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Evaluation of breed effects on *n*-3 PUFA metabolism with dietary flaxseed oil supplementation in dogs

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Abstract

PUFA are important for human and animal health. To our knowledge, previous studies investigating the metabolism of PUFA in dogs have not examined breed differences. The aim of the present study was to evaluate the potential to elongate PUFA in two pure breeds of dogs. Plasma fatty acid composition (%) was measured in dogs during 3 weeks supplementation with flaxseed oil (57 % α-linolenic acid (ALA) and 17 % linolenic acid (LA)) at the rate of 100 ml/kg food following 4 months of feeding an identical standard basal diet. Plasma extracted at fasting state from five beagles and five greyhounds was analysed by GC. Plasma ALA, EPA and LA increased steadily and significantly from days 0 to 22 ($P<0{\cdot}05$); however, no significant breed differences were shown. Plasma DHA levels, on the other hand, showed no significant increase over time, but a significant breed difference was observed, with beagles having higher plasma level from day 0 ($P=0{\cdot}002$). This breed difference requires further investigation. Levels of ALA and EPA were still rising significantly between days 15 and 22, indicating that PUFA levels in plasma had not stabilised in 3 weeks. These findings together suggest that flaxseed oil could be a useful source of PUFA in dogs, especially ALA and EPA, and that breed differences may be important.

Key words: Flaxseed oil: PUFA: Fatty acids: Breeds: Dogs

The conversion of short-chain PUFA to long-chain PUFA is rate limiting, and competition exists for the Δ6-desaturase enzyme[1]. The extent of conversion varies between species[2], and a study[3] has shown that canines have a differential metabolising ability compared with rodents. Within the canine species, it is not known whether breeds have a distinct differential ability.

Fish oil, known to be rich in *n*-3 long-chain PUFA, has been reported to have health benefits in human subjects[4]. In dogs, less inflammatory mediators were produced when fed diets with fatty acid ratios (*n*-6:*n*-3) of 5:1 and 10:1 in comparison with being fed an *n*-6-rich diet with a fatty acid ratio of 100:1[5]. The need for a vegetarian source of *n*-3 due to the reduction of global marine sources[6] has led to studies in dogs[3,7] using plant sources such as flaxseed.

The present study evaluates the plasma composition of fatty acids following supplementation with flaxseed oil in two dog breeds and investigates the time course following oil supplementation to determine an effective supplementation period for future experiments.

Experimental methods

Animals

For the study, five beagles and five greyhounds were used. All were females and aged between 2 and 9 years. The dogs weighed an average of 12·4 (SD 1·2) kg (beagles) and 25·6 (SD 2·0) kg (greyhounds) at the start of the study. All dogs were borrowed with written consent from their owners and returned at the end of the trial. Health checks were performed at the start of the trial, and only healthy dogs were accepted. Dogs were housed at the University of New England dog research facilities at Armidale, NSW, Australia. The care and use of animals followed the guidelines set by the University of New England Animal Ethics Committee (authority no. AEC09/072), in accordance with section 25 of the Animal Research Act (1985).

Basal diet and flaxseed supplementation

A nutritionally complete and balanced commercial dog food (substantiated by the Association of American Feed

Abbreviations: ALA, α-linolenic acid; LA, linolenic acid.

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Table 1. Composition of the basal and supplemented diets per kg

	Basal diet	Supplemented diet
ME (MJ)	8·1	10·6
DM (g)	433·3	393·9
Crude protein (g)	132·7	120·6
Crude fat (g)	100	181·9
Crude fibre (g)	30	27·3
Ash (g)	55	50·0
PUFA (g)		
LA	17·16	34·16
AA	0·56	0·56
ALA	1·07	58·07
EPA + DHA	0·41	0·41

ME, metabolisable energy; LA, linolenic acid; AA, arachidonic acid; ALA, α-linolenic acid.

Control Officials feeding protocol) was fed for 4 months before supplementation. Beagles and greyhounds were fed once a day, and *ad libitum* water was available at all times. From day 1 of the supplementation period, beagles were fed 300 g and greyhounds were fed 600 g of the basal diet daily to which Melrose® (Mitcham, VIC, Australia) flaxseed oil (57 % α-linolenic acid (ALA) and 17 % linolenic acid (LA)) was added at the rate of 100 ml/kg food, which equates to 2·4 (SD 0·2) ml/kg body weight for 3 weeks (30 ml/beagle and 60 ml/greyhound). The supplementation represented a fifty-two times increase in ALA and a two times increase in LA compared with the basal diet alone. The basal and supplemented diet compositions are summarised in Table 1.

Sample analyses

Whole blood (5 ml) was collected on three occasions: day 0 (before supplementing oil) and on days 15 and 22 post-supplementation. Plasma was extracted by centrifugation at 1900 ***g*** for 15 min. Fatty acid components in plasma were determined by GC (< 6 % CV for the analysis).

Statistics

Initial analysis with generalised linear model showed no significant interaction between the breed effect and the day effect. Hence, for each individual fatty acid constituent in the plasma, the breed effect and the day effect were analysed using fully nested ANOVA, wherein the breed effect was the fixed effect and the day effect was the random variable. Windows MINITAB version 14 (Minitab Limited, Coventry, WMD, UK) was used to analyse the data. The level of significance was considered at $P<0·05$. Significant differences between means were detected by the *t* test. The relationship between body-weight gain and plasma fatty acid concentrations was determined using regression analysis.

Results

Dogs remained healthy throughout the 3-week study, and there were no refusals of food or supplements. Average body-weight gains over this period were 2·4 % for the beagles and 3·1 % for the greyhounds. Regression analysis showed no relationship between body-weight gain and concentrations of plasma fatty acid constituents (R^2 values: ALA, 0·29; EPA, 0·002; DHA, 0·16; LA, 0·03; arachidonic acid, 0·43).

Plasma ALA, EPA and LA increased steadily and significantly from days 0 to 22 with no significant breed difference. Plasma arachidonic acid showed neither a significant breed effect nor a day effect.

Plasma DHA, on the other hand, showed no significant changes over time, but a significant breed effect ($P=0·02$) was observed at all time points, including at day 0 which is before supplementing flaxseed oil (*P* values: day 0, 0·008; day 15, 0·036; day 22, 0·002). Beagles maintained a higher plasma level of DHA than greyhounds throughout the study. Plasma fatty acid data are presented in Table 2.

Discussion

Following supplementation with flaxseed oil, a steady rise in the concentrations of ALA and EPA was observed but not of DHA, which agrees with previous studies in dogs[3] as well as in human subjects[8]. It was observed that fifty-two times more ALA was consumed with the supplemented diet than with the basal diet, which explains the increase in ALA and EPA in plasma. The extent of efficiency of the canine system in elongating C_{18} PUFA to C_{20} PUFA was also demonstrated. Greyhounds showed a consistent numerically higher increase in ALA and EPA from days 0 to 22 compared with beagles, but this difference was not significant.

Table 2. Plasma fatty acid composition (%) in dogs supplemented with flaxseed oil
(Mean values and standard deviations)

	Beagles (*n* 5)						Greyhounds (*n* 5)						Pooled *P**	
	Day 0		Day 15		Day 22		Day 0		Day 15		Day 22		Day	Breed
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
ALA	0·36	0·07	4·12	1·41	4·35	0·72	0·33	0·04	5·00	1·00	5·47	0·78	0·007	NS
EPA	0·63	0·16	2·48	0·29	3·01	0·52	0·67	0·11	3·34	1·11	3·58	0·92	0·015	NS
DHA	3·94	0·51	3·64	0·59	3·87	0·69	2·97	0·44	2·61	0·42	2·56	0·22	NS	0·002
LA	24·98	2·10	28·89	4·91	27·87	2·36	26·24	0·83	28·93	2·72	29·38	1·56	0·045	NS
AA	19·68	2·52	18·03	1·47	17·63	2·01	18·33	1·58	15·66	2·41	15·17	1·46	NS	NS

ALA, α-linolenic acid; LA, linolenic acid; AA, arachidonic acid.
* There were no significant diet × breed interaction.

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Bauer *et al.*[3] explained from their study that the unchanged level of DHA could be due to some resistance exhibited in the conversion of ALA to DHA in the liver, which is a rate-limiting step. Most of the DHA is produced in the neurological tissue, which could explain why the plasma levels of DHA remain not elevated in the present study and previous reports.

Similarly, LA levels rose significantly, which may be due to the increased levels in the supplemented diet. Arachidonic acid levels showed no significant change, perhaps resulting from competition for the Δ6-desaturase enzyme, from the conversion of ALA to EPA.

Breed differences were observed with respect to DHA. Dunbar *et al.*[7] concluded that hepatic conversion of docosapentaenoic acid to DHA is slow in the canine, and that even after docosapentaenoic acid is produced from ALA, it is likely to be relocated to the neurological tissues for DHA synthesis as stated previously. This is similar in other species such as cats as reported by Pawlosky *et al.*[9]. It is possible that different breeds may have a different rate at which this conversion and transportation to the neurological tissue occurs. Extensive studies are required to confirm how different breeds metabolise PUFA and what this means in terms of dietary recommendations for specific breeds.

Levels of ALA and EPA were still rising significantly between days 15 and 22, indicating that plasma levels had not stabilised in 3 weeks. In the study of Bauer *et al.*[3], plasma fatty acid enrichment attained a steady state after 4 weeks. This needs to be taken into account when choosing time frames of supplementation in future studies. In conclusion, flaxseed oil could be used as an alternative source to fish oil for some essential fatty acids, but not DHA, in dog foods. There was an apparent breed difference between greyhounds and beagles in their plasma DHA levels. Further breed studies are warranted, as are fatty acid measurements in tissues or erythrocytes, for further clarification.

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