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The effect of dietary corn oil and fish oil supplementation in dogs with naturally occurring gingivitis

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Abstract

The aim of this randomized, double-blinded, placebo-controlled study was to evaluate if downregulation of the inflammatory response due to ingestion of high levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) can slow down gingivitis development, and thus delay the progression of periodontal disease (PD) in dogs. To this aim, 44 client-owned adult dogs (>1 and <8 years old) with naturally occurring PD (stages 1 and 2) were submitted to a plaque, gingivitis and calculus scoring followed by a dental cleaning procedure and collection of blood samples. The animals were then fed a canine adult maintenance diet, supplemented with either corn oil (0.00 g EPA and 0.00 g DHA) or fish oil (1.53 g EPA and 0.86 g DHA, both per 1,000 kcal ME) over the following 5 months. At the end of this period, the PD scoring and the blood sampling were repeated. The animals consuming fish oil had higher plasma levels of the longer chain (C ≥ 20) omega 3 fatty acids ($p < 0.01$) and similar plasma levels of alpha-linolenic acid ($p = 0.53$), omega 6 fatty acids ($p > 0.63$) and C reactive protein ($p = 0.28$) then the ones consuming corn oil. There were no differences between fish oil and corn oil diet supplementation on plaque (18.2 vs. 17.8, $p = 0.78$), calculus (10.1 vs. 11.5, $p = 0.18$) or gingivitis (19.3 vs. 19.0, $p = 0.77$) indexes. The authors conclude that supplementation with EPA + DHA does not slow down progression of PD in dogs.

KEYWORDS
docosahexaenoic acid, eicosapentaenoic acid, gingivitis, long-chain fatty acids, omega 3, periodontal disease

1 | INTRODUCTION

Periodontal disease (PD) is the most prevalent disease in dogs and was reported to be approximately 80% in dogs aged 2 years or older (Niemiec, 2008). PD results from an inflammatory process that develops in two phases: gingivitis (reversible process) and periodontitis (irreversible process) (Wiggs & Lobprise, 1997). This disease has a multifactorial nature involving host–microbial interactions, environmental and genetic factors (Kornman, 2008). Gingivitis is the first stage of the disease and is characterized by an inflammation confined to the gingiva. In a clinical manner, gingivitis is seen as reddening and swelling of gingiva up to ulceration and spontaneous bleeding as it progresses in severity (Lindhe & Rylander, 1975; Schroeder, Beer, & Attström, 1975; Vrieling, 2010). Although gingivitis is reversible, if the inflammation persists, the disease can progress to periodontitis. Periodontitis refers to the inflammation of other periodontal tissues: the periodontal ligament, the cementum and the alveolar bone and its progression eventually leads to tooth loss, bone reabsorption and allows pathogenic bacteria to gain access to the blood stream, from where it can cause organ damage.

The pathogenic mechanism that leads to gingivitis starts with the formation of a thin layer of glycoprotein on the tooth surfaces called pellicle. When left undisturbed for more than 24 hr, this layer gets a more organized structure and is colonized by bacteria present in the mouth, forming the plaque layer (Harvey, 2005; Niemiec, 2008; Vrieling, 2010). The interaction of the bacteria and their toxic products with the gingiva can promote the expression of proinflammatory cytokines and adhesion molecules, which increase vascular permeability and migration of leucocytes into the gingival tissues (Bartold, Walsh, & Narayanan, 2000; Page, Offenbacher, Schroeder, Seymour, & Kornman, 1997). The initial inflammatory response, activated by the pathogenic bacteria, is mainly mediated by neutrophils and activates oxygen-dependent and oxygen-independent mechanisms (Kantarci, Oyaizu, & Van Dyke, 2003) that will eventually damage the host tissues (Nussbaum & Shapira, 2011). The next step in the inflammatory process is the recruitment of macrophages which further enhances the inflammatory response by the release of more proinflammatory cytokines causing a cascade effect that exacerbates the inflammatory response (Sorsa et al., 2006). This cascade can result in an osteoblast/osteoclast imbalance that leads to bone loss, a main feature of periodontitis (Sun et al., 2003).

Calcium salts in the salivary fluid mineralize the plaque and, in 2–3 days, form calculus which is not easily removed by homecare methods. It is believed that calculus is not pathogenic and that the presence of pathogenic bacteria and their toxic products in the plaque above and beneath the gingiva are the trigger of PD. Nevertheless, the rough surface of the calculus can irritate the gingiva and provide additional places for the bacteria to adhere to, enhancing further plaque development (Harvey, 2005; Tatakis & Kumar, 2005).

The golden standard to prevent PD is based on the control of the bacterial colonies that drive the inflammatory response by mechanical debridement of the pellicle and plaque by regular tooth brushing (Harvey, Serfilippi, & Barnvos, 2015); however, dog owners compliance is low (Haws & Anthony, 1996; Miller & Harvey, 1994). Therefore, easier and still effective alternatives, among which dietary solutions, are intensively researched.

The clinical benefits of omega 3 (ω3) polyunsaturated fatty acids (PUFAs), in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in conditions where inflammation plays a major role, are currently supported by the literature. In dogs, those effects have been documented in inflammatory skin disorders (Abba, Mussa, Vercelli, & Raviri, 2005; Logas & Kunkle, 1994; Mueller et al., 2004), cardiovascular disorders (Freeman et al., 1998), renal disease (Brown, 2008; Brown et al., 1998, 2000), osteoarthritis (Curtis et al., 2002; Fritsch et al., 2010; Hansen et al., 2008; Roush, Cross, et al., 2010; Roush, Dodd, et al., 2010; Watkins, Li, Lippmann, & Seifert, 2001) and wound healing (Mooney et al., 1998). Although the cellular and molecular mechanisms by which EPA and DHA may exert their effects are only partially understood, they include their incorporation into cell membrane phospholipids (Browning et al., 2012; Silva, Barazzoni, & Singer, 2014), the changing of lipid mediators (Calder, 2015), the changing of protein mediators (Hansen et al., 2008; Rahman, Bhattacharya, & Fernandes, 2008), the alteration of gene expression and the modulation of T-cell reactivity (Calder, 2015; Cleland, James, & Proudman, 2006). Together these mechanisms can exert a significant impact in both phases of the inflammatory process, that is, not only in its initiation phase but, as more recently found, in its resolution phase (Hasturk et al., 2007; Schwab, Chiang, Arita, & Serhan, 2007).

The significance of inflammation in the development of gingivitis and its progression to periodontitis (Chapple, 2009; Logan, Finney, & Hefferren, 2002; Lommer & Verstraete, 2003) makes the host modulation a target for the therapeutic approach to PD. Among the possible host modulatory agents, EPA and DHA are particularly interesting due to (a) their anti-inflammatory effects (Alam, Bergens, & Alam, 1991; Campan, Planchand, & Duran, 1997; El-Sharkawy et al., 2010; Naqvi et al., 2014; Rosenstein, Kushner, Kramer, & Kazandjian, 2003); (b) their inhibition of putative periodontal pathogens, such as *Porphyromonas gingivalis* (Choi et al., 2013; Kesavalu et al., 2007); (c) their role as substrates of endogenous lipid mediators involved in the resolution phase of PD inflammation (Van Dyke, 2008); and (d) their lack of significant adverse effects (Lenox & Bauer, 2013).

Although EPA and DHA have been in the spot light as future therapies for the treatment of PD diseases their effects on gingival inflammation and progression of PD in dogs have not been evaluated yet. In this study, we hypothesized that downregulation of the inflammatory response of the host, which is expected to occur after ingestion of high levels of EPA and DHA, can slow down gingivitis development, and thus delay the progression of PD in dogs.

2 | MATERIALS AND METHODS

2.1 | Animals

Forty-four adult dogs were selected (22 in the Netherlands and 22 in Portugal). Inclusion criteria were: (a) male or female dog with >1 and <8 years of age, (b) natural occurring PD stages 1 and 2 (AVDC, 2009; Wolf, Rateitschak, Rateitschak, & Hassell, 2005) and (c) willingness of the owners to cooperate in this study and commitment to the protocol. Exclusion criteria were: (a) severe PD (stages 3 and 4; AVDC, 2009; Wolf et al., 2005), (b) any concurrent disease, (c) outdoor unattended access (d) use of other diet or supplement during the study, (e) use of hygienic dental measures during the study. The 44 recruited animals, characterized in Table 1 were randomly assigned to either group "A" or group "B."

2.2 | Diet and treatment

The diet of all dogs was changed to the same adult dog dry formula (Hill's VetEssentials Canine Adult) at the beginning of the experiment (day 1 of phase 1). This diet is designed to allow mechanical cleansing of the tooth surface (manufacturer claim) and contained 0.124 g of ω3 PUFAs and 0.968 g of ω6 PUFAs, both per 1,000 kcal ME and a negligible amount of EPA + DHA (0.0051 g per 1,000 kcal ME). The diet was supplemented with either corn oil (0.00 g EPA and

TABLE 1 Characteristics of the recruited dogs

Breeds	18 Crossbreed/undetermined
	11 Beagles
	4 Dachshunds
	2 Stabyhouns
	2 Labrador retrievers
	1 English cocker spaniel
	1 Jack Russell
	1 Pug
	1 Poodle
	1 Boxer
	1 Epagneul Breton
Gender	23 Females
	12 Castrated females
	6 Castrated males
	2 Males
Body weight (kg ± *SD*)	13.7 ± 6.76
Animals BW <12 kg (*n*)	21
Animals BW >12 kg (*n*)	22
Body condition score (±*SD*)	5.6 ± 0.95
Age (years ± *SD*)	4.8 ± 1.68

TABLE 2 Gingivitis, plaque and the calculus four-point scales

Plaque scale
0 = no plaque detected on the tooth surface
1 = plaque coverage of the tooth surface <25%
2 = plaque coverage of the tooth surface 25%–50%
3 = plaque coverage of the tooth surface 50%–75%
4 = plaque coverage of the tooth surface >75%
Gingivitis scale (Logan et al., 2002)
0 = normal gingiva
1 = mild inflammation and slight redness
2 = moderate inflammation and redness and no bleeding on probing
3 = moderate inflammation, severe redness and bleeding on probing
4 = severe inflammation, redness, oedema, ulceration and spontaneous bleeding
Calculus scale
0 = no calculus on the tooth surface
1 = calculus coverage of the tooth surface <25%
2 = calculus coverage of the tooth surface 25%–50%
3 = calculus coverage of the tooth surface 50%–75%
4 = calculus coverage of the tooth surface >75%

0.00 g DHA, per 1,000 kcal ME) or fish oil (1.53 g EPA and 0.31 g DHA, both per 1,000 kcal ME). To prevent oxidation, the oils were enriched with 10 mg/ml of alpha-tocopherol. The oils were supplied by Nutriceuticoils, Oelegem, Belgium with equal appearance (i.e., corn oil with fish smell and fish oil), in identical vials, labelled as oil "A" and oil "B" and delivered with a dosage syringe and a locked key. The daily amount of food was calculated for each dog according to FEDIAF (2014) maintenance energy requirements recommendation (95–130 kcal metabolizable energy/kg$^{0.75}$ body weight) and the individual amounts of food and oil were indicated to the owners.

2.3 | Study protocol

The study was conducted as a randomized double-blinded controlled study. The clinical time line was identical for each dog and started at day 0 (phase 1) with an oral condition examination and PD scoring (Table 2), blood sampling, a complete professional dental cleaning and random assignment of the dogs to either group "A" or group "B."

During the 5 months of the trial period, starting at day 1 (phase 1), the dogs were fed the adult commercial dog diet supplemented daily with oil "A" or oil "B" depending on the group they were randomly assigned to. The trial period ended on the last day of the 5th month (phase 2) with an oral examination and PD scoring and blood sampling. For health check purposes, a physical exam of the dogs was performed at beginning and end of the study. At the same consult, a questionnaire regarding living conditions and health and eating habits was completed by the owners. All owners signed an informed consent document previous to their dog's inclusion in the study and agreed to not feed or treat their dogs with anything else but the commercial experimental diet and the oil supplement. All dogs recruited for the study came to the clinics for medical care, thus they were not exclusively anesthetized for this study.

2.4 | Oral examination

The buccal/labial and palatal/lingual of each tooth was scored for calculus, plaque and gingivitis according to the criteria mentioned in Table 2. A periodontal probe was used to score the gingivitis. The plaque was made visible with a disclosing agent (erythrosine).

All results were recorded on a dental chart. After PD scoring, a professional dental hygiene, with scaling and polishing was performed. Calculus and plaque were removed using instruments and ultrasonic scalers. If present, fractured teeth were extracted. The treatment was finished by polishing the teeth using polishing paste and a slow moving hand piece with a rubber cup.

Before the start of the study, a workshop was held at Utrecht University to synchronize all procedures.

2.5 | Blood sample collection and analysis

Blood samples were taken after an overnight fasten period by jugular venipuncture at the beginning (day 0) and at the end of the study period (5 months after), and collected into EDTA and serum tubes. These samples were used for health check and for the study purposes. The serum tubes were centrifuged and the serum was analysed for urea, creatinine, glucose, alkaline phosphatase, bile acids,

total protein and albumin determination. Half of the EDTA blood was used for a complete blood cell count using an automated cell counter. The other half of the EDTA blood was immediately centrifuged and stored at −20°C for determination of the fatty acid profile of the cholesteryl ester fraction of the plasma and C reactive protein.

Cholesteryl esters in plasma were isolated by a modified "Bligh & Dyer" extraction according to Retra et al. (2008), followed by a solid-phase extraction method according to Hamilton and Comai (1988). Thereafter, the cholesteryl esters were saponified according to the modified method described by Kates (1986), where petroleum ether was replaced by hexane. Polyunsaturated fatty acid analysis was performed by high-pressure liquid chromatography/mass spectrometry (HPLC/MS) according to Retra et al. (2008) in which the Synergi 4 lm MAX-RP 18A column was replaced by a Kinetex 2.6 lm C18 100A column (150 3 mm; Phenomenex, Torrance, CA, USA). Internal standards were used for comparison.

The C reactive protein analysis was performed with the C reactive protein test and the vet reader (both from LifeAssays®, Lund, Sweden). For C reactive protein analysis values below the reader threshold (<10 mg/L) were assumed to be 5 mg/L.

2.6 | Indexes calculation

The gingival inflammation, the plaque and the calculus were scored after an overnight fasten period according to the five-point scale (Table 2). These scores were used to calculate the gingivitis index, the plaque index and the calculus index respectively. The oral cavity was divided into 12 sections: (a) buccal side of the upper right premolars and molars (110–105 element), (b) labial side of the upper canines and incisors (104–204 element), (c) buccal side of the upper left premolars and molars (205–210 element), (d) buccal side of the lower left premolars and molars (411–405 element), (e) labial side of the lower canines and incisors (404–304 element), (f) buccal side of the lower right premolars and molars (304–311 element), (g) palatal side of the upper right premolars and molars (110–105 element), (h) palatal side of the upper canines and incisors (104–204 element), (i) palatal side of the upper left premolars and molars (205–210 element), (j) lingual side of the lower left premolars and molars (411–405 element), (k) lingual side of the lower canines and incisors (404–304 element), (l) lingual side of the lower right premolars and molars (304–311 element). Average scores were calculated for each region and indexes were calculated as the sum of all regions averages (index range: 0–48).

2.7 | Statistical analysis

The power analysis equation was used to determine sample size. With the variables values $\alpha = 0.05$, $\beta = 0.90$ and $\delta = 0.5$, the result was $n \geq 18$ dogs per group. Due to the relative long-term of the trial (5 months), compliance with the inclusion criteria by the owners was expected to diminish and the likelihood that a health issue would lead to the exclusion of an animal was expected. Thus, to insure a minimum of 18 dogs per group, 44 dogs entered the study.

The normal distribution of the index data was tested by the Shapiro–Wilk test. A one-way ANCOVA model was performed using oil ("A" vs. "B") as independent variable and phase 1 index data as covariable. Tukey test was used to verify differences in means. A p-value of <0.05 was considered significant.

Plasma fatty acids and C reactive protein concentrations were also tested for normality with a Shapiro–Wilk test. The data that was not normally distributed were compared using a Wilcoxon rank sum χ^2 test. The level of significance was set at $\alpha = 0.05$. All statistical analyses were carried out using JMP software (SAS) 16.0 for Windows (SPSS, Chicago, IL, USA).

All procedures described in this article were approved by the University of Utrecht and University and Trás-os-Monte e Alto Douro Committees of Experimental Use of Animals. Animal experiment permission number: DEC.2014.III.03.030. These procedures were also performed with written consent from the dog owners.

3 | RESULTS

The unlock of the oil key, performed only when the final results were known, revealed that oil "A" was the fish oil and the oil "B" was the corn oil.

One dog assigned to the fish oil group was lost to follow-up, thus 43 dogs (21 in fish oil group and 22 in the corn oil group) completed the study. All dogs remained healthy during the study and there were no significant changes in BW ($p > 0.05$).

The plaque, gingivitis and calculus indexes were normally distributed. Table 3 shows the indexes scores (phase 2) results of the ANCOVA using oil as independent variable in the model and phase 1 plaque, gingivitis or calculus indexes as covariable.

The phase 1 index data used as a covariable was not statistically significant for the plaque or the gingivitis indexes results ($p = 0.41$ and $p = 0.57$ respectively) but was statistically significant for calculus ($p = 0.01$), showing that the calculus present on the teeth surface before the initial cleaning procedure exerted an effect on the calculus observed at the end of the trial.

There were no differences between fish oil and corn oil diet supplementation on plaque, calculus or gingivitis indexes ($p > 0.05$; Table 3).

Plasma fatty acids and C reactive protein data were not normally distributed. The nonparametric analysis of these data at the beginning of the trial (phase 1; Table 4) shows that there were no

TABLE 3 Indexes scores

	Oil			*p*-Value	
	A	B	*SEM*	Cov	Oil
Plaque	18.2	17.8	1.02	0.69	0.78
Gingivitis	19.3	19.0	0.90	0.29	0.77
Calculus	10.1	11.5	0.75	0.03	0.18

Note. A: fish oil; B: corn oil.

TABLE 4 Plasma fatty acids (nmol/ml) and C reactive protein levels (CRP, mg/L)

	Phase 1			Phase 2		
	Fish oil (A)	Corn oil (B)	χ^2	Fish oil (A)	Corn oil (B)	χ^2
n	21	22		21	22	
Omega 6 PUFA						
C18:2 (LA)	33.2 ± 18.95	30.0 ± 12.31	0.96	49.9 ± 22.45	46.8 ± 13.93	0.88
C20:4 (ARA)	6.1 ± 8.20	5.7 ± 6.05	0.48	14.6 ± 6.62	13.4 ± 5.76	0.63
Omega 3 PUFA						
C18:3 (ALA)	5.97 ± 7.93	5.11 ± 4.41	0.66	17.6 ± 11.65	14.4 ± 8.75	0.53
C20:5 (EPA)	0.6 ± 0.71	1.0 ± 1.23	0.09	7.8 ± 6.54	2.0 ± 2.13	0.001
C22:5 (DPA)	1.2 ± 1.54	1.2 ± 1.07	0.24	5.11 ± 2.91	3.0 ± 2.00	0.01
C22:6 (DHA)	1.6 ± 1.92	1.6 ± 1.58	0.81	8.3 ± 6.02	3.6 ± .0	0.01
C20:5 + C22:6	2.2 ± 2.60	2.6 ± 2.67	0.41	16.1 ± 12.38	5.7 ± 5.1	0.001
Acute phase protein						
CRP	14.5 ± 5.12	12.5 ± 4.08	0.15	6.67 ± 4.55	5.45 ± 2.13	0.28

Note. The shown values are mean ± *SD*.
LA: linoleic acid; ARA: arachidonic acid; ALA: alpha-linolenic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; CRP: C reactive protein; χ^2: chi-square.

statistical differences on fatty acids profile among treatment groups, reflecting the randomized distribution of the dogs to group "A" or "B." As expected, at the end of the trial (phase 2, Table 4) the animals consuming fish oil had higher plasma levels of the longer chain (C ≥ 20) omega 3 PUFA ($p < 0.01$) and similar plasma levels of alpha-linolenic acid ($p = 0.53$) and omega 6 fatty acids ($p > 0.63$) compared to the animals consuming corn oil.

The nonparametric analysis of C reactive protein data shows that there were no statistical differences between the two treatment groups ($p = 0.15$) at the beginning of the trial (phase 1, Table 4) and also no effect of oil ($p = 0.28$) at the end of the trial (phase 2, Table 4). At the end of the trial (phase 2, Table 4) all C reactive protein levels were far below the inflammation threshold of 10 mg/L (Nakamura et al., 2008).

4 | DISCUSSION

The intense research on PD prevention in dogs did not find, till present, the perfect solution. This research has been overlooking the possibility that the anti-inflammatory effects of the long-chain fatty acids found in fish oil could delay the progression of gingivitis and thus of the PD. This study was designed to evaluate this possibility in dogs.

The initial plaque and gingivitis status of the animals did not have an influence on the results at the end of the trial, as there was no significant effect of the phase 1 scores as a covariable in the model for these 2 indexes, but the calculus status presented by the animals before the cleaning mouth procedure exerted an effect, shown by the statistical significance of the covariable ($p = 0.01$) in the calculus results. Thus, although the use of the phase 1 calculus as a covariable in the model allowed the isolation of the animals previous calculus effect from the treatment effect on the calculus scores, it is suggested for future trials that the calculus status of the dogs will be used as a factor to take into account in the treatment group's formation.

Other factors that may influence calculus (and plaque and gingivitis) indexes and should be taken into account during randomization are: (a) weight and breed differences, dogs under 10 kg and brachycephalic breeds are more prone to PD (Logan, Wiggs, Scher, & Cleland, 2010), probably reflecting the skull conformation impact on the extension of the teeth surface cleaning and (b) life style differences (Logan et al., 2010), although all dental cleaning "toys" where not accessible to the dogs during the trial it is possible that some dogs could have had more easily access to chewing materials that would have helped to promote some cleaning of the plaque and consequently diminished the chance of plaque mineralization and calculus formation.

The results clearly show an increase in the plasma EPA and DHA concentrations as consequence of the fish oil supplementation of the diet. This increase in plasma levels was expected and even necessary if immunomodulation and clinical improvement of gingiva inflammation were to be expected. On the other hand, the plasma omega 6 PUFA concentrations were not different among treatment groups. This might be explained by the high concentration of omega 6 PUFA in the diet offered to both groups in similar amounts. In fact, this concentration seems to be high enough to mask the expected increase in the serum levels as a result of the omega 6 PUFA supplementation from the corn oil.

To the author's best knowledge, the evaluation of EPA and DHA effects in gingiva inflammation in dogs were not reported in the literature; but a tendency for lower gingivitis index was observed in humans receiving fish oil instead of olive oil (Campan et al., 1997) or fish oil instead of borage oil (Rosenstein et al., 2003). One of the

main mechanisms by which EPA and DHA would reduce the gingiva inflammation resides in their ability to compete with arachidonic acid. This competition can take place at two levels: at the incorporation into cell membrane phospholipids, reducing the proportion of substrate available for arachidonic acid-derived eicosanoids and as a substrate for the cyclooxygenase and lipoxygenase pathways (Bagga, Wang, Farias-Eisner, Glaspy, & Reddy, 2003). The overall result would be the reduction of proinflammatory lipid mediators derived from arachidonic acid such as prostaglandin-E2 and leukotriene-B4 (Kelley, Taylor, Nelson, & Mackey, 1998; Peterson et al., 1998). These lipid mediators are implicated in a wide range of pathophysiologic processes associated with PD, including inflammatory cell recruitment, oedema, pain, collagen destruction, and bone resorption (Pouliot, Clish, Petasis, Van Dyke, & Serhan, 2000). Also in rats, EPA and DHA reduced the gingiva inflammation induced by *P. gingivalis* when compared to rats fed a corn oil diet (Kesavalu et al., 2007). In this study, a lower proinflammatory cytokine gene expression (interleukin 1 beta, tumour necrosis factor alpha) and enhanced interferon gamma, catalase and superoxide dismutase messenger RNA expression were identified, supporting a diet-induced modulation of host inflammatory reactions (Kesavalu et al., 2007). In mice supplemented with fish oil, Sun et al. (2003) observed suppressed expression of inflammatory cytokines, nuclear factor kappa B activation and osteoclast formation and activation, resulting in less bone resorption. This last effect can only be evaluated in more advanced stages of the PD, which were not included in the present study.

Reviewing the veterinary literature Bauer (2011) estimated that to achieve clinical benefits an amount of EPA and DHA of 125 mg/kg$^{0.75}$ body weight was needed for inflammatory skin disorders, 115 mg/kg$^{0.75}$ BW for cardiovascular disorders, 140 mg/kg$^{0.75}$ BW for renal disease and 310 mg/kg$^{0.75}$ BW for osteoarthritis. Bauer (2011) also raises the possibility that in more severe or more chronic cases higher dosages than the aforementioned might be required, and suggests NRC safe upper limit of 370 mg EPA + DHA/kg$^{0.75}$ BW as the upper reference for the dosage. In the present study, an amount of 270 mg of EPA + DHA/kg$^{0.75}$ BW was fed in total (diet plus supplement) to the fish oil group. This dosage is higher than the dosages expected to show clinical effects in most inflammatory clinical disorders (Bauer, 2011), and the gingiva inflammation was not severe; thus, a lack of effect of fish oil on gingivitis index, when compared to the corn oil control, observed in this study was not expected. Nevertheless, it could be that only a dosage similar to the one required to diminish inflammation in osteoarthritis (310 mg/kg$^{0.75}$ BW; Bauer, 2011) will be effective in lowering the gingiva inflammation. It is also possible that the use of a base diet without the dental cleaning technology of the diet fed would have allowed a more extensive development of the PD and thus a more effective evaluation of the potential benefits of the EPA + DHA in the gingivitis development.

Another explanation for the lack of effect in gingivitis index might be the effect of the EPA and indirectly DHA on reducing the thromboxane A2 synthesis and the consequent decrease in platelet aggregation (Ågren, Väisänen, Hänninen, Muller, & Hornstra, 1997). Therefore, one effect of long-chain omega 3 PUFAs supplementation would be a prolonged bleeding time (Bang & Dyerberg, 1980). In the present study, a lower concentration of thromboxane A2 leading to easier bleeding could result in an increase in gingivitis index, as score 3 correspond to bleeding on probing and score 4 to spontaneous bleeding. Thus, it is possible that in the fish oil supplemented group, a lower inflammation would be required to achieve the same gingivitis index because higher gingivitis index could be reached without a true higher grade of inflammation. Decreased platelet aggregation was reported in cats fed a omega 6:omega 3 PUFA ratio of 1.3:1 (Saker, Eddy, Thatcher, & Kalnitsky, 1998) and decreased epithelialization of wounds was also reported in dogs fed a diet with a omega 6:omega 3 PUFA ratio of 0.3:1 (EPA and DHA from fish oil, Scardino et al., 1999). Thus, because the eventual lower haemostatic capacity would increase the bleeding probability and this might translate in an increase in the gingivitis scores, it is possible that with a high daily intake of DHA and/or EPA the gingivitis scoring is not the best indicator of the true inflammation of the gingiva.

5 | CONCLUSION

The results show that there was no benefit of using EPA and DHA supplementation on gingivitis scores associated to PD, thus neither on PD progression.

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