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Vitamin E Requirement of Adult Cats Increases Slightly with High Dietary Intake of Polyunsaturated Fatty Acids[1,2]

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EXPANDED ABSTRACT

KEY WORDS: • cats • vitamin E • fish oil • requirements • polyunsaturated fatty acids

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Over the last 50 y there have been numerous reports of vitamin E deficiency in cats with the main cause of these deficiencies being attributed to ingestion of diets high in polyunsaturated fatty acids (PUFAs[4]). Steatitis ("yellow fat disease") is attributed to vitamin E deficiency and has been experimentally induced in kittens after feeding a commercial cat food with a high fish content (1) and after feeding vitamin E–deficient purified diet containing tuna oil and stripped safflower seed oil (2,3).

Fish oil is a rich source of PUFAs, which are highly susceptible to oxidation, and an increased intake of fish oil has been associated with increased vitamin E requirements. Currently the minimum dietary vitamin E requirement of cats has been set at 30 IU/kg dry matter with dietary levels expected to increase 3–4 times with a diet high in PUFAs (4). To prevent vitamin E deficiency in cats fed commercially sold diets, the Association of American Feed Control Officials (AAFCO) recommends that fish oil–containing diets for cats should be supplemented with 10 IU of vitamin E for every g of fish oil per kg diet (5). However, there is no direct evidence to substantiate the latter value or the increases recommended (4,5) in adult cats.

The main objective of this study was to determine the vitamin E requirements of adult cats fed high dietary levels of PUFAs from fish oil.

MATERIALS AND METHODS

Animals and diets

Thirty-two (16 male, 16 female) 5- to 8-y-old domestic cats (*Felis catus*) from the Feline Unit at Massey University (Palmerston North, New Zealand) with an initial body weight range of 2292 to 5505 g (mean ± SEM, 3587 ± 146 g) were randomly allocated according to gender to one of four experimental diets varying in vitamin E content. The experimental diets were formulated from a basal diet, which contained (g/100 g): salmon protein meal, 24; salmon oil, 23; rice, 18; frozen salmon, 16; soy protein concentrate, 14; and minor ingredients, 5. Analysis showed the basal diet to contain (g/100 g dry matter): crude protein, 39; crude fat, 30; ash, 7.6; and (MJ/kg dry matter) ME, 19.5. The basal diet was supplemented with DL-α-tocopheryl acetate so that the four experimental diets contained (after heat processing) approximately 0, 1.5, 3.0 and 4.5 IU/g dry matter, respectively. The cats were group housed in large outdoor pens and fed to appetite with water being available at all times. The animals were weighed weekly until the end of the study and feed intake of each pen was recorded daily over the 26-wk study. The experimental procedures and care of the cats were approved by the Massey University Animal Ethics Committee.

Whole blood and blood plasma assays

A blood sample (±2.5 mL) was collected from each cat at biweekly intervals up to wk 18 and at wk 26. The blood samples were subjected to α-tocopherol, lymphocyte proliferation to concanavalin A (Con A), red blood cell hydrogen peroxide hemolysis (RBCH), the ferric-reducing ability of plasma (FRAP), triglycerides and alkaline phosphatase analyses. Plasma α-tocopherol RBCH analysis and whole blood lymphocyte proliferation to Con A were similar to procedures previously described (6–8), with minor modifications. The FRAP was determined by a previously described method (9). Plasma alkaline phosphatase (EC 3.1.3.1) and triglycerides were determined using commercially available kits (Roche Products NZ, Auckland, New Zealand).

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[4] Abbreviations used: Con A, concanavalin A; FRAP, ferric-reducing ability of plasma; PUFAs, polyunsaturated fatty acids; RBCH, red blood cell hemolysis.

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Data analysis

Data were tested for homogeneity of variances using Barlett's test. The plasma α-tocopherol, FRAP and plasma triglyceride data were $\log_{10}$ transformed, whereas the in vitro stimulation index of lymphocytes to Con A data were square-root transformed before ANOVA. All the data were subjected to ANOVA using the general linear model procedure in SAS with diet and gender as variables, and time as a repeated factor. The Tukey test was used to determine significant differences of plasma α-tocopherol, FRAP and RBCH between dietary groups at each time point. All statistical analyses were performed using the SAS statistical package (SAS version 6, SAS Institute, Cary, NC).

RESULTS

All cats remained healthy throughout the study and no signs of vitamin E deficiency were observed. A female cat on diet 0 was excluded from the study after wk 3 because of severe loss of body weight (27% of initial weight). The four diets were found to contain 24, 1197, 2830 and 4314 IU/kg dry matter α-tocopherol, and free of peroxides and the synthetic antioxidants, butylated hydroxytoluene, butylated hydroxyanisole and ethoxyquin.

There was no difference ($P > 0.05$) in the plasma α-tocopherol concentrations, RBCH, FRAP value, stimulation index of lymphocytes to Con A, plasma triglyceride and alkaline phosphatase concentrations of the four groups of cats at the beginning of the study. Plasma α-tocopherol concentrations of the cats on diet 0 remained low, whereas the levels of the cats on diet 1.1, 2.8 and 4.3 increased (**Fig. 1**). There was an effect ($P < 0.001$) of diet, time and the interaction between diet and time on plasma α-tocopherol levels. The RBCH of the cats on diet 0 remained high until wk 16 and decreased abruptly at wk 18, after which it remained constant until the end of study (**Fig. 2**). RBCH abruptly decreased after wk 2 in the cats fed the supplemented diets and remained relatively low until the end of the study. There was an effect ($P < 0.001$) of diet, time and interaction between diet and time on RBCH. The FRAP of the cats on diet 0 was significantly decreased after wk 2 of feeding and thereafter increased to reach a plateau at wk 10. In general, the FRAP of the cats on the α-tocopherol–supplemented diets increased throughout the study (**Fig. 3**). There was an effect ($P < 0.001$) of diet, time and the interaction between diet and time on the FRAP status of the cats.

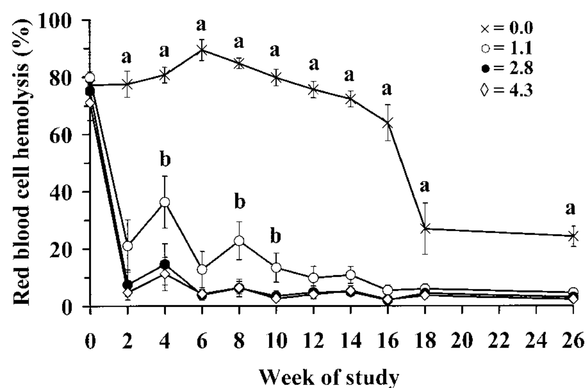

FIGURE 2 Red blood cell hemolysis of the adult cats fed a high fish oil–containing diet with 0, 1.1, 2.8 and 4.3 IU α-tocopherol/g. Values are means ± SEM, $n = 7–8$ cats per group. Throughout the study period there was an effect of diet, time and the interaction between diet and time on red blood cell hemolysis (repeated-measures ANOVA, $P < 0.001$). Those at a time with different superscripts are significantly different, $P < 0.05$.

Lymphocyte proliferation to Con A, plasma triglyceride and plasma alkaline phosphatase levels in the cats on diet 0, 1.1, 2.8 and 4.3 were not different ($P = 0.73$, 0.14 and 0.91, respectively). The response to Con A increased at wk 2, and thereafter became suppressed throughout the entire period except for a slight increase at wk 18. There was an effect ($P < 0.001$) of time on lymphocyte proliferation to Con A and plasma triglycerides concentration, with the latter being reduced by 44% at the end of study (mean of all groups).

DISCUSSION

No clinical signs of vitamin E deficiency were observed in any of the adult cats fed the four experimental diets during the 26-wk study. The mean (± SEM) plasma α-tocopherol concentration of the adult cats on the vitamin E–unsupplemented diet (0) at the end of the study was 8.8 ± 0.6 μg/mL. Although there have been many reported cases of clinical signs of vitamin E deficiency in cats, only a few report plasma

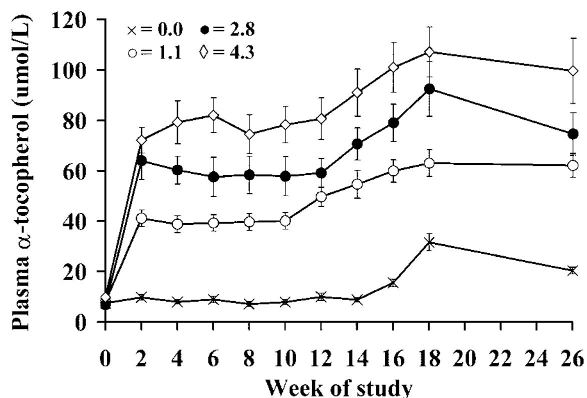

FIGURE 1 Plasma α-tocopherol concentration of adult cats fed a high fish oil–containing diet with 0, 1.1, 2.8 and 4.3 IU α-tocopherol/g. Values are means ± SEM, $n = 7–8$ cats per group. Throughout the study period there was an effect of diet, time and the interaction between diet and time on plasma α-tocopherol levels (repeated-measures ANOVA, $P < 0.001$).

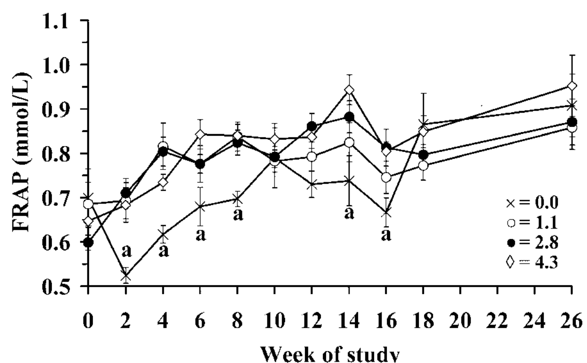

FIGURE 3 Ferric-reducing ability of plasma of the adult cats fed a high fish oil–containing diet with 0, 1.1, 2.8 and 4.3 IU α-tocopherol/g. Values are means ± SEM, $n = 7–8$ cats per group. Throughout the study period there was an effect of diet, time and the interaction between diet and time on red blood cell hemolysis (repeated-measures ANOVA, $P < 0.05$).

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or serum α-tocopherol concentrations. In a previous study (2), serum α-tocopherol concentrations in vitamin E–deficient cats were reported to range from 0.3 to 5.0 μg/g. Others (3) have found levels of plasma tocopherol in vitamin E–deficient cats of 1.2–3.5 μg/mL. Based on these studies, the cats fed the unsupplemented diet in the present study maintained an adequate plasma α-tocopherol concentration to prevent vitamin E deficiency symptoms. It is interesting to note that the average plasma α-tocopherol concentration of the cats at the start of the study was 3.4 ± 0.2 mg/mL, which is close to levels reported in vitamin E–deficient kittens (2,3).

The reason for the relatively high plasma α-tocopherol levels in the cats fed diet 0 may be the result of several factors. All experimental diets were found to be free of the synthetic antioxidants butylated hydroxytoluene, butylated hydroxyanisol and ethoxyquin. Other dietary factors, however, are well known to have a sparing effect on α-tocopherol. It has been shown in growing dogs fed a low vitamin E diet that supplementation of the diet with selenium at 0.5 ppm can prevent the development of clinical signs of vitamin E deficiency (10). The sparing effect of selenium on vitamin E is thought to result from the reduction of hydroperoxides through the action of the Se-dependent enzyme gluthathioneperoxidase. The diets in the present study contained a relatively high level of selenium: the analyzed selenium level in the four diets ranged from 0.95 to 1.03 mg/kg. These dietary values are within the normal range found in commercial cat foods, but approximately 10 times higher than the minimum requirements (4) for growing cats. Other compounds with antioxidant properties such as ascorbic acid, retinol and carotenoid may have further spared the α-tocopherol from being oxidized. Additionally, the cats in the present study may have had a low in vivo oxidation rate, given that the fish oil used in the present study was of a high quality, as evident by the failure to detect lipid peroxides. Several studies have shown that tissue α-tocopherol can be more rapidly depleted when animals consume diets containing partially oxidized oil (11). Finally, the time for the adult cats in the present study to develop vitamin E deficiency symptoms may have been longer than 26 wk. In a previous study involving growing cats, the first signs of vitamin E deficiency were observed at approximately 240 d after the start of feeding a vitamin E–free diet (2). Others noted vitamin E deficiency symptoms after 9–12 mo of feeding cats an unsupplemented vitamin E diet (3). However, the average plasma α-tocopherol level of the cats fed diet 0 in the present study was lower at the start compared to that at the end of the study (3.4 vs. 8.8 μg/mL). It is, therefore, unlikely that the cats in the present study would have developed clinical signs of vitamin E deficiency when fed the diet for a longer period of time.

Cats fed the α-tocopherol–supplemented diets had a lower RBCH than that of animals on diet 0. The FRAP assay measures the water-soluble antioxidant status of plasma, and the FRAP in the cats fed diet 0 was found to be compromised. No difference was found in plasma triglyceride levels among the four groups of cats in the present study. The latter is not surprising, given that all four diets contained similar levels of fish oil and the dietary intake of the cats on the four diets was also similar. The data in the present study showed no beneficial effect of dietary α-tocopherol on lymphocyte proliferation when levels were 150% of the recommendations of AAFCO (5). The reason for the increase in plasma α-tocopherol at approximately 10 wk in all four groups may be related to the replacement of oxidized body fat with high-quality fat from the diet and the associated reduction in α-tocopherol required.

Studies in other animals have demonstrated that lymphocyte proliferation to B-cell and T-cell mitogens are influenced by the dietary level of vitamin E. The dietary vitamin E requirement to obtain optimal immunological health is approximately 4–10 times higher than the dietary vitamin E level to prevent vitamin E deficiency. In rats, a dietary vitamin E level of 15 IU/kg has been found to be adequate to prevent myopathy (12). However, to optimize T- and B-lymphocyte responses to Con A and pokeweed mitogen, levels were found to be much higher (50 to 200 IU/kg). In another study, the effect of vitamin E supplementation on the immune response of young and older cats using diets containing 52, 272 and 500 IU/kg diet was measured (13). These authors found that vitamin E supplementation of diets to supraphysiological levels does not provide the added benefit, as is generally found in other species. The present study provides further impetus for this finding, given that there was no improvement in immune status, as measured by the stimulation of lymphocytes to Con A in adult cats fed diets containing α-tocopherol up to 4.3 IU/g diet dry matter.

The National Research Council (4) recommends that moderate to low-fat (<10% dry weight) diets for growing cats should contain 30 IU of α-tocopherol per kg diet with a three- to fourfold increase in dietary α-tocopherol if the diet contains a high level of PUFAs. AAFCO (5) recommends that 10 IU of vitamin E should be added per g of added fish oil per kg diet. However, the quality of fish oil used in the formulation of diets for cats is likely to affect the vitamin E requirements, and lower-quality fish oil will most likely increase in vivo oxidation in cats, thereby increasing vitamin E requirements. Estimates of vitamin E requirements in cats vary, therefore, depending on the quality of fish oil used. The present study shows that when a high-quality fish oil is used, the dietary vitamin E requirement of adult cats fed high dietary levels of PUFAs from fish oil is <5 IU per g of added fish oil per kg diet.

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