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THE EFFECT OF SUPPLEMENTATION OF MICROALGAE *Schizochytrium sp.* AS A SOURCE OF DOCOSAHEXAENOIC ACID (DHA) ON DOGS WITH NATURALLY OCCURRING GINGIVITIS

(Efeito da suplementação de microalga *Schizochytrium sp*. como fonte de ácido docosahexanóico (DHA) em cães com ocorrência natural de gengivite)

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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 dogs with gingivitis. Two diets containing 0 and 0.4% of microalgae *Schizochytrium* sp. were offered for 30 days to 12 randomly distributed adult Beagles to determine gingivitis. Gingivitis score and area were analyzed on days 0 and 30. Prior to the analysis, the area was gently cleaned with cotton wool on the surface of the crown of the teeth. There was no change in the gingivitis score ($P>0.05$). However, there was a reduction ($P<0.05$) in the area affected by gingivitis (day 30 - day 0) in animals supplemented with 0.4% microalgae *Schizochytrium* sp. The addition of 0.4% dietary microalgae reduced the area of gingivitis on dogs.
Keywords: Inflammation; lipids; microalgae; omega 3.

RESUMO: O objetivo desse estudo foi avaliar os efeitos da microalga *Schizochytrium* sp., como fonte dietética de ácido docosahexaenóico (DHA), em cães com gengivite. Duas dietas contendo 0 e 0.4% de microalga *Schizochytrium* sp. foram ofertadas por 30 dias para 12 cães Beagle adultos distribuídos em delineamento inteiramente ao acaso para determinar a gengivite. O escore e a área de gengivite foram analisados no dia 0 e 30. Antes das análises, foi realizado a higienização com um algodão sobre a superfície da coroa do dente. Não foi observado alteração no escore de gengivite ($P>0.05$). Entretanto, houve redução ($P<0.05$) na área afetada pela gengivite (dia 30 - dia 0) em animais suplementados com 0.4% de microalga *Schizochytrium* sp. A adição de 0.4% da microalga *Schizochytrium* sp. reduz a área da gengivite em cães.
Palavras-chave: Inflamação; lipídios; microalga; ômega 3.

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INTRODUCTION

Gingivitis, considered a silent and progressive disease, has a high occurrence rate in dogs due to its chewing habit. Its cause is associated with the presence of plaque (Gorrel et al., 2007) and dental calculus (Sete and Figueredo, 2013). The interaction of bacteria and their toxic effects with the gingiva may promote the expression of proinflammatory cytokines and the adhesion of molecules, which trigger an inflammatory response in the gingival tissues (Moura et al., 2016).

Considering this, the use of compounds with an anti-inflammatory effect in dogs diet may help to control gingivitis, such as docosahexaenoic acid (DHA, C22:6, n-3) (Naqvi et al., 2014). DHA acts as a substrate of endogenous lipid mediators involved in the resolution phase of inflammation in periodontal disease (Sete et al., 2013).

DHA is mainly present in the oil of some fishes and seaweeds, as the microalgae *Schizochytrium* sp.. This microalgae may represent a promising organism for DHA supplementation in diets, since it has a high concentration of this fatty acid (approximately 20%) readily available (Souza et al., 2019), helping to delay the development of inflammations caused by the incidence of gingivitis (Lourenço et al., 2018).

However, there is still little scientific information on the efficacy and adequate dosage of the microalgae *Schizochytrium* sp., and although DHA has been used in research for the treatment of periodontal disease in dogs (Lourenço et al., 2018) its effects on the gingival inflammation in this species are lacking. Thus, the objective of this study was to evaluate the supplementation of microalgae *Schizochytrium* sp. as a source of DHA on the score and area of naturally occurring gingivitis in dogs.

MATERIALS AND METHODS

Animals and facilities

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

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, ALL-G RICH®, Alltech Inc, Lexington, USA). The diet contained the following ingredients: poultry by-product 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 analyzed 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.

Gingival index

The gingival index was determined by evaluating the score and area of gingivitis in the oral cavity on days 0 and 30 of the experiment. Prior to the analyzes, the area was cleaned gently with cotton wool on the surface of the crown of the teeth.

Table 1. Analyzed chemical composition (%, dry matter basis) of experimental diets.

Item	Microalgae	
	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

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).

Item	% Dry matter
Moisture	3.70
Crude protein	9.53
Ether extract in acid hydrolysis	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

Gingival inflammation score was always performed by the same evaluator, who was unaware of the treatments. The marks from 0 to 3 were attributed, being: 0 = absence of inflammation; 1 = mild inflammation (slight change in color and texture, no bleeding at probing); 2 = moderate inflammation (redness, edema, and hypertrophy, bleeding at probing); 3 = severe inflammation (marked redness and hypertrophy, spontaneous bleeding, ulceration) according to Loe and Silness (1963).

To measure the gingivitis area, photos with a resolution of 2364x1728

were taken, with the distance of the camera relative to the teeth proportional to the desired visual field. The scanned images were treated by the ImageJ® program (Wayne Rasband, National Institutes of Health, Bethesda, MD, USA) using mainly threshold and tools to measure the inflamed gingiva area (characterized by more intense red).

Calculations and statistical analyses

The experiment followed a completely randomized design, with two treatments and six replicates (dogs) each. Data were previously analyzed for their normality by the Shapiro Wilk test at 5% probability. Data with normal distribution were evaluated by the Student t-test (P<0.05) and the non-parametric data were analyzed by the Mann-Whitney test at 5% probability.

RESULTS

There was no change in the gingivitis score (P>0.05). There was a reduction (P<0.05) in the area affected by gingivitis (day 30 - day 0) in animals supplemented with 0.4% microalgae *Schizochytrium* sp. (Table 3).

Table 3. Medians (1st and 3rd quartiles) of the score and means of the gingivitis area of dogs fed diets containing 0 and 0.4% of microalgae *Schizochytrium* sp. on days 0 (initial) and 30 (final) and their difference over time (day 30 - 0).

	Score[1]			Area (cm²)[2]		
Diets	**Day 0**	**Day 30**	**Day 30 - 0**	**Day 0**	**Day 30**	**Day 30 – 0**
0% microalgae	2.0(2.0/2.25)	1.0(1.0/1.0)	1.0(1.25/1.0)	0.069	0.038	0.031
0.4% microalgae	3.0(2.0/3.0)	1.5(1.0/2.0)	1.0(2.0/1.0)	0.252	0.042	0.210
SEM[3]	-	-	-	-	-	0.032
P[4]	-	-	1.000	-	-	<0.010

[1]Gingivitis score: 0 = no inflammation; 1 = mild inflammation (slight change in color and texture, no bleeding at probing); 2 = Moderate inflammation (redness, edema, and hypertrophy, bleeding at probing); 3 = Severe inflammation (marked redness and hypertrophy, spontaneous bleeding, ulceration); 3SEM: standard error of the mean; [4]Gingivitis score: Mann-Whitney test (P<0.05), Area: t-Student test (P<0.05).

DISCUSSION

In the present study, there was a reduction in the gingivitis area of dogs fed a diet containing microalgae *Schizochytrium* sp. The level of inflammatory derivatives of arachidonic acid (AA) can be controlled through diet. Levels of eicosanoids, including prostaglandins, thromboxanes, leuko-trienes and lipoxins derived from the enzymatic oxidation of AA, clearly increase during gingival and periodontal inflammation response (Ongaro et al., 2017). Thus, the incorporation of n-3 fatty acids (EPA and DHA) via diet leads to a reduction of the substrate for the synthesis of eicosanoids from AA (Moura et al., 2016). In addition, it gives rise to mediators with less inflammatory potential, modulating inflammation. This is because EPA and DHA use the same metabolic pathways and enzymes that metabolize AA (De Oliveira et al., 2013).

According to Kesavalu et al. (2007), a diet rich in n-3 provides a decreased expression of genes responsible for the secretion of inflammatory cytokines and an increase in antioxidant enzymes (catalase and superoxide dismutase). The antioxidant action of n-3 is expressed with positive regulation of antioxidant enzymes and non-enzymatic antioxidant factors, as well as the production of metabolites

(such as protectins and resolvins) that have the capacity to reduce oxidative stress, inhibit neutrophil infiltration, and reduce the expression of cytokines in cells (Raffaelli et al., 2008; Souza et al., 2019).

Studies have shown that n-3 PUFA influences the reduction of gingival inflammation in humans and some animals (Sete et al., 2013; Oba et al., 2014; Elwakeel and Naza, 2015; Naqvi et al., 2017). Neqvi et al. (2010) reported that adult humans who consumed medium or high amounts of fish with a high concentration of n-3 became less likely to develop gingivitis. These same authors report that DHA is associated with less periodontal disease.

Another hypothesis for the reduction of inflammation with dietary DHA supplementation would be the newly discovered class of bioactive products from the transformation of n-3, the resolvins. Its association with the reduction of inflammation is characterized by the paralysis of neutrophil infiltration and by directing neutrophils to apoptosis, attracting nonphlogistic monocytes, which differentiate within the macrophages of resolution. Resolving macrophages present increased phagocytosis of apoptotic neutrophils and increased removal of bacteria from mucosal surfaces, promoting healing of the wound and return of homeostasis (Schwab et al., 2007). The rapid resolution of acute resolvin induced inflammation is, at least in part, based on the beneficial actions of EPA and DHA-rich diet (Van Dyke, 2011). In this way, DHA helps in the control of inflammatory processes in the body and may be a therapeutic approach to assist inflammatory processes in the gingiva.

CONCLUSION

The addition of 0.4% of microalgae S*chizochytrium* sp. in the diet, as a source of DHA, reduces the area affected by gingivitis, being a potential tool, in complement with veterinary care, to help oral health of dogs.

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INFORMATION NOTES

The experiment was approved by the Committee of Ethics on Animal Use of the sector of Agrarian Sciences of the Federal University of Paraná, Curitiba, PR, Brazil, under protocol n. 027/2017.

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