α-Linolenic Acid Metabolism in Dogs

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Abstract

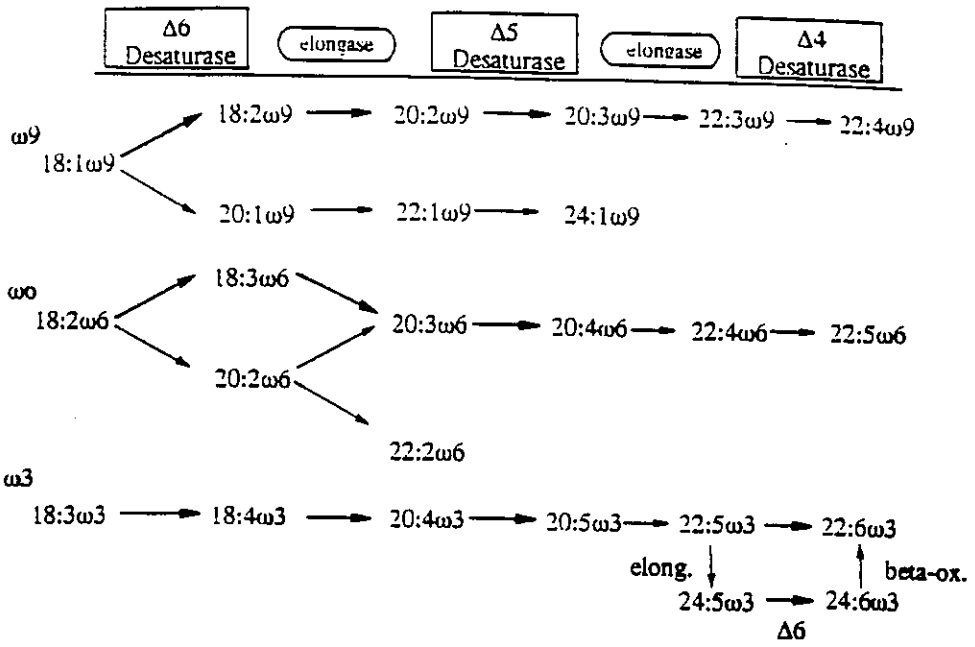
Aims. The objective of this study was to investigate the effect of feeding a 1 and later a 3% flax diet on the presence and metabolism of 18:3ω3 in the plasma of adult pure bred beagles. The animals were raised on their basal diet, containing 2.5% 18:2ω6, until the time of the study at which time 1 and 3% (0.2 and 0.6% 18:3ω3) flax wt/wt was added to the diet. The 1% diet was fed for the first 42 days and the 3% diet was fed for the next 42 days or the 84 day sample. Bloods were drawn at baseline, 42 and 84 days. Fatty acid compositions were determined for phospholipid (PL), free fatty acids (FFA) and triglycerides (TG) classes. **Results.** Elevated baseline levels of 20:3ω9 in PL and TG indicate essential fatty acid deficiency that was corrected by feeding flax. PL, FFA and TG levels of 18:3ω3, 20:5ω3 and 22:5ω3 increased. In PL and TG, 20:3ω9 fell linearly as 18:3ω3 increased. Total PUFA levels increased from baseline at days 42 and 84 in PL and TG. 22:6ω3 was unaffected by flax feeding in all lipid classes tested. **Conclusions.** Dietary flax improves the fatty acid profiles of dogs by decreasing 20:3ω9 and increasing 18:3ω3 and its metabolites.

Introduction

The essential nature of fat was discovered by Burr in the late 1920's (**1-2**). His experiments showed that when young animals were fed a fat free diet they ceased to grow, developed dermatitis, dry skin, thickened and brittle hair, kidney malfunction and failure to reproduce. This condition was reversed by the addition of corn or flaxseed oil to the diet. The main components of these oils, linoleic (18:2ω6) and linolenic acid (18:3ω3), were determined to be the fatty acids responsible for this recovery and thus termed essential fatty acids. Since this time, several studies have documented the unique requirements of PUFA in human and animal nutrition (**3-5**). These fatty acids are essential because most mammals are unable to desaturate or introduce double bonds in the last 9 carbons from the carboxy terminus end of the fatty acid. If supplied by the diet, linolenic and linoleic acids can be desaturated and elongated to the long chain highly unsaturated fatty acids as depicted in figure 1. These molecules are unique in structure and function and are essential for such things as immunity, membrane fluidity and brain structure (**6-7**).

Deficiency of essential PUFA in the diet results in essential fatty acid deficiency (EFAD) (**1**). The extent of deprivation is correlated with the degree of symptoms. Extreme deficits of PUFA result in symptomology similar to what Burr originally had described. However, smaller deficits of PUFA may not result in acute and blatant symptomology but rather may acculminate and insidiously culminate in disease over a period of several years. In 1960, Holman identified the decrease of arachidonic acid (20:4ω6) and the increase of Mead's acid (20:3ω9) as a marker of EFAD and referred to this as the triene:tetraene ratio(**8**). A triene:tetraene ratio of 0.4 indicated EFAD in his experimental animals, but today it is known that EFAD can occur at levels below 0.4. The mere presence of 20:3ω9 above trace amounts typically indicates deficit of PUFA because 20:3ω9 is usually only produced in response to inadequate dietary PUFA.

Figure 1.



Pets can be at risk for developing deficiency of PUFA as they are completely dependent on their care providers for food. EFAD in pets usually results from poor quality food, metabolic disorders, fat restrictive diets, improperly stored foods, dry foods or foods that are not oxidatively stabilized (**9, 20-22**). Deficient animals have coats that are brittle, dull and dry. Hair that breaks off will not regrow and result in areas of alopecia. Deficiency of EFAs also effects the epidermis and can result in serious skin disorders which can be corrected by feeding EFAs (**10,18**). In addition to EFAs, important nutrients for the skin include vitamins A and E and the trace element zinc. Zinc is believed to be a cofactor for desaturase activity that is required for the metabolism of 18:3ω3 to the long chain highly unsaturated ω3 PUFA. In rats however, zinc deficiency did not effect the elongation and desaturation of 18:3ω3 (**11**). Deficiency of EFAs can usually be corrected by changing to a food that is high in EFAs (**12**). Fat supplements can also be provided if food changes are not desired, but this is expensive.

The ω3 fatty acids have also been shown to improve symptomology in disease states such as arthritis, lupus, cystic fibrosis and other inflammatory type diseases (**13**) They have also been shown to prevent atherosclerosis in animal and human studies (**14**).

Dogs have a simple digestive tract that is efficient at digesting food (**15**). Fat is a dense source of energy and is the preferred fuel for energy synthesis during prolonged exercise in trained dogs. As much as 50 to 90% of a dog's energy may be derived from plasma FFA during exercise (**16**). During the fasted state, the composition of plasma FFA in dogs reflects the composition of the diet which is an effective tool in investigating dietary and nutritional status (**17**).

The fatty acid content of many dog foods are typically overlooked especially for the ω3 EFAs (**18**). Most dog food is made with grain or animal byproducts as the source of fat and protein which provide mostly ω6 and little ω3, resulting in dog food that is high in ω6 and low in ω3. Diets deficient in ω3 acids will result in ω3 deficiency. This will not only effect the individual animal but will also effect the offspring at a time when the requirement of ω3 is highest for retinal and neurological development (**5**).

Dietary flax in dog food would serve to bolster the level of ω3 EFAs in the diet as flax seed contains significant amounts of α-linolenic acid. Conceivably, supplementation with only low levels of flax would be needed because ω3 PUFA are typically preferentially sequestered from the diet, incorporated and conserved in tissues. Dietary supplementation of flax in dog food would serve several factors by maintaining EFA status, improving coat quality, supporting immune function, potentiating reproductive ability, in neurological development and function and for overall health. The following study was conducted to investigate the presence and metabolism of 18:3ω3 in beagles fed a diet containing 1% flax over a 42 day period and then feeding a 3% flax diet for an additional 42 days.

Materials and Methods

Pure-bred adult beagles, 5 males and 7 females, from Dr. Popp's breeding colony were used for this study. The dogs were maintained on their basal diet throughout their lifetime until the time of the study. The basal diet contained 2.5% 18:2ω6 and 0.08% 18:3ω3 with no other PUFA (data not shown). Stabilized ground flax, ENRECO Nutrition, Manitowoc, WI, was added to the diet at 1 and then 3% wt/wt. Fresh food was prepared daily and the dogs were allowed to eat and drink *ad libitum*. Blood samples were obtained in the morning during the fasted state at 0, 42 and 84 days. Plasma was separated and frozen until fatty acid analysis could be performed. Fatty acids were analyzed as described previously (19). Briefly, 2mls of plasma were extracted with chloroform:methanol (2:1). Plasma lipids, including PL, FFA and TG, were separated by thin layer chromatography in a solvent system consisting of petroleum ether: diethyl ether: acetic acid (80:20:1). Resulting lipid bands were transesterified with boron trifluoride 12% in methanol. The fatty acid methyl esters were analyzed on a Packard model 427 gas chromatograph utilizing a 50m FFAP007 capillary column (Quadrex). Data were analyzed using EZChrom software and are expressed in % composition ± SEM. The mole fraction mean melting point (MMP) was calculated for PL. MMP is a mathematical estimation of membrane fluidity. It is calculated by determining the mole fraction of each fatty acid, multiplying this value by respective melting points and summing the resulting temperatures for each profile. This serves as a correlation of fluidity only and is not a actual physical measurement of a membrane. Statistical analysis between groups was performed with the Student's t-test. Values were considered different for $p<.05$.

Results

Dietary supplementation with 1 or 3% flax resulted in a significant reduction in 20:3ω9 with significant increases in 18:3ω3, 20:5ω3 and 22:5ω3 for plasma PL and TG, figure 2. Numerical values for PL, TG and FFA are shown in table 1. No changes in plasma 22:6ω3 were observed in the lipid classes tested. Baseline values of 20:3ω9 were elevated in PL and TG suggesting initial deficiency of PUFA.

PL 20:3ω9 was elevated at baseline and decreased significantly as 18:3ω3, 20:5ω3 and 22:5ω3 increased. PL 18:2ω6 and 20:4ω6 tended to decrease from baseline to 84 days but these changes were not significant, as a result however, total ω6 significant decreased on 42 and 84 days. Total PL PUFA increased initially from baseline to 42 days and remained significantly elevated through 84 days, figure 3. PL MMP exhibited a significant increase at 42 days that returned to baseline at 84 days, figure 3. PL total saturated acids increased significantly on day 42 and 84. FFA 18:3ω3 increased significantly throughout the 84 day period but had no effect on 20:3ω9 which was low initially, figure 2. Small increases in 20:5ω3 and 22:5ω3 were also observed for FFA, table 1. No significant effects were observed for FFA and TG 18:2ω6, 20:4ω6 and total ω6 during the 84 day period. Total PUFA increased in TG but not in FFA.

Table 1

	Phospholipids			Triglyceride			Free Fatty Acids		
EFAs	0	1% -42d	3% -84d	0	1% -42d	3% -84d	0	1% -42d	3% -84d
18:3ω3	.11±.01	.46±.06*	.92±.13*	.38±.05	1.45±.10*	3.31±.25*	.49±.04	1.57±.09*	3.84±.38*
20:5ω3	.40±.01	1.08±.18*	2.20±.27*	.60±.14	2.0±.35*	4.14±.49*	.16±.03	.31±.05*	.69±.20*
22:5ω3	1.42±.13	3.07±.27*	5.16±.24*	.64±.14	1.78±.15*	3.27±.21*	.31±.06	.36±.07	.53±.10*
22:6ω3	.44±.05	.28±.03*	.39±.04	.05±.01	.04±.01	.19±.09*	.06±.02	.04±.04	.15±.05
18:1ω9	9.65±.55	9.44±.44	8.76±.37	33.86±1.8	32.37±1.74	33.88±1.12	31.43±2.0	34.31±1.14	32.25±1.10
20:3ω9	.90±.12	.53±.10*	.41±.08*	2.72±.22	2.55±.24	2.00±.15*	.29±.05	.25±.05	.30±.09
18:2ω6	17.31±1.2	15.03±1.12	16.88±1.0	15.39±.73	15.41±.90	16.35±.66	14.7±.46	13.42±.3*	14.84±.72
20:4ω6	21.89±1.5	18.06±1.47	19.10±1.2	6.45±1.24	7.69±1.27	5.63±.64	4.7±1.1	2.73±.34*	2.41±.19*
16:0	16.52±.48	17.87±.60	14.7±.40*	16.87±.75	16.19±1.0	13.19±.76	25.06±.78	23.8±.99	23.62±.58
18:0	20.24±.64	24.89±.62*	22.93±.54*	7.72±.79	5.53±.57*	4.84±.27	12.53±.92	9.92±.43*	9.61±1.2*
total sat	37.95±.71	44.6±.48*	40.6±.66*	27.3±1.12	26.8±1.47	26.53±1.14	40.22±1.2	36.9±1.13*	36.4±1.02*
total ω6	43.83±1.1	36.58±.72*	39.25±.80*	24.65±2.1	25.90±2.06	25.84±1.44	20.68±1.7	17.58±.54	18.51±.94
total ω3	2.0±.16	3.8±.24*	6.6±.28*	1.21±.11	3.39±.20*	7.01±.39*	1.01±.09	2.29±.13*	4.71±.43*
PUFA	40.10±.67	46.8±1.11*	46.3±0.96*	28.58±2.2	31.89±2.26	34.86±1.2*	20.13±.58	21.98±1.78	23.57±.13
T:T	.04±.01	.03±.01	.02±.01	.42±.11	.33±.18	.35±.08	.06±.01	.09±.01	.12±.01

Table 1. Fatty acids are expressed in % composition ± SEM, *= $p<0.05$ vs. 0 days.

Figure 2

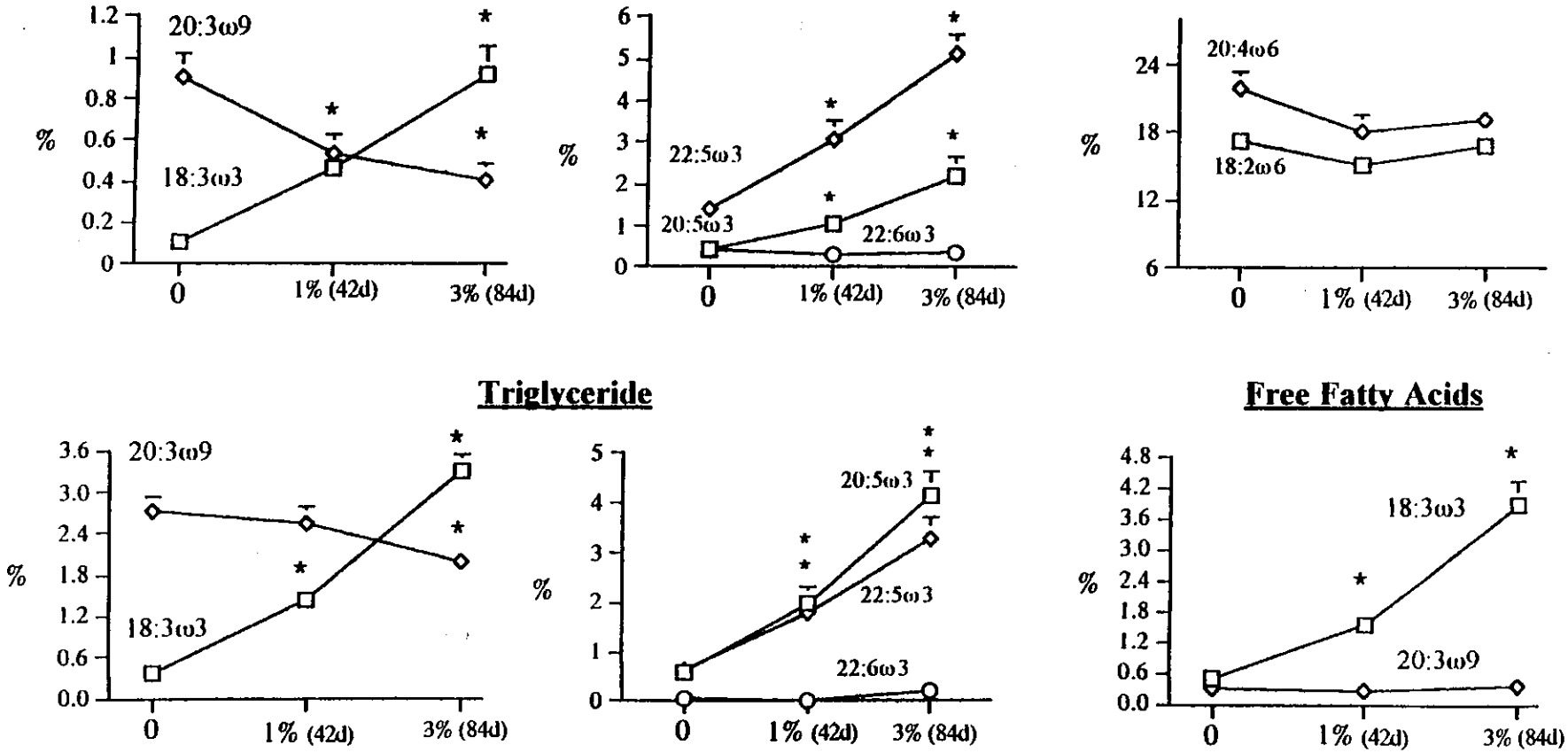
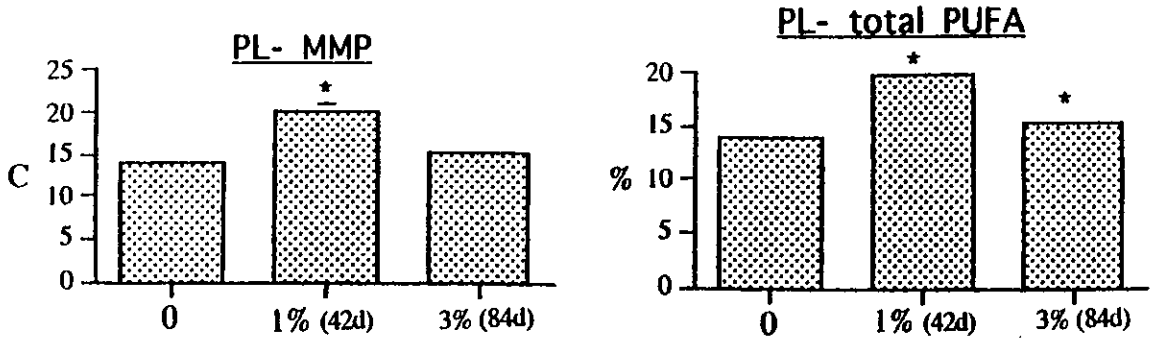


Figure 3



Discussion

The presence of high concentrations of 20:3ω9 at baseline suggest that these dogs were not being provided with sufficient EFAs, initially, and were thus EFA deficient. The addition of 1 and 3% flax to the diet alleviated the deficit of PUFA as evidenced by the significant decrease of 20:3ω9 and increased total PUFA in PL and TG. Feeding diets high in only 18:2ω6 has been shown to suppress ω3 acids which may also be responsible for the elevation of 20:3ω9(**23**). Anderson et al., found similar incorporation of 18:3ω3 to 22:5ω3 and not 22:6ω3 in poodles fed linseed oil(**24**). Data for 20:3ω9 was not reported by Anderson.

The presence of plasma 20:3ω9 was shown to increase in dogs fed a diet containing 14% olive oil (high 18:1ω9) when compared to 14% sunflower oil (**25**). This study found no effect on total cholesterol and erroneously concluded that the olive oil diet would be better for dogs as this type of diet has been shown to increase HDL cholesterol. This recommendation failed to acknowledge the large increase of 20:3ω9 that occurred by feeding the olive oil diet. This study also found no effect on ω3 acids or 20:4ω6 for olive versus sunflower oil diets. Giron et al. also confirmed increased delta-9 desaturase activity in dogs fed olive versus safflower in a later study but found no difference on delta-6 desaturase activity(**28**). Interestingly, they found that feeding of safflower oil significantly increased 20:5ω3 and 22:6ω3 in the sphingomylein lipid fraction compared to the olive oil fed dogs.

Simoens et al. demonstrated that infusion of soybean and fish oil resulted in tissue incorporation of ω3 and ω6 that was diminished by the inclusion of medium chain triglyceride to the parenteral formula. Incorporation of ω3 from the fish oil preparation was most pronounced in the liver which is thought to be the source of 22:6ω3 for the brain and retina during development(**34**). Pitas et al. showed that feeding dogs with tallow and cholesterol increased 20:3ω9 in platelet phospholipids when compared to safflower oil (**27**).

The present study and Anderson's show that dogs have a high level of delta-5 desaturase activity and little delta-4 products in plasma. Other studies have also found significant delta-5 desaturase activity in dogs that may have a higher delta-5 activity than humans (**18, 35**). The failure in the elongation/desaturation to 22:6ω3 in plasma may be due to a lower level of delta-4 desaturase activity in dogs (**29**). This is assuming that 22:6ω3 is indeed formed by the action of delta-4 desaturase and not of delta-6 desaturase that has been proposed by Sprecher's lab(**30**). In this scenario, 22:6ω3 may not increase as a result of competitive inhibition from 18:2ω6 and 18:3ω3 for the delta-6 desaturase (**31**). Plasma levels of 22:6ω3 also may not indicate the tissue status of retina and brain in the dog as it has been shown that 22:5ω3 is converted into 22:6ω3 in poodle retina independent of plasma concentrations (**24,32**). Linseed oil supplementation in rats however, does result in significant increases in plasma 22:6ω3 (**33**).

In summary, this study found that the addition of milled flax to the diets of adult beagles was effective at increasing 18:3ω3, 20:5ω3 and 22:5ω3 and decreasing 20:3ω9 for the period tested.

References

1. Burr, G.O. and Burr, M.M. A new deficiency disease produced by the rigid exclusion of fat from the diet. J. Biol. Chem. 1929; 82:345-367.

2. Burr, G.O. and Burr, M.M. On the essential nature and role of the fatty acids essential in nutrition. J. Biol. Chem. 1930; 86:587-621.

3. Holman, R.T. Omega 3 deficiencies in humans. Proc. 55th Flax Institute USA ed. Jack Carter NDSU, Fargo, ND. 1994: p4-11.

4. Holman, R.T. and Johnson, S.B. Essential fatty acid deficiencies in man. In "Dietary fats and health" Eds. Perkins, G. and Visek, W.J. American Oil Chemists' Society, Champaign, IL. 1983; p247-266.

5. Crawford, M.A. The role of essential fatty acids in neural development: implications for perinatal nutrition. Am. J. Clin. Nutr. 1993; 57(Suppl.):703S-710S.

6. Kinsella J. and Lokesh B. Dietary lipids, eicosanoids, and the immune system. Critical Care Medicine. 1990;18(2): S94-S113.

7. Stubbs, C.D. and Smith, A.D. The modification of mammalian membrane polyunsaturated fatty acid composition in relation to membrane fluidity and function. Biochem. Biophys. ACTA 1984; 779:89-137.

8. Holman. R.T. The ratio of trienoic:tetraenoic acids in tissue lipids as a measure of essential fatty acid requirement. J. Nutr. 1960:60:405-410.

9. Muller. G.H.. Kirk. R.W. and Scott. D.W. Small animal dermatology III. Philadelphia. ed. Saunders. W.B. 1983.)

10. Campbell. K.L. Fatty acid supplementation and skin disease. Vet. Clinics North Am.: Sm. Anim.Prac. 1990; 20(6):1475-1486.

11. Eder. K. and Kirrchgessner. Levels of polyunsaturated fatty acids in tissues from zinc-deficient rats fed a linseed diet. Lipids 1994; 29:839-844.)

12. Miller. W.H. Nutritional considerations in small animal dermatology. Vet. Clinics North Am. 1989; 19(3):497-511.

13. Endres. S., Singha. B. and Eisenhut. T. ω3 fatty acids in the regulation of cytokine synthesis. World Rev. Nutr. Diet. 1994; 76:89-94.

14. Dolecek. T.A. Epidemiological evidence of relationships between dietary polyunsaturated fatty acids and mortality in the multiple risk factor intervention trial. Proc. Soc. Exp. Biol. Med. 1992; 200:177-182.

15. Kararli. T.T. Comparison of the gastrointestinal anatomy. physiology and biochemistry of humans and commonly used laboratory animals. Biopharm. Drug Dis. 1995; 16:351-380.)

16. Paul, P. and Holmes. W.L. Free fatty acid and glucose metabolism during increased energy expenditure and after training. Med. Sci. in Sports. 1975; 7(3):176-184.)

17. McClelland. G.. Zwiingelstein. G., Taylor. C.R. and Weber. J.M. Effect of exercise on the plasma nonesterified fatty acid composition of dogs and goats: Species with different aerobic capacities and diets. Lipids 1995; 30(2):147-153)

18. Bauer. J.E. The potential for dietary polyunsaturated fatty acids in domestic animals. Aust. Vet. J. 1994; 71:342-345.

19. Holman. R.T.. Johnson. S.B. and Ogburn. P.L. Deficiency of essential fatty acids and membrane fluidity during pregnancy and lactation. 1991; 88:4835-4839.

20. Defretin. V.L. Ebergy requirements of cats and dogs - what goes wrong? Inter. J. Obesity 1994;18(Suppl. 1):S8-S13.

21. Buffington. C.A. Management of obesity - the clinical nutritionist's experience. Inter. J. Obesity 1994;18(Suppl.1):S29-S35.

22. Munday, H.S. Assessment of body composition in cats and dogs. Inter. J. Obesity 1994;18(Suppl. 1):S14-S21.

23. Campbell. K.L. and Dorn. G.P. Effects of oral sunflower oil and olive oil on serum and cutaneous fatty acid concentrations in dogs. Res. Vet. Sci. 1992; 33:179-183.

24. Anderson. R.E. Maude. M.B.. Acland. G. and Aguirre. G.D. Plasma lipid changes in PCRD-affected and normal miniature poodles given oral supplements of linseed oil. Indications for the involvement of n-3 fatty acids in inherited retinal degenerations.

25. Giron. M.D., Mataix. F.J. and Suarez. M.D. Long-term effects of dietary monounsaturated and polyunsaturated fatty acids on plasma lipids in dogs. Arch. Inter. Physiol. de Biochem. de Biophy. 1992; 100:321-326.

26. Simoens. C.H.. Richelle. M.. Derluyn. M. Deckelbaum. R.J. and Carpentier. Y.A. Manipulation of tissue fatty acid profile by intravenous lipids in dogs. Clin. Nutr. 1995; 14:177-185.