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A prospective, randomized, double blind, placebo-controlled evaluation of the effects of an *n*-3 essential fatty acids supplement (Agepi® ω3) on clinical signs, and fatty acid concentrations in the erythrocyte membrane, hair shafts and skin surface of dogs with poor quality coats

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ABSTRACT

Canine haircoat quality, the time course of incorporation of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) into the canine erythrocyte membrane, changes in total lipids content on hair shafts and of neutral lipids on the skin surface, were investigated after administrating an *n*-3 fatty acids supplement. Twenty-four dogs with poor haircoat received a placebo or *n*-3 oil capsules (110 mg EPA/68 mg DHA) daily for 90 days. Clinical assessments and tissue sampling were performed monthly from day (D)0 to D180. The clinical score in treated dogs was significantly reduced from D60, then attained a plateau and returned to baseline levels on D180. EPA and DHA contents in the erythrocyte membrane increased significantly from D30 and decreased rapidly after supplement withdrawal in treated dogs. Total lipids on the hair shaft increased progressively in the supplemented group. EPA/DHA supplements impact blood and hair fatty acids important for haircoat quality in dogs.

1. Introduction

The skin is the body's largest organ and with haircoat, uses a large proportion of the daily nutrient intake. Modifying nutrition can lead to visible effects on skin and haircoat quality. Fatty acids (FA) are one of the most common dietary ingredients that seem to benefit such quality [1]. In the skin, FA not only form part of the cell membranes but also have an extracellular function and contribute to normal haircoat luster, skin smoothness and epidermal barrier integrity [1, 2]. Some FA, such as eicosapentaenoic acid (EPA, C20:5 *n*-3) and docosahexaenoic acid (DHA, C22:6 *n*-3), are called "essential" because of the limited ability of individuals to synthesize them. In consequence, the most efficient way to increase their tissue concentrations is to incorporate such *n-3* FA into the diet. Essential fatty acids (EFA) deficiency has also been linked with other clinical signs such as infertility, coronary heart disease, weak cutaneous blood vessels, poor growth, cognitive deficiency and ocular retinopathies [3-5].

Among other things, EFA deficiency in dogs causes matted coat and unkempt appearance, discolored coat, scaly skin, sebaceous gland hypertrophy, increased epidermal turnover rate and delayed wound healing [6-8]. EFA supplements are known to have beneficial effects not only on hair and skin quality [1, 6], but also in dogs suffering from pruritic and inflammatory skin conditions [6, 9, 10]. FA supplements are known to be able to modify the composition of FA in canine serum and skin [11]. EPA and DHA are generally considered to be the *n-3* FA with the most beneficial effects [12]. Dog owners are concerned about hair and skin quality and many over-the-counter diets are marketed for this purpose [13]. Unfortunately, their compositions vary considerably and do not always fit with established recommendations [14]. Furthermore, although many commercial maintenance foods are now well-balanced in terms of FA composition, increasing the total dietary fat has been shown to contribute to coat gloss and skin softness, even in the

Abbreviations: SSI, Skin seborrheic index; EPA, Eicosapentaenoic acid; DHA, Docosahexaenoic acid; EM, Erythrocyte membrane; RBC, Erythrocyte; *n*-3, Omega-3 fatty acids; *n*-6, Omega-6 fatty acids; FA, Fatty acids; EFA, Essential fatty acids; ARA, arachidonic acid

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absence of FA deficiency [15]. To increase the total amount of ingested *n*-3 FA, the owner may choose a special FA-enriched diet or opt to supplement the dog's current diet. Although the benefit of EFA on hair and skin quality is generally acknowledged, little is known about the following aspects: the most beneficial type of EFA and the required dose, the optimal *n*-6 to *n*-3 EFA ratio in the diet, the improved coat quality expected over time following supplementation, the duration and frequency of EFA supplementation required to maintain an optimal haircoat or how such supplementation modifies FA concentrations within the organism.

The purpose of the study was to assess the benefit of daily addition (for three consecutive months) of a commercial veterinary food supplement (Agepi® ω3, MP Labo, Grasse, France) containing 160 mg of EPA and 100 mg of DHA (in triglyceride form) per capsule, in dogs with poor quality haircoat, all receiving a medium quality diet and living in the same environmental conditions. Effects on *(i)* clinical signs, and *(ii)* the amount of FA incorporated into the erythrocyte (RBC) cytoplasmic membranes, the total lipids content on hair shafts and the neutral lipids on the skin surface, were determined.

2. MATERIAL AND METHODS

2.1. Animals

Unrelated hound dogs with poor coat condition, as objectivized with a specific scoring system (table 1) - see 2.3, but otherwise healthy, all belonging to a sole owner were selected. The dogs were permanently housed in outdoor, partially-covered runs and were fed once daily. Water was provided *ad libitum*. The dogs were in good clinical condition and had no history of skin disease. All dogs had been treated against fleas and dewormed with a combination of spinosad and milbemycine oxyme (Trifexis®, Elanco Animal Health, UK) for one year prior to inclusion in the study. All dogs had been receiving the same dry pet food [Pasqui Energie, agriPasquier, Les Cerqueux, France - table S1 (supplementary data)] for the past twelve months and this diet was not modified during the study. Exclusion criteria were parasitic infestation, fungal or bacterial skin infection, poor general condition, EFA supplementation during the two months prior to the study or administration of any anti-inflammatory treatment.

2.2. Design

This was a prospective, randomized, double blind, placebo-controlled study. It was approved by the Toulouse veterinary school (Université de Toulouse, ENVT) Ethical Committee and a written informed consent was obtained from the owner. Before beginning the study, the included dogs were randomly assigned to two groups with thirteen dogs per group. Each dog was allocated a number from 1 to 26 for the duration of the study. The dogs in both groups received one capsule per 10 kg of body weight once daily for 90 days. The placebo capsule (group A, placebo) contained only microcrystalline cellulose. The tested product (TP) capsule (Agepi® ω3, group B, EPA-DHA) had a gross weight of 968 mg and contained 746.5 mg of fish oil including 230 mg of *n*-3 (with 160 mg of EPA and 100 mg of DHA in a triglyceride form) and 4.5 mg of vitamin E. The fish oil, provided by MP Labo, Grasse, France, was processed in France, from fish caught in the North Atlantic and certified "Friend of the Sea". The specifications of the fish oil used were as follows: EPA min 18%, DHA min 12 %, total Omega 3 approx 39 %, EPA (as FA) approx 160 mg/g and DHA (as FA) approx 100 mg/g. The two capsule containers could only be distinguished by their label (A or B). The owner and investigators were blinded to the groups until the end of the study. The owner was instructed to administer the daily dose individually, once every morning at feeding

Table. 2
Timeline presenting an overview of the study's protocol. Dogs received the capsules (placebo or tested supplement) once daily for three months then capsules were withdrawn for the rest of the study. Clinical scoring was performed once monthly from D0 to D180. Analyses were performed on blood samples once monthly from D0 to D120, on hair samples once monthly from D0 to D150 and on skin surface samples on D0 and D90.

	D0	D30	D60	D90	D120	D150	D180
supplementation	Yes				No		
clinical score	X	X	X	X	X	X	X
blood sample	X	X	X	X	X		
hair sample	X	X	X	X	X	X	
skin surface sample	X			X			

Table. 3
Characteristics of the canine cohort – TS: Tested supplement.

Gender	Age (years)	Breed	Weight (kg)	Group
male	8	Bruno du Jura	21	TS
male	2	Griffon	32	TS
male	7	Griffon	30	TS
male	3	Bleu de Gascogne	23.7	TS
male	8	Bruno du Jura	28.2	TS
male	5	Griffon	27	TS
female	3	Bleu de Gascogne	23	TS
female	7	Griffon	24	TS
female	1	Griffon	19	TS
male	4	Griffon	25.5	TS
male	3	Bruno du Jura	28	TS
female	7	Griffon	26	TS
male	9	Griffon	31	TS
male	7	Griffon	37.1	placebo
female	8	Bruno du Jura	27	placebo
male	8	Bruno du Jura	27	placebo
female	13	Griffon	20	placebo
male	3	Griffon	29	placebo
male	2	Bruno du Jura	24	placebo
male	1	Griffon	25	placebo
male	1	Griffon	25	placebo
male	1	Griffon	28	placebo
male	2	Griffon	22.3	placebo
male	1	Griffon	26	placebo
male	5	Griffon	31	placebo
male	1	Griffon	20	placebo

Table. 1
Grading scale used to evaluate skin lesions in dogs with poor quality haircoat. The SSI was obtained by adding the different scores and ranged between 0 and 21.

parameter/score	0	1	2	3
malodor	absent	perceptible in proximity to the animal	perceptible at some distance from the animal	strong even at some distance
affected area	< 20% of the body surface	20 - 50% of the body surface	50 - 75% of the body surface	> 75% of the body surface
pruritus	absent	mild	moderate	severe
erythema	absent	mild	moderate	severe
scaling	absent	mild	moderate	severe
secondary lesions (papules, pustules)	absent	mild	moderate	severe
lichenification	absent	mild	moderate	severe

time, for three consecutive months. He was also instructed to monitor any adverse event and to report to the investigator in due course. The study was performed during the closed season.

2.3. Clinical assessment

After a general clinical examination, the dogs were dermatologically evaluated, always by the same investigator, on day (D)0, D30, D60, D90, D120, D150 and D180. A seborrheic scoring index (SSI), adapted from Viaud *et al.* [16] and allowing attribution of a clinical score, was used. Briefly, the following parameters were assessed on a 0-3 scale: body odor, coat dullness and dryness, affected surface, pruritus severity, erythema, scales, secondary lesions and lichenification (table 1).

2.4. Biologic samples

Two mL of whole blood was collected from a peripheral vein of each dog into ethylenediaminetetra-acetic acid (EDTA) tubes (Vacumed®, Torreglia, Italy) on D0, D30, D60, D90 and D120. The blood samples were stored at 4°C and sent to the laboratory for analysis within less than 24 hours.

Hair samples were collected on the dorsum of each dog by plucking hair shafts with a hemostat on D0, D30, D60, D90, D120 and D150. Approximately 40 hair shafts were sampled and cut to 1.5 cm-lengths for each dog and each sampling time. Samples were conserved in plastic vials (Greiner Bio-one CELLSTAR®, Greiner Bio-One, Kremsmünster, Austria) at -20°C until analysis. Finally, skin surface samples for neutral lipid analysis were taken on D0 and D90 by rubbing a 5-cm line on the skin with two swabs (Dryswab® MWE, Medical Wire & Equipment, Wiltshire, England) previously soaked in an aqueous non-ionic surfactant solution (Synelvia, Labège, France - proprietary method). The swab heads were then removed, placed in dry Eppendorf tubes (Safe-Lock Tubes 1.5 mL, Eppendorf AG, Hamburg, Germany) and frozen at -20°C until analysis. An overview of the protocol is presented in table 2.

2.5. Measurements and analyses

2.5.1. Fatty acids in RBC

Before analysis, RBC were separated by centrifugation (1500 g for 15 min at 4°C). RBC were lysed and FA, especially DHA, were converted to the corresponding methyl esters with 3N HCl in methanol. The FA methyl esters were then extracted with n-hexane and analyzed using an Agilent 7890A gas chromatograph equipped with a 100 m SLB-IL111 fused silica capillary of 0.2 µm thickness and coupled to an Agilent 5975C mass spectrometer. The column was temperature programmed and the injection port temperature was 250°C. The detector port temperature was 255°C, the Helium gas carrier flow 1.5 mL/min with a split ratio of 10:1, and the injection volume was 2 µL. FA were identified using the MS Database, FA concentration was calculated using an external calibration and FA content was expressed in µg per 100 mg of RBC.

2.5.2. Fatty acids on hair fibers

Before analysis, 20 mg of hair fibers were cut and ground to a fine powder in a Retch mill. FA were analyzed as described for FA in RBC. FA concentration was calculated using an external calibration and FA content was expressed in µg per 100 mg of hair fibers.

2.5.3. Neutral lipids in skin surface samples

Before analysis, total lipids were extracted from swabs by the method of Bligh & Dyer [17]. The neutral lipids (free fatty acids, squalene, cholesterol, cholesterol esters and glycerides) were then analyzed using an Agilent 7890A gas chromatograph equipped with a 30 m 5HT fused silica capillary of 0.1 µm thickness and coupled to an Agilent 5975C mass spectrometer. The column was temperature programmed and the injection port temperature was 315°C. The detector port temperature was 380°C, the Helium gas carrier flow 1.0 mL/min and the injection volume was 2 µL. Neutral lipids were identified using the MS Database. Squalene and cholesterol concentrations were calculated using an external calibration and the corresponding contents were expressed in µg per collected sample. The other neutral lipids (free fatty acids, cholesterol esters and glycerides) were estimated as response factors of the analyte (peak area of analyte of interest/peak area of identifiable compounds).

2.6. Statistical analysis

All data were tested for normality distribution using the Shapiro-Wilk normality test. When data were normally distributed, homogeneity of variance was verified by Barlett's test. In the case of EPA and DHA measurements on erythrocyte membranes (EM), the data were normally distributed and the parametric ANOVA 1 test was used to compare concentrations between the different sampling days. For data that showed statistically significant differences over time the Bonferroni's Multiple Comparison Test was computed to compare individual time points within the group. When data were not normally distributed at all-time points, non-parametric tests (Kruskal-Wallis) were used.

Statistical analyses were performed using SAS® software 9.3 (SAS Institute Inc., Cary, NC, USA) and a two-sided p-value <0.05 was considered statistically significant.

3. RESULTS

3.1. Animals (table 3)

Twenty-six dogs, thirteen in each group, met the inclusion criteria. Group A (placebo group) included 11 males and 2 females (10 griffons and 3 Bruno du Jura dogs). The median age was 4 years (range 1-13) and median weight 26.3 kg (range 20-37.1). Group B (EPA-DHA group contained 9 males and 4 females *i.e.* 8 griffons, 3 Bruno du Jura and 2 Bleu de Gascogne dogs with a median age of 5.2 years (range 1-9) and a median weight of 26.0 kg (range 19-32). The differences in breed, sex, age or weight between the two groups were not statistically significant. Dogs received an average combined EPA and DHA supplement of 20.4 mg/kg/day. One dog in each group did not complete the study because of health issues (dog bites) not related to the study and both were excluded from the analyses.

3.2. Clinical score

The baseline clinical scores were similar in both groups (median 2, min 0, max 3 and 4, group EPA/DHA and placebo, respectively) and clinical scores remained steady throughout the study in the placebo group (median 2, min 0 or 1 and max 3 or 4), $p > 0.05$. Conversely, a statistically significant reduction of SSI was observed in the treated group on D60 when compared to D0 (median 1, min 0, max 3) ($p = 0.007$), reaching the lowest index on D90 (median 0, min 0, max 1) ($p < 0.003$). Although the SSI had already shown a reduction on D30, this difference was not statistically significant ($p = 0.08$). The observed reduction of SSI in the treatment group between D60 and D90 was not statistically significant ($p = 0.102$). After withdrawal of the supplement on D90, the SSI remained stable for one month then progressively increased to attain baseline levels on D180 (Fig. 1).

3.3. EPA and DHA on EM (Fig. 2A and 2B)

EPA and DHA levels did not differ significantly between the groups on D0 and were not significantly modified in the placebo group during the study (Fig. 2A and 2B). In the treated group, EPA content significantly increased from D30, remaining at a plateau until D90 ($p < 0.0005$). No statistically significant differences were observed

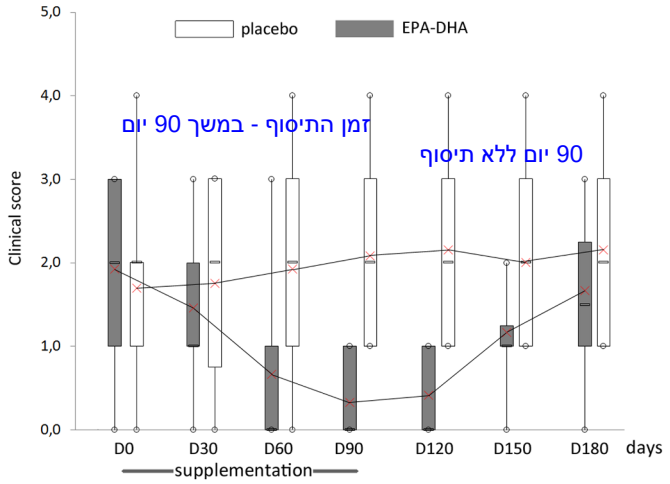

Fig. 1. Kinetics of clinical scores (SSI) in dogs with poor coat condition that received either EPA-DHA (gray boxes) or placebo (white boxes) supplementation once daily for three consecutive months (from D0 to D90) at different time points during the study. Box and whiskers plot interpretation: Top of box is the upper quartile, the line in the middle is the median, the bottom of the box is the lower quartile and the upper and lower whiskers represent minimum and maximum scores. × represents the mean and the line connecting the dates represents the mean line.

between D30 and D60 or between D60 and D90 (Fig. 2A). DHA levels in the treated group significantly increased on D30 compared to D0 ($p < 0.0005$) and on D60 compared to D30 ($p < 0.05$). The increase in DHA content of the EM between D60 and D90 was not statistically significant (Fig. 2B). In the treated group, supplement withdrawal led to a significant decrease of both EPA and DHA levels on D120 ($p < 0.0005$). The arachidonic acid (ARA) content of EM was also calculated. The ARA/(EPA + DHA) ratio was reduced from the first month of supplementation (see supplementary material, Fig. S1). Details of the various FA are given in table S2 (supplementary data).

3.4. *Total lipids on hair samples (Fig. 3)*

Total FA contents on hair samples were significantly higher on D0 in the placebo group, when compared to the EPA-DHA group ($p = 0.006$). In the treated group, the total lipids content increased progressively, reaching statistical significance at D60 ($p = 0.045$). The total content then decreased slightly on D90 and D120, remaining higher than baseline although this difference was not statistically significant. Some variations in the total lipids content of hair shafts were observed in the placebo group during the study but were not statistically significant. Neither EPA or DHA were detected on hair samples. Details of the various FA are given in table S3 (supplementary data).

3.5. *Squalene, cholesterol and total neutral lipids on the skin surface (Fig. 4A and 4B)*

No statistically significant differences were observed in the contents of squalene, cholesterol, cholesterol esters and total neutral lipids between the groups at baseline. Squalene content on skin surface samples decreased significantly in the treatment group on D90 when compared to D0 ($p = 0.001$) whereas no difference was observed in the placebo group (Fig. 4A). Although some increase in the average amount of cholesterol present on the skin surface of treated dogs was observed on D90, when compared to D0, this increase was also observed in the placebo group and was not, in either case, statistically significant (Fig. 4B). No significant changes from baseline were observed in the other neutral lipids tested on D90. Details of the various FA are given in table S4 (supplementary data).

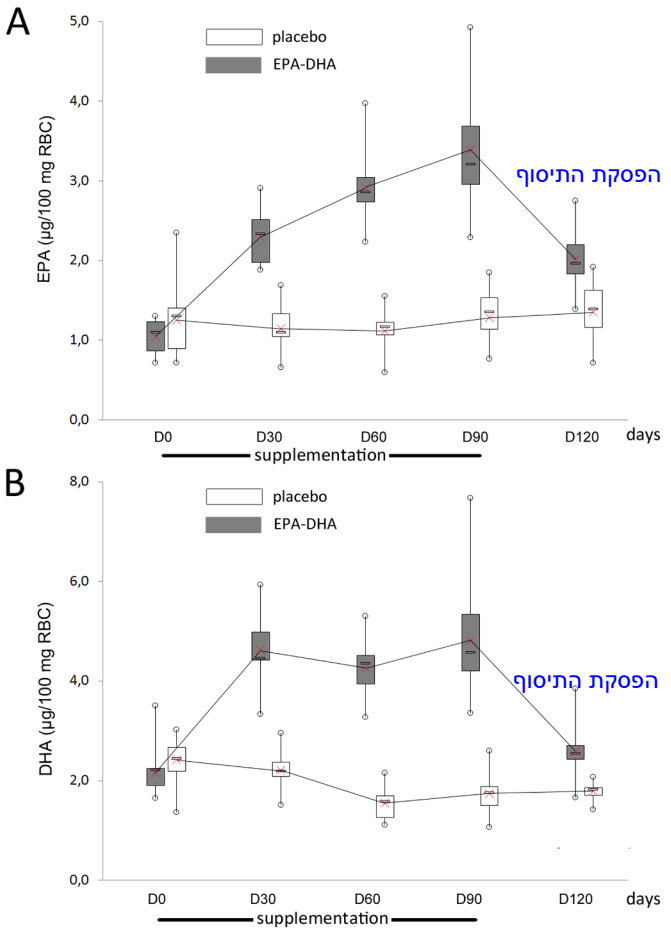

Fig. 2. Kinetics of EPA (A) and DHA (B) levels on erythrocyte membranes in dogs with poor coat condition that received either EPA-DHA (gray boxes) or placebo (white boxes) supplementation once daily for three consecutive months (from D0 to D90) at different time points during the study. Box and whiskers plot interpretation: Top of box is the upper quartile, the line in the middle is the median, the bottom of the box is the lower quartile and the upper and lower whiskers represent minimum and maximum scores. × represents the mean and the line connecting the dates represents the mean line.

3.6. *Adverse events*

No diarrhea or vomiting was reported by the owner.

4. DISCUSSION AND CONCLUSION

Daily supplementing of the diet with a commercial preparation based on fish oil (average dose 20.4 mg/kg of EPA and DHA) significantly improved haircoat and skin quality in the dogs. This positive effect was observed from the first month of treatment and reached a plateau after two months of supplementation. The beneficial clinical effect was observed to remain stable for one month after withdrawal and only deteriorated after the second month. In consequence veterinarians and owners should be recommended to administer this supplement to dogs for at least two months to attain the maximum beneficial effect and subsequently not to stop for more than one month in order to maintain the clinical benefit. To the best of the authors' knowledge this is the first study *(i)* correlating clinical improvement with the incorporation of EPA-DHA in EM, *(ii)* showing the time needed, after withdrawal of the supplement, for the clinical score to get back to baseline levels and *(iii)* evaluating the "Agepi® ω3" capsules as a supplement for improving hair coat and skin quality of dogs.

The EM has been widely studied as a cell membrane prototype *(i)* because of its ready availability [18], *(ii)* because its FA composition correlates

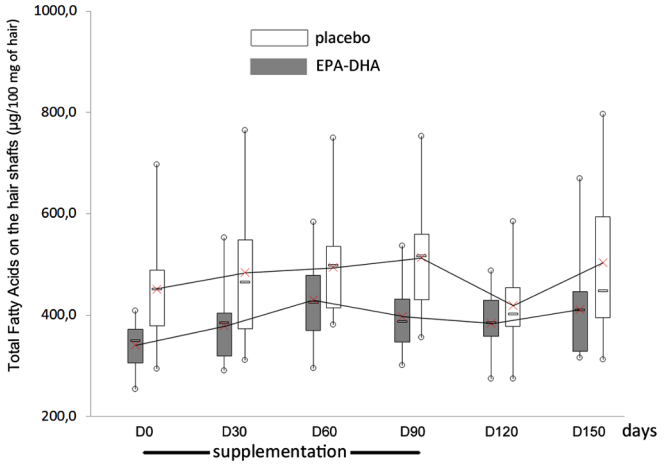

Fig. 3. Kinetics of total lipids content on hair shafts in dogs with poor coat condition that received either EPA-DHA (gray boxes) or placebo (white boxes) supplementation once daily for three consecutive months (from D0 to D90) at different time points during the study. Box and whiskers plot interpretation: Top of box is the upper quartile, the line in the middle is the median, the bottom of the box is the lower quartile and the upper and lower whiskers represent minimum and maximum scores. × represents the mean and the line connecting the dates represents the mean line.

with that of other cell membranes in the body [19] and *(iii)* there is a significant correlation between FA intake and its incorporation into EM [20]. Measurement of the incorporation of *n*-3 FA into EM therefore provides a suitable model for monitoring FA intake. It also enabled us to evaluate owner compliance in the two groups throughout the study.

Blood was first sampled on D30 after beginning FA supplementation. DHA and EPA levels already showed a considerable increase at that time, although the increase of FA content in EM has been shown to occur progressively over the first weeks [12]. This rapid increase of FA content in EM cannot be explained by the entry of new RBC into the blood flow, but would instead be indicative of a rapid exchange between RBC and plasma FA [12, 21]. On D120, *i.e.* one month after stopping FA supplementation, the DHA content in EM in the treated group was still significantly higher than on D0, supporting previous reports that a washout period of more than 4 weeks is required in studies dealing with dietary *n*-3 FA in dogs [12]. We also observed a reduction of the ARA/(EPA + DHA) ratio after the first month of supplementation. Thus the supplement could be used to decrease the amount of ARA available as a substrate for inflammatory eicosanoid production, thereby supporting the interest of using *n*-3 EFA to manage inflammatory skin diseases like atopic dermatitis [5].

The levels of total lipids extracted from plucked hairs were found to increase until D60 in the *n*-3 supplemented group. Thereafter, in contrast to the EM observations, the total lipids content remained higher than baseline even 60 days after supplement withdrawal. It is possible that lipids incorporated into the hair shaft, as opposed to the RBC, last longer *in situ* because of the reduced likelihood of lipids exchange with the microenvironment. Although the groups of dogs did not differ significantly in terms of age, sex or breed and all dogs were living under the same conditions, the total lipids content on hair shafts was higher in the placebo group at baseline. No valid explanation has been found for this. EPA and DHA were not found in hair samples. Interestingly, the authors found only one publication describing dog's hair lipid content [22]. In that article, EPA and DHA were not represented as one of the most common 12 FA. However, as 5% of the FA were described as "others" [22], it remains unknown if EPA or DHA were found in small amounts or not present at all, as in our study.

Although many techniques can be used to sample epidermal lipids [23, 24], some are invasive and cannot be applied in conscious dogs [23]. We used a non-invasive, solvent-free method (Synelvia, Labege, France) and specific lipid markers such as squalene, sterols, free fatty

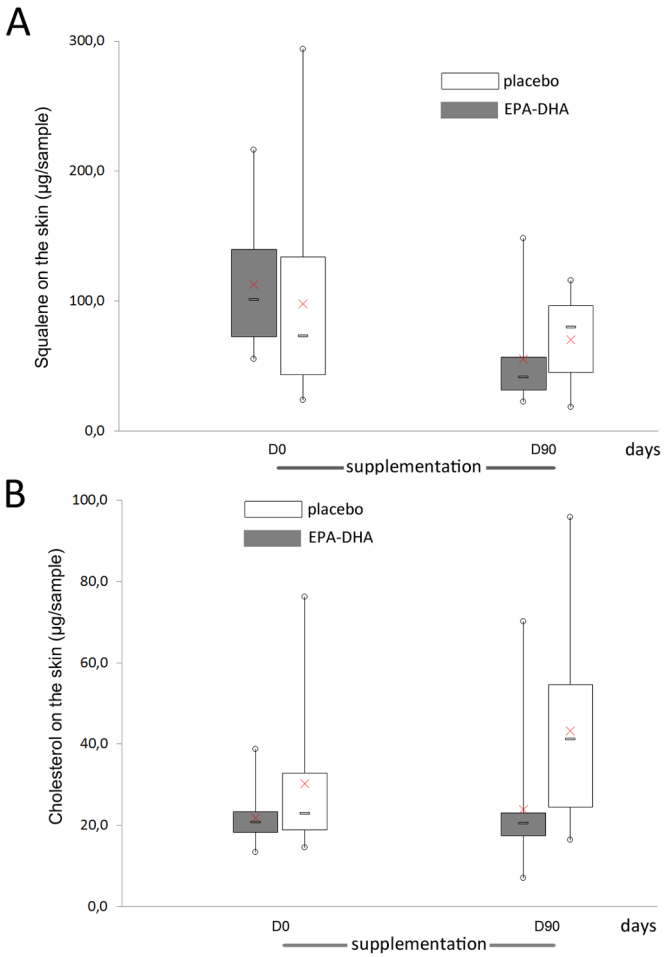

Fig. 4. Kinetics of squalene (A) and cholesterol (B) contents on skin surface in dogs with poor coat condition that received either EPA-DHA (gray boxes) or placebo (white boxes) supplementation once daily for three consecutive months (from D0 to D90) at different time points during the study. Box and whiskers plot interpretation: Top of box is the upper quartile, the line in the middle is the median, the bottom of the box is the lower quartile and the upper and lower whiskers represent minimum and maximum scores. × represents the mean.

acids, waxes, glycerides and total neutral lipids. The secretion produced by sebaceous glands is mainly composed of cholesterol, squalene, wax esters and triglycerides [25]. These lipids are then distributed over the skin and hair shafts during fur growth and may influence haircoat sheen, glossiness and softness [15]. Furthermore, glandular secretory activity may be modified by increasing FA intake [15]. In the present case, squalene was the only neutral lipid in the DHA-EPA group that was significantly modified during the study. The significance of this finding is unclear. As the dogs were kept in semi-open kennels with clay courts some contamination with dust particles present in the fur might have occurred during skin swabbing and could have impaired the results. Concerning surface sampling, it would have been interesting to measure ceramide contents, because of their importance in skin barrier function, as well as other time points in the study.

Although *n*-3 FA supplementation can be useful in many cases, clinicians should remember that numerous dermatoses, including ectoparasites, infectious and allergic diseases, can affect hair and skin quality. Addressing the primary cause remains a key step in clinical resolution.

Altered platelet function [26], gastro-intestinal signs like diarrhea and vomiting [27], weight gain, nutrient excess and toxin exposure or nutrient-drug interactions have been described in the literature as adverse effects associated with *n*-3 FA, although most of them have never been reported in dogs [28]. It would have been interesting, in the present study, to assess these possible effects by monitoring dog weight and platelet function. No

adverse effects were observed in this study. Unfortunately, it is difficult to compare the *n*-3 FA dose given in this study with those reported in the literature, as most studies used the *n*-3: *n*-6 ratio rather than specify the total *n*-3 FA dosage or DHA and EPA concentration. However, this ratio does not reflect the total amount or the type of *n*-3 FA present in the diet, and should therefore be used with caution [28].

The relatively small number of dogs in the trial (12 dogs/group) could be perceived as a limitation. However, any bias was reduced and the results made more statistically powerful by the homogeneity of the two groups at baseline in terms of food, environment and breeds and the randomized and double-blinded nature of the experiment.

In conclusion, our findings suggest that daily ingestion of a food supplement containing fish oil (Agepi® ω3) is safe for dogs and improves skin and haircoat quality. Maximum improvement seems to occur after two months and to last for one month after supplement withdrawal. Reduction of the clinical score is associated with a marked increase of EPA and DHA contents in EM and an increase of total lipids content on hair shafts. The long-term effects of supplementation with this product require further investigation.

Declaration of Competing interest

DC, ECC, LAL and CP declare that they have no competing interests. MCC has previously been a consultant for MP Labo.

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Authors' contributions

DC assisted with study design and drafted the manuscript. ECC, LAL, CP participated in the design of the study and assisted the clinical evaluations. NA participated in the study's design, did the laboratory evaluations and assisted with review of the manuscript. MCC conceived the study, participated in its design and coordination, performed clinical evaluations and reviewed the manuscript.

Study approval

The study was approved by the Toulouse veterinary school (Université de Toulouse, ENVT) Ethical Committee and a written informed consent was obtained from the owner.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.plefa.2020.102140.

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