Prostaglandins, Leukotrienes and Essential Fatty Acids 109 (2016) 1–7

Contents lists available at ScienceDirect

Prostaglandins, Leukotrienes and Essential Fatty Acids

journal homepage: www.elsevier.com/locate/plefa

A prospective, randomized, double blind, placebo-controlled evaluation of the effects of eicosapentaenoic acid and docosahexaenoic acid on the clinical signs and erythrocyte membrane polyunsaturated fatty acid concentrations in dogs with osteoarthritis ☆

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ARTICLE INFO

Article history:
Received 21 November 2015
Received in revised form
28 March 2016
Accepted 29 March 2016

Keywords:
Dogs
Osteoarthritis
Polyunsaturated fatty acid
Arachidonic acid
Eicosapentaenoic acid
Docosahexaenoic acid
Alpha-linolenic acid

ABSTRACT

Background: Osteoarthritis (OA) in dogs is a prevalent and serious condition. The most common treatment for the clinical signs of OA in dogs is the administration of nonsteroidal antiiflammatory pharmaceuticals. Omega-3 ($n-3$) fatty acids have been shown to reduce the clinical signs of osteoarthritis in dogs.
Objective: The primary goals of this study were 1) to determine the effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on the clinical signs of OA in dogs, 2) to evaluate the effects of supplementation on the arachadonic acid (ARA)/ (EPA+DHA) algorithm and 3) to correlate alterations in the ARA/(EPA+DHA) with changes in the clinical signs of canine OA.
Methods: Seventy-eight client owned dogs were enrolled in a prospective, randomized, double-blind, placebo controlled clinical trial. Dogs were randomized to placebo oil or triglyceride n-3 oil (providing an average dose of 69 mg EPA+DHA/kg/day). Orthopedic examinations and blood analyses were performed at baseline, day 42, and day 84. A single investigator confirmed a diagnosis of OA of the coxofemoral joints and/or stifle joints in all dogs.
Results: Seventy-four dogs completed the trial. All clinical outcomes for measuring discomfort, lameness, and joint severity at day 84 and all blood metrics at day 42 and day 84 significantly ($p < 0.05$) improved compared with placebo. No major side effects were observed.
Conclusion and clinical relevance: This study demonstrated that the daily supplementation of a dogs diet with EPA and DHA shifts the blood fatty acid concentrations correlating to relief of clinical signs associated with OA in dogs.

1. Introduction

OA affects up to 20% of dogs >1 year of age [1]. The disease afflicts dogs of all breeds and ages. Common approaches to the disease include attempts at prevention, slowing progression, and managing the clinical signs associated with OA. These are accomplished with appropriate nutrition, body- weight control, exercise, physical therapy, and anti-inflammatory and analgesic medications [2,3]. Nonsteroidal anti-inflammatory drugs (NSAID) are effective modes of treatment, but have potential negative systemic side effects such as gastrointestinal ulceration, liver and kidney damage, and accelerated cartilage degeneration [3,4]. There is a pressing need for safe alternatives to manage OA. Previous studies in dogs have supported the efficacy of the marine $n-3$ fatty acids in OA [2,3,5,6–8.]

Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; ALA, α-Linolenic acid; ARA, arachidonic acid; EM, erythrocyte membrane, $n-3$ omega-3 fatty acids, $n-6$ omega-6 fatty acids; OA, osteoarthritis; iFATS, inflammatory fatty acid target score

☆The authors thank Dr. Nancy Soares, Dr. Stephanie Finley, Dr. Carrie Woodcock, for their assistance with case recruitment and Dr. Joe Hauptman for his statistical expertise. SM and ZS designed the research; SM and CK conducted research; SM, ZS, and WH analyzed data; and SM, ZS, WH, and LM wrote the paper. SM had primary responsibility for final content. All authors read and approved the final manuscript.

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http://dx.doi.org/10.1016/j.plefa.2016.03.015

The primary $n-3$ fatty acids are EPA (eicosapentaenoic acid, C20:5$n-3$), DHA (docosahexaenoic acid, C22:6$n-3$), and ALA (alpha-linolenic acid, C18:3$n-3$). Both EPA and DHA are found in high concentrations in fish oils. Both EPA and DHA have potent anti-inflammatory properties, and DHA is a major component of the central nervous system. ALA is found in some seed oils (e.g., flaxseed oil) and requires the enzymes delta-6 and delta-5 desaturases to convert it into EPA and DHA in the body [5,7–11]. Dogs have a limited ability to accomplish this conversion [5,9,10,12], therefore providing preformed EPA and DHA is the most efficient way to increase tissue concentrations of these fatty acids. The other family of essential fatty acids are the $n-6$ fatty acids including LA (linoleic acid, C18:2$n-6$), GLA (gamma-linolenic acid, C18:3$n-6$), and ARA (arachidonic acid, C20:4$n-6$). The former two, especially LA, are found primarily in vegetable oils and food products made with them. ARA is metabolized through multiple pathways (cyclooxygenase, lipoxygenase, cytochrome P450 monooxygenases, etc.), and some of its metabolites are inflammatory mediators [13]. EPA and/or DHA, can be utilized to decrease the amount of ARA available as a substrate for inflammatory eicosanoid production. The body needs episodic inflammation to heal wounds and fight infection, but chronic inflammation can lead to chronic disease.

Consuming fish oils results in the partial replacement of ARA in cell membranes by EPA and DHA. This leads to decreased availability of ARA for conversion into leukotrienes and prostaglandins [14,15]. EPA and DHA give rise to lesser inflammatory molecules (e.g., prostaglandin E3, leukotriene B5) with a resultant competitive inhibition of ARA metabolism [11]. The net effect is a reduction in the anti-inflammatory environment systemically and within the joint.

The primary goals of this study were (1) to determine the effects of daily supplementation of a natural triglyceride form of EPA and DHA (in a ratio of 3:2; Canine Omega Benefits, Veterinarian Recommended Solutions, Plymouth Meeting, PA) on the clinical signs of OA in dogs, (2) to evaluate the effects of supplementation on the blood ARA/EPA+DHA ratio (Inflammatory Fatty Acid target Score, iFATS™), and (3) to correlate changes in the iFATS with changes in OA clinical signs.

2. Materials and methods

2.1. Design

This was a prospective, randomized, double blind, placebo controlled study. Any dog greater than 2 years of age with naturally occurring OA of the stifle or coxofemoral joint as previously diagnosed by a veterinarian physical examination and radiographs was eligible to participate. Exclusion criteria included any dog with a disease in which surgery was recommended (cruciate ligament instability, grade two medial or lateral patella luxation, hip luxation or significant subluxation), concurrent active neurologic conditions, any comorbidity causing weakness or discomfort in the limbs not directly related to osteoarthritis, or current administration of an $n-3$ supplement or diet supplemented with $n-3$. Patients previously taking NSAIDs were enrolled if the pet owner refrained from administering the NSAID for two weeks or more before enrollment. If dogs required rescue analgesia during the trial, they were removed from the study and referred to their primary veterinarian. The data from these dogs was not included in the final analysis. Dogs were recruited from and evaluated at four primary care practices[1]. All pet owners were educated on the study, the product and placebo, and each owner signed a consent form prior to enrolment. All examinations, blood work, and product were administered at no cost to the owner. All animals in this study were the property of a responsible adult pet owner that consciously consented to the participation of their pet in the study. No Animal Use Committee was consulted with for the study; however, a standardized pet owner consent and acknowledgment form was described to and read and signed by every pet owner and each medical director of the participating clinic read and acknowledged the study protocol prior to initiating the trial. All physical examinations and blood collections were performed in a routine manner and in the presence of the pet owner. The ARRIVE guidelines were complied with during the entire clinical trial.

2.2. Interventions

The active treatment group received the $n-3$ product and the placebo group received a medical grade mineral oil. The $n-3$ product contained EPA and DHA, derived from a mixture of anchovy, sardine, and mackerel, in a ratio of 3:2 in a natural triglyceride form. Both oils were distributed in 500 mL, tinted glass bottles with a pump that delivers 3 mL per pump. The label on the outside of the bottle contained a randomized study number, assigned by the study statistician, who also maintained all blinding, held the key for randomization, and performed all data analysis. No block randomization was performed. The number of pumps to deliver to the patient's food dish per day was based on body weight and written on the bottle label. The pet owner was instructed to administer the daily dose once per day either with the morning or evening feeding but that the frequency and time of dosing was consistent throughout the study period. Each mL of the $n-3$ product contained 240 mg of combined EPA and DHA. Dosing schedules for the study were as recommended by the manufacturer: 1 pump (720 mg EPA and DHA) for dogs weighing 4.5–13.6 kg, 2 pumps (1440 mg of EPA and DHA) for 13.7–27.2 kg; 3 pumps (2160 mg of EPA and DHA) for 27.3–40.8 kg, and 4 pumps (2880 mg of EPA and DHA) for dogs weighing greater than 40.8 kg.

2.3. Assessments

Compliance was assessed by having an independent study technician weigh each bottle at day 42 and day 84 and compare that information to the amount of doses recommended to be administered per day.

A single investigator (SJM) blinded to treatment assignment conducted all clinical examinations. Radiography and physical examination were used to confirm a diagnosis of OA. Evaluations by the investigator included a physical examination, a lameness/discomfort visual analogue scale (VAS), a lameness/discomfort severity grade, and an individual and total joint score; and at day 42 and 84 an investigator improvement VAS.

The Lameness/Discomfort VAS is a 10 cm scale on the vertical axis. At the bottom is a description of a normal dog and at the top of the scale is a description of a dog debilitated by OA. A horizontal line is drawn on the scale that best describes the pet on that day. A ruler is used to measure from baseline to the horizontal line and is recorded in centimeters.

The Lameness Grade is derived from a series of numbers (0–10) spaced evenly across the top of the evaluation form. The investigator circles the number that best describes the pet on that day. Zero represents a normal dog and 10 represents a dog debilitated by OA.

[1] Macungie Animal Hospital, Macungie, PA; VCA Wellington, Newtown Square, PA; West Chester Veterinary Rehabilitation Specialty Center, West Chester, PA; Leigh Valley Veterinary Dermatology Center, Allentown, PA.

The Individual Joint Score is derived from an evaluation of the left and right stifle and left and right coxofemoral joints. All four joints were scored in each dog. For each joint, three variables are measured at each time period: effusion, pain on palpation or range of motion, and crepitus. Each variable for each joint was scored 0–3 (no pathology to severe). Points for each variable, each joint, and total points per patient were computed.

The Improvement Scale is a 10 cm scale on the vertical axis, with "no improvement" at the bottom and "complete return to normal" at the top. The investigator placed a horizontal line to best describe the patient on that day. The distance (cm) was recorded from zero (the base of the vertical line) to the drawn horizontal bar.

2.4. Laboratory methods

A 0.2 mL of whole blood was collected using a peripheral vein from each patient at baseline, day 42, and day 84. An aliquot of 25 uL was placed on an anti-oxidant treated card and allowed to air dry. Cards were then sent to OmegaQuant Analytics, LLC (Sioux Falls, SD) where the blood fatty acid composition was measured using capillary column gas chromatography as previously described [15]. From the fatty acid data, the iFATS was calculated.[b]

2.5. Pet foods

All 22 diets were purchased from local pet stores or from an online distributer. If the manufacturer was willing to release label information of fatty acid profile of the specific diet, it was recorded. For any company that would not disclose such information, the sealed bag of pet food was submitted for fatty acid analysis at an independent laboratory (Table 1).

Table 1
The partial fatty acid profile and total omega-6 to omega-3 ratio (*N*-6:*N*-3) representing the 22 diets that dogs in the study were reported to be consuming during the trial period. C18:2*n*6 is ARA, C18:3*n*3 is ALA, C20:5*n*3 is EPA, and C22:6*n*3 is DHA. The individual data presented in this table represent the percentage of a particular fatty acid present within the cell membrane of the erythrocyte in relationship to total fatty acid content of the membrane. The *N*-6:*n*−3 ratio is unitless.

Diet	C18:2*n*6	C18:3*n*3	C20:5*n*3	C22:6*n*3	Total *N*-6:*N*-3
a	31.83	1.36	2.00	1.98	6.17
b	18.88	1.02	1.88	0.63	5.53
c	8.45	0.44	0.28	0.21	9.44
d	26.71	3.05	0.01	0.03	8.73
e	12.82	4.27	0.01	0.02	3.04
f	31.39	2.19	0.01	0.03	14.22
g	7.83	0.38	0.00	0.01	19.91
h	25.55	2.90	2.08	1.53	4.01
i	25.60	1.53	0.04	0.07	16.06
j	19.75	14.05	4.42	2.86	0.95
k	11.11	0.78	0.10	0.22	10.33
l	8.23	2.80	0.20	0.14	2.69
m	18.00	3.44	0.28	0.21	4.67
n	24.20	3.05	0.04	0.05	7.81
o	30.20	1.88	2.00	1.98	5.30
p	18.20	4.70	1.88	0.63	2.62
q	13.00	4.12	0.12	0.10	3.07
r	31.10	2.19	0.05	0.07	13.63
s	22.20	3.88	0.08	0.10	5.50
t	19.20	5.30	1.00	1.20	2.64
u	14.84	3.37	0.62	0.30	3.56
Average	19.96	3.18	0.81	0.59	7.14

2.6. Statistical methods

The data were not normally distributed, Wilcoxon rank sum test were performed on all data, testing for differences between groups at each time, and differences between times for each group (Wilcoxon signed rank test). There were two treatment time points, days 42 and 84, and the changes from time 0 (baseline) were compared between groups. The Type I error rate was protected by means of Bonferroni *P*, *P*/*m* where $P=0.05$ and $m=$the number of comparisons. Data were presented as median (Interquartile Range) and $P<0.05$ was considered significant. Pearson's product moment correlation coefficient, *r*, was used to measure the strength and direction of the linear relationship between two normally distributed variables, in this case, the relationship between the iFATS and specific orthopedic outcome variables at day 0 and day 84. The value of *r* is assumed to be between $+1$ and -1. All statistical analysis was performed using a SAS 9.3 (Cary, NC).

3. Results

One hundred dogs were evaluated for inclusion in the trial. Twenty-two dogs, fifteen in the placebo group and seven in the treatment group, were not included in the final analysis of the study because they had a surgical orthopedic condition diagnosed at the base line examination or between the baseline and the 42-day evaluation. Another four dogs were removed from the final analysis; two dogs in the placebo group required rescue analgesia (in the form of an NSAID) between days 42 and 84 and were removed from the trial. One dog in the active group developed acute left thoracic limb lameness and was diagnosed with metastatic squamous cell carcinoma of the nail bed and was euthanized between the 42-day and the 84-day evaluation. One dog in the treatment group did not present for the 84-day evaluation and was removed from the study. There were 41 dogs in the treatment group and 33 dogs in the placebo group that completed the study. The treatment group included 10 Labrador retrievers, 2 golden retrievers, 3 pit-bull terriers, 2 bulldogs, 9 mix breed dogs, and one each of a cockapoo, smooth coated collie, miniature poodle, standard poodle, wire pointing griffon, giant schnauzer, pug, German shepherd, German shorthair pointer, basset hound, bichin frise, old English mastiff, Shetland sheepdog, Chihuahua, and an Australian shepherd. In the placebo group there were 8 Labrador retrievers, 3 pit-bull terriers, 9 mix breed dogs, 2 German shepherds, and one each of a Welsh corgi, King Charlers Cavalier, miniature schnauzer, Great Dane, Saint Bernard, Bernese mountain dog, Pomeranian, Shiba Inu, husky, basset hound, and a Bouvier des Flanders. Mean ages of the two groups were 7.3 (2–12.5) and 8.5 (2–14) years, respectively. The treatment group included 25 spayed females and 14 castrated males; for the placebo group, 14 spayed females, 18 castrated males and 1 intact male. There was no relationship between breed, sex, age, or weight and any of the outcome metrics. The average dose of combined EPA and DHA received by dogs in the study was 68.9 mg/kg/day (33–103.4 mg/kg/day).

3.1. Bottle weights and compliance

All bottles were weighed at baseline, day 42, and day 84 for each dog. Expected weights of bottles were calculated for each weight class and compared to the measured weights. There were no significant differences in the weights of the bottles for each weight class between the treatment and placebo group.

3.2. Clinical outcomes

Investigator assessment results are represented in Figs 1–4. Compared with the placebo group, there was a significant improvement in the treatment group at day 42 and day 84 for the Total Joint Score ($p < 0.001$) and the Improvement VAS ($p < 0.0001$). On day 42, there was no significant difference between the placebo and treatment groups for the Lameness/Discomfort VAS and the Lameness Severity Score; however, both outcome variables demonstrated a significant difference at the day 84-time point ($p < 0.001$).

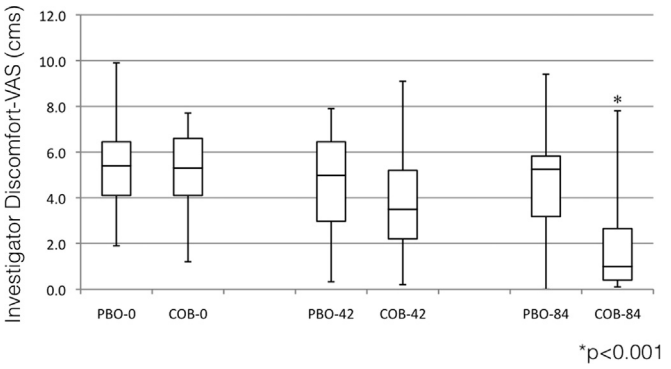

Fig. 1. Box and whisker plot Investigator Visual Analogue Scale for Discomfort/Lameness. This is a 10 cm vertical scale. The plots represent the median, 25–75 quartiles, and minimum and maximum data points. A significant decrease in discomfort and lameness occurs in the treatment group compared to the placebo group at the 84 day time point. $^{*}p < 0.001$.

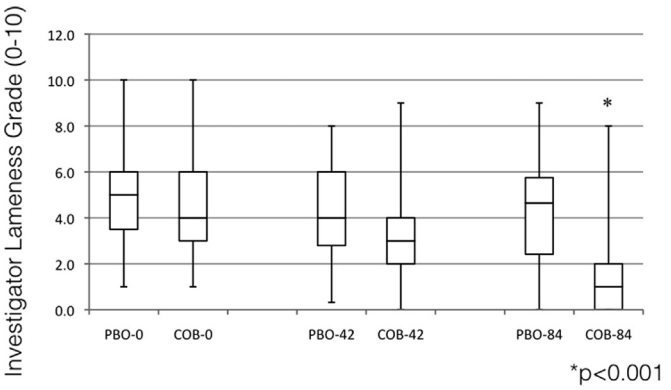

Fig. 2. Box and whisker plot Investigator 10 Point Severity Grade for Lameness. The plots represent the median, 25–75 quartiles, and minimum and maximum data points. A significant decrease in the lameness grade occurs in the treatment group compared to the placebo group at the 84 day time point. $^{*}p < 0.001$.

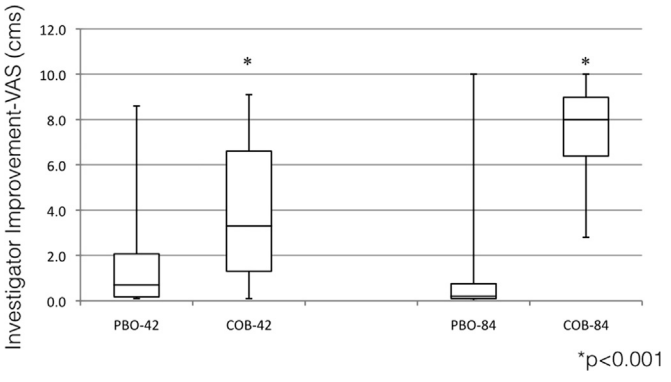

Fig. 3. Box and whisker plot Investigator Visual Analogue Scale for overall Improvement. The plots represent the median, 25–75 quartiles, and minimum and maximum data points. A significant improvement in overall lameness, function, and comfort was observed in the treatment group compared to the placebo group at day 42 and day 84. $^{*}p < 0.001$.

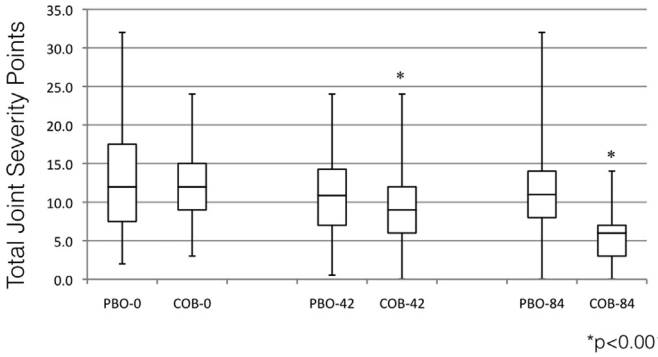

Fig. 4. Box and whisker plot The Total Joint Severity Score. The plots represent the median, 25–75 quartiles, and minimum and maximum data points. The total score was calculated by adding the scores from the assessment of three variables, pain, crepitus, and effusion rated from 0 (normal) to 3 (most severe) for each joint. There is a statistically significant improvement in the total point score for all joints at day 42 and day 84 in the treatment group compared to the placebo group. $^{*}p < 0.0001$.

3.3. Blood fatty acid metrics

The individual EPA, DHA, and ARA values are listed in Table 2, as is the individual iFATS. The iFATS was significantly decreased ($p < 0.001$) in the treatment group (Fig. 5) at days 42 and 84. The effects of $n-3$ treatment on the iFATS were similar at days 42 and 84.

3.4. Correlation between the change in the iFATS and Clinical Outcomes

At baseline, for the 74 dogs that completed the trial, a correlation coefficient of $r=0.74$ was calculated between the iFATS and the Lameness Severity Score and a correlation coefficient of $r=0.73$ was calculated between the iFATS and the Lameness/Discomfort VAS. The correlation coefficient calculated between the iFATS and the Total Joint Score was $r=0.03$. The 84-day data was also evaluated for an existing correlation to each outcome variable and the iFATS. The correlation coefficient between the Lameness Severity Score in the 74 dogs and the iFATS at day 84 was $r=0.75$, and for the Lameness/Discomfort VAS and the iFATS was $r=0.67$. The correlation coefficient identified between the Total Joint Score and the iFATS at day 84, was $r=0.37$. These results indicate a strong positive correlation between the iFATS at baseline and at day 84 with the Lameness Severity Score and the Lameness/Discomfort VAS but no correlation exists between the iFATS and the Total Joint Score.

3.5. Adverse events

Four adverse events were reported during the trial, two in each group. One dog from each group developed diarrhea during the first week of the study. Both were prescribed metronidazole for 1 week and were able to continue in the study. Two other dogs developed food aversion at day 2–3 of the study. In both cases, normal dry food was replaced with canned food and this resolved the aversion.

Table 2
The median, interquartile range (IQR) low and high for EPA, DHA, ARA, and iFATS for both the placebo and active treatment groups at day 0 and day 84.

		EPA		DHA		ARA		ARA/ EPA+DHA	
		Day 0	Day 84	Day 0	Day 84	Day 0	Day 84	Day 0	Day 84
Placebo	Median	0.50%	0.40%	0.98%	0.95%	21.00%	20.50%	14.77	14.88
	IQR Low	0.20%	0.20%	0.69%	0.43%	17.80%	17.25%	7.93	7.29
	IQR High	1.00%	0.80%	1.92%	1.64%	23.15%	22.90%	24.29	24.46
Active	Median	0.20%	2.70%	0.64%	2.13%	22.40%	18.50%	28.31	3.48
	IQR Low	0.10%	1.85%	0.33%	1.81%	19.40%	15.45%	15.12	2.86
	IQR High	1.10%	3.40%	0.99%	2.58%	24.40%	20.65%	45.03	4.83

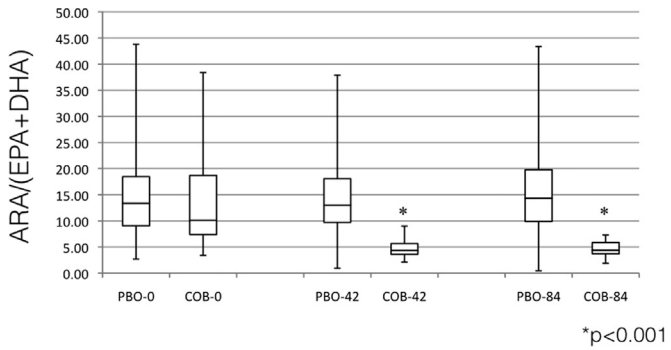

Fig. 5. Box and whisker plot of the ARA/(EPA+DHA) ratio. The plots represent the median, 25–75 quartiles, and minimum and maximum data points. There is a statistically significant decrease in the ARA/(EPA+DHA) ratio in the treatment group compared to the placebo group at day 42 and day 84. * $p < 0.0001$.

4. Discussion

The main findings of this study were that treatment with a triglyceride form of EPA and DHA (average dose of 68.9 mg/kg/day) for 84 days improved all investigator-assessed clinical markers of OA. The methods of clinical evaluation were chosen based on recent publications validating the use of these techniques to assess improvements in lameness and pain in dogs with OA [2,6–8,16,17–25]. The crepitus, pain, and effusion scores and the overall improvement scales had significantly improved in dogs in the treatment group compared to the placebo group by day 42 but the discomfort scales and lameness grades did not significantly improve until day 84. Blood concentrations of ARA changed rapidly in dogs receiving EPA and DHA supplementation and may be associated with a more rapid decrease in the clinical signs associated with inflammation (effusion and pain) of the joint and an early and obvious overall improvement. While the clinical signs associated with compensatory mechanisms, chronic nerve stimulation, and muscle atrophy, that contribute to clinical lameness and discomfort, will likely take longer to improve and may be the reason why these particular parameters improved at 84 days and not at 42 days. The clinical significance of the statistical findings was readily apparent during the trial. Patients receiving daily supplementation of fish oil had consistent clinical improvements in their pain, dysfunction, and overall well-being by the conclusion of the trial.

There are numerous reports documenting the biochemical benefits of diets enriched or supplemented with EPA and DHA in dogs with OA [2,5,7,8,25,26–30]. The mechanisms in which EPA and DHA interact with the inflammatory system have been reported and include the suppression of proinflammatory mediators IL-1, IL-2, and tumor necrosis factor in cartilage [31]. modification of COX-2 and 5-LOX metabolism of ARA [32], a decrease in expression of pro matrix metalloproteinases (MMP's) and urokinase plasminogen activator, and increases in tissue inhibitors of MMP-2 [15] Clinical improvements have also been documented, including improvements in lameness, weight-bearing scores, and force-plate analysis [8]. In a multicenter study, dogs fed a diet enriched with EPA and DHA had a significantly higher serum concentration of total $n-3$ fatty acids and a significantly lower serum concentration of ARA at 6, 12, and 24 weeks and, according to owners, dogs fed the test food had a significantly improved ability to rise from a resting position and play at 6 weeks and improved ability to walk at 12 and 24 weeks [2]. In another multicenter study evaluating the effect of dietary supplementation with $n-3$ fatty acids on carprofen dosage in dogs with OA, the investigators found that the intervention allowed for a reduction in carprofen dosage over time [7]. Other studies have evaluated the effect of an EPA and DHA enriched diet on the clinical signs of OA and plasma concentrations of fatty acids [2,8] but the application of EPA and DHA to a variety of commercial diets in dogs with OA has not been evaluated in combination with using whole blood ratios of ARA/(EPA+DHA).

The hypothesis of this study was that dogs with clinical signs of OA would benefit clinically and would experience an increase in the blood ARA/(EPA+DHA) ratio after supplementation of a triglyceride form of EPA and DHA. In the absence of these two fatty acids, cells incorporate ARA (whether consumed preformed in the diet or produced from dietary LA) into cell membranes resulting in the ultimate production of a suite of inflammatory mediators at sites of injury or disease. Increased consumption of EPA and DHA raise their own tissue concentrations and, at the same time, lowers ARA concentrations by inhibiting its synthesis from LA and by competition for esterification into a finite number of plasma phospholipid molecules. Thus, higher EPA+DHA intake lowers ARA and markedly lowers the iFATS. The manufacturers recommended dose is lower than the dose historically recommended to treat OA in dogs. The discrepancy in dosage recommendations is related to form. It is known in mammals that absorption, and therefore bioavailability, of a natural or reesterified triglyceride form of fish oil is significantly more efficient than the ethyl ester form of fish oil [5,33]. The historically studied and reported form of fish oil used in dogs to treat OA has been in the ethyl ester form and therefore a higher dose of combined EPA and DHA is required to achieve plasma or erythrocyte membrane levels similar to a lesser dose of a triglyceride form of EPA and DHA.

It was also hypothesized that a correlation would exist between the iFATS and the orthopedic clinical outcome variables. At baseline and at day 84 of the trial, the iFATS was positively correlated with the Lameness Severity Score and the Lameness/Discomfort VAS but not with the Total Joint Score at either time point. If there is less ARA in the tissues of the body, an indirect measure of the iFATS, then there should be less inflammation produced by the ARA cascade yielding less clinical signs associated with OA. This is likely a part of the mechanism correlating a lower iFATS with a less severe lameness and discomfort. One justification for the lack of a

correlation between the Total Joint Score and the iFATS is due to the definition of the Total Joint Score. A dog with a severely arthritic left stifle joint may have a severe lameness and discomfort in that leg and a relatively high iFATS, and because only one joint is affected in this case, the Total Joint Score will be high for that joint but low for the other joints. This is in comparison to a dog with minimal OA in 5 different joints. This second patient may have a high Total Joint Score because of the number of effected joints involved in the scoring is 5 compared to 1, but the patient is expected to have minimal lameness and a relatively low iFATS.

In clinical experience, OA can occur in dogs that are on a variety of individual diets representing a wide range of $n-6$:$n-3$ ratios. Because of the addition of flaxseed oil (which contains 55% ALA) or even soybean oil (7% ALA) to pet foods [34], the $n-6$:$n-3$ ratio in the food can be rather easily manipulated, but ALA is not EPA or DHA, and conversion of ALA to these long chain metabolites is quite inefficient in dogs. Hence, the claim that a product has a given $n-6$:$n-3$ ratio is relatively meaningless unless the specific fatty acids that comprise the ratio are listed. A ratio of 4:1, for example, would be considered strongly anti-inflammatory if the majority of the $n-3$ was in the form of EPA and DHA, but it would likely have minimal effect on the inflammatory processes if the $n-3$ was predominantly ALA. This is why blood levels of ARA in comparison to EPA and DHA have come to be recognized as more physiologically relevant than $n-6$:$n-3$ ratios [35]. There were 22 different commercial diets represented in the study. Although no specific diet or diet type could be directly linked to a level of baseline $n-3$ deficiency in our study, many of the diets with added $n-3$ represented in our study used ALA. The results of our study demonstrated that, even without knowledge of the background diet's $n-6$:$n-3$ ratio, the daily administration of EPA and DHA to dogs in the treatment group significantly lowered systemic ARA concentrations and elevated EPA and DHA concentrations and improved their clinical signs associated with OA.

The ARA/(EPA+DHA) ratio of whole blood correlates strongly with that of erythrocyte membranes ($r=0.94$, $n=50$ unpublished data), which are themselves a valid reflection of the polyunsaturated fatty acid composition of other cell membranes throughout the body [36,33,37]. This method of assessment was chosen because there is considerably more day-to-day variation in plasma fatty acid levels than in whole blood or erythrocyte membrane [38,39.]

Baseline concentrations of EPA and DHA in the dogs enrolled in the study were low, and ARA concentrations were elevated compared to the post treatment levels indicating a possible pattern in the fatty acid ratio of the dogs' diets. Patients in the treatment group had a correction of this imbalance with daily supplementation with EPA and DHA. The significant decrease in ARA (about 17%), and the significant increase in EPA (13.3-fold) and DHA (3.3-fold) correlated with significant changes in the comfort and function of patients in the treatment group.

The $n-3$ triglyceride supplement evaluated in this study appeared to be safe for daily ingestion. A total of 4 dogs developed gastrointestinal side effects, two of which were in the treatment group, which may have been attributable to the administration of fish oil. In all 4 cases, the signs resolved within a few days.

One of the limitations in this study was a relatively small number of patients and the intervention was applied over a relatively short time period. Longer and larger studies are needed to confirm the prolonged improvement in clinical signs observed here. Strengths of the study include the methods of assessing discomfort, lameness, and joint health which were based on previously validated techniques [2,6–8,16,17–25]. Having outcomes assessed by a single (blinded) investigator eliminated bias [2,8,18]. The use of a refined visual analogue and scoring scale, a validated composite score, and force plate analysis may be helpful in future studies to examine differences in investigators' and owners' assessments. Finally, variability in diets before and during the trial could be considered a limitation or weakness; however, it may also be viewed as a strength since the diets represented here are characteristic of the clinical environment. This variability presumably contributed to differences in baseline fatty acid levels, but the majority of patients demonstrated patterns that were associated with worse clinical features of OA that were significantly altered in the treatment group by day 84.

5. Conclusion

Our findings suggest that the daily ingestion of EPA and DHA is safe for dogs with OA and improves the clinical signs, and this improvement is associated with a marked reduction in the ratio of ARA to EPA and DHA in the blood. Additional studies are needed to assess the longer-term effects of supplementation with this product on OA. Additional endpoints in such studies could include synovial fluid analysis of the enzymes known to damage articular cartilage, force plate analysis, and possibly a head-to-head comparison with NSAID agents and/or other forms of EPA and DHA.

Disclosures

At the time of study design, implementation, data collection, and document preparation SJM was a compensated member of the Veterinarian Recommended Solutions (VRS) scientific advisory board. WSH is the owner of OmegaQuant Analytics, LLC.

Funding source

The research was partially funded by Veterinarian Recommended Solutions.

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