of coefficients of total tract apparent digestibility and metabolizable energy (ME, kcal/g) of diets and fecal characteristics of dogs fed with 0 and 0.4% of microalgae *Schizochytrium* sp

	Microalgae (%)			
Items	**0**	**0.4**	***SEM*[c]**	***p*-value[d]**
Coefficients of total tract apparent digestibility (%)				
Dry matter	76.9	80.9	1.04	.049
Ether Extract in acid hydrolysis	87.5	88.9	0.76	.379
Crude protein	74.8	82.3	1.40	.001
Gross energy	81.5	84.8	0.85	.049
Organic matter	81.7	85.8	1.08	.051
Metabolizable energy (kcal/g)	3.5	3.7	0.045	.005
Fecal characteristics				
Sialic acid (μmol/g)	1.98	1.96	0.075	.380
Fecal dry matter (%)	32.78	34.50	0.780	.292
pH	6.11	6.10	0.036	.881
Ammonia (%)	0.640	0.750	0.060	.356
Fecal production[a]	0.115	0.097	5.052	.062
Fecal score[b]	3.8	3.6	0.081	.154

[a]Fecal production (g feces/g DM intake/5 days).
[b]Fecal score = 1 (liquid stools) to 5 (dry stools).
[c]*SEM*: Standard error of the mean.
[d]P: Probability by Student *t* test ($p < .05$).

repair of tissue damage (Geissmann et al., 2010). Thus, DHA reduces inflammatory markers while inducing phagocytosis by means of M2 macrophages/monocytes (Hjorth et al., 2013).

Although the number of phagocytic granulocytes increased in blood, the activity of these cells was reduced with DHA from microalgae supplementation. The role of DHA on the granulocyte phagocytosis intensity is controversial. Positive and negative effects of DHA on granulocyte phagocytic activity have been described (Pisani et al., 2009). The DHA are precursors of molecules called resolvins and protectins, which reduce the infiltration of granulocytes at sites of inflammation. This process triggers the end of the inflammatory phase of immunity (Serhan & Savill, 2005) and it seems reasonable to expect granulocytes at this stage to be less active.

As with monocytes/macrophages, there are two subtypes of granulocytes, N1 and N2, of which N2 granulocytes are present in the resolution of inflammation. However, while M2 monocytes resolve inflammation through increased phagocytic activity, the same does not occur with N2 cells. In fact, one of the characteristics of the resolution phases of inflammation is that M2 monocytes/macrophages phagocyte and remove N2 granulocytes (De Oliveira, Rosowski, & Huttenlocher, 2016). Thus, the results found in the present study in relation to phagocytic activity may be consistent with the expected characteristics of DHA in the resolution of inflammation.

The DHA supplementation did not induce differences in the circulating percentage of suppressor T lymphocytes. These cells exert negative regulation on other lymphocytes and are defined by the expression of the CD4+ (helper lymphocyte) and FoxP3 (Kim, 2009) markers. The analysis of these cells is widely used in the study of DHA functions, since DHA is believed to have an anti-inflammatory action (Chapkin, Kim, Lupton, & McMurray, 2009). In mice, it has been shown that DHA is able to reduce signs of inflammatory diseases by increasing the amount of suppressor T lymphocytes (FoxP3+; Han et al., 2015). This was confirmed in another study, which demonstrated that the use of n-3 increased the amount of CD4 + FoxP3 + lymphocytes in the liver of mice by stimulating the proliferation of these cells. In addition, these lymphocytes produce

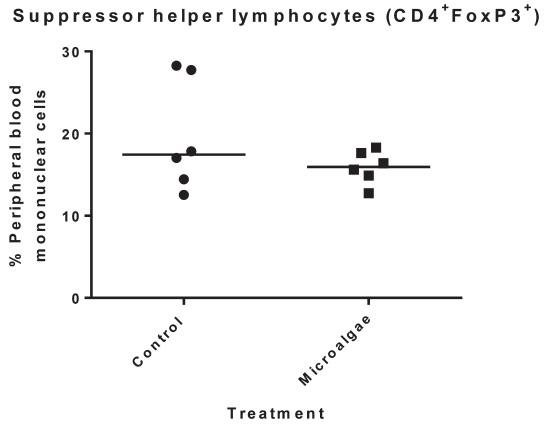

FIGURE 1 Circulating percentage of suppressor helper T lymphocytes in dogs fed with control diet (0%) or containing 0.4% of microalgae *Schizochytrium* sp. (DHA). The vertical axis shows the percentage of suppressor lymphocytes in relation to peripheral blood leukocytes. Each point indicates an animal, and the center bar shows the median ($p > .05$)

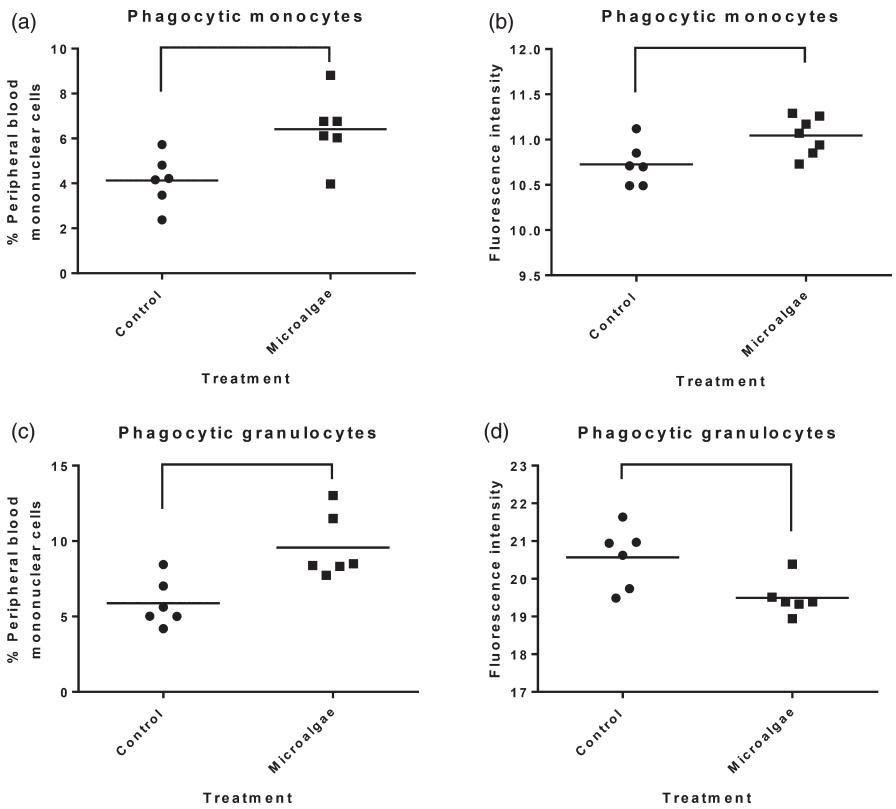

FIGURE 2 Number of phagocytic cells in blood and the phagocytosis intensity of monocytes and granulocytes in dogs fed a control diet (0%) or containing 0.4% of microalgae *Schizochytrium* sp. (DHA). (a) Percentage of phagocytic monocytes relative to total blood leukocytes; (b) Intensity of monocyte phagocytosis; (c) Percentage of phagocytic granulocytes in relation to total leukocytes in the blood; (d) Intensity of granulocyte phagocytosis. Each point indicates an animal and the center bar shows the median. The line connecting the groups indicates significant difference by the Mann–Whitney test ($p < .05$)

more anti-inflammatory cytokines, such as interleukin-10 (IL-10) and beta-transforming growth factor (TGF-β; Lian, Luo, Sui, Li, & Hua, 2015). Contrastingly, another study has shown that although the amount of FoxP3 is increased in DHA-treated suppressor lymphocytes, the function of the suppressor cell itself is inhibited by this treatment (Yessoufou, Plé, Moutairou, Hichami, & Khan, 2009).

Supplementation with the microalgae *Schizochytrium* sp. for 30 days did not alter the biochemical profile, erythrogram and leukogram of the dogs. This result corroborates those found by Le Blanc, Bauer, Hoosgood, and Mauldin (2005), in which supplementation with fish oil (7 g) in healthy dogs had no effect on blood parameters. Similar results were observed by Hammond et al. (2001) in studies with rats evaluating diets with microalgae *Schizochytrium* sp. and fish oil. The authors did not observe changes in hematological parameters related to the supply of up to 4 g kg day^{-1} of DHA, except for cholesterol, which was reduced in both treatments. The researchers report that these changes are more likely because the PUFA present in these sources increase hepatic fatty acid oxidation and reduce lipogenesis. Reduced serum levels of triglycerides and cholesterol esters have also been observed previously in rats fed diets with DHA (Demoz, Asiedu, Lie, & Berge, 1994). However, in the present study, these changes were not observed, which may be related to the time and amount supplied.

4.3 | Experiment III: Oxidative stability

Fish oil fatty acids contains high degree of unsaturation and number of carbon atoms (Ackman & Lamothe, 1989). The DHA esters (6 double bonds) has a high rate of oxidation reaction. This is because the oxidation rate of polyunsaturated compounds and methylene compounds is much higher than of compounds having only one double bond. Since this methylene group is activated by the two adjacent double bonds, thus increasing the rate of oxidation reaction (Rovellini, Cortesi, & Fedeli, 1997).

Although the microalgae studied also have a high content of PUFA, these fatty acids are possibly more protected from the action of free radicals and oxidation catalysts than fish oil. This is due to the natural encapsulation of the biomass of microalgae cells, which confers protection to their fatty acids against oxidizing agents (Ganuza et al., 2008), thus providing a longer product life (Ahn, Kim, Seo, Choi, & Kim, 2008).

In addition, the oxidative stability of samples containing algae can also be attributed to structural components of algae with antioxidant activity. For Fujimoto and Kaneda (1980) the fact that some algae are stored for a long period without the danger of oxidative deterioration, even though they present more than 30% of their total fatty acids in the form of polyunsaturated chains, is related to the antioxidant mechanism in these microalgae.

Junwei et al. (2015) report in a study with oil of the microalgae *Schizochytrium aggregatum* the presence of high concentration of beta-carotene, vitamin E, sterols, phenolics and flavonoids in its composition, demonstrating that these compounds not only enrich the oil but also improve its oxidative stability, due to the great antioxidant activity. In this way, the higher oxidative stability for the microalgae *Schizochytrium* sp. when compared to fish oil, brings great practical advantages to the industry, ensuring even greater food safety.

The addition of 0.4% of microalgae *Schizochytium* sp. in the diet as a source of DHA is palatable to dogs and increases the digestibility of nutrients and ME. In addition, it increases phagocytic cells and the phagocytosis intensity of monocytes in the blood and exhibits superior oxidative stability to anchovy oil. However, it does not alter the fecal characteristics, the biochemical profile and the blood hemogram of dogs.

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How to cite this article: Souza CMM, de Lima DC, Bastos TS, de Oliveira SG, Beirão BCB, Félix AP. Microalgae *Schizochytrium* sp. as a source of docosahexaenoic acid (DHA): Effects on diet digestibility, oxidation and palatability and on immunity and inflammatory indices in dogs. *Anim Sci J*. 2019;00:1–8. https://doi.org/10.1111/asj.13294