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# A randomized, controlled study to evaluate the steroid sparing effect of essential fatty acid supplementation in the treatment of canine atopic dermatitis

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**Abstract** A randomized, double blind, placebo-controlled multicentre clinical trial of 12 weeks' duration was undertaken in 60 dogs with atopic dermatitis to evaluate the steroid sparing effect of essential fatty acid supplementation. The dogs were randomly assigned to receive either a combination of borage seed oil and fish oil or a placebo, in addition to prednisolone tablets. All dogs received a standardized basal diet. Owners of the dogs recorded pruritus daily using a 10 cm visual analog scale and the dosage of prednisolone was established based on the pruritus score, according to written instructions. The dosage of prednisolone and the use of any concurrent treatment (shampoo and/or ear-cleanser) were recorded by the owner on a daily basis. The investigators graded the skin lesions at days 0, 42 and 84. The use of prednisolone during the test period was lower in the active group, but the difference was not statistically significant ($P = 0.32$). The test period was sequentially divided into 43–84, 50–84, 57–84, 64–84, 71–84 and 78–84 days. On day 64, the difference between the active group and the placebo group reached statistical significance ($P = 0.04$) with an increasing difference towards the end of the study. A statistically significant reduction in the pruritus scores and the total clinical scores from day 0 to day 84 was apparent in both groups ($P < 0.0001$). At the end of the study, both the pruritus score and the total clinical score were lower in the active group. Our findings indicate a steroid sparing effect of essential fatty acid supplementation in canine atopic dermatitis and, furthermore, that there is a time lag before the effect is attained.

*Keywords:* atopic dermatitis, atopy, canine, essential fatty acid supplementation, randomized controlled trial, steroid sparing effect.

## INTRODUCTION

Essential fatty acids were proposed for the treatment of canine atopic dermatitis in the mid-1980s. Since then a large number of studies reporting the efficacy of essential fatty acid supplementation have been performed (reviewed by Olivry *et al.*[1]). Numerous inflammatory cells play a role in the pathogenesis of canine atopic dermatitis.[2,3] Moreover, the inflammation and dermatitis in atopic dermatitis may be partially caused by inappropriate eicosanoid synthesis. The proposed beneficial mechanism of dietary supplementation of essential fatty acids in atopic individuals is to direct fatty acid metabolism towards the production of less inflammatory eicosanoids.[4–6] Essential fatty acids have also been reported to inhibit cellular activation and excretion of various cytokines in humans and rats.[7–9] Lastly, the n-6 fatty acid linoleic acid (LA) is important for epidermal lipid barrier function, and supplementation with diets enriched with LA can result in a significant decrease in transepidermal water loss.[10]

The reported efficacy of essential fatty acid supplementation in canine atopic dermatitis is quite variable, and could reflect differences, as well as deficiencies, in the design of previously performed studies (reviewed by Olivry *et al.*[1]). Of special importance is the lack of standardization of the regular diet used in those studies. The levels of fatty acids vary greatly from one diet to another and the variability in background fatty acid intake could be at least as great as the level of supplemental fatty acids provided.[11,12] To the best of

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our knowledge, none of the placebo-controlled studies evaluating the effect of essential fatty acid supplementation in canine atopic dermatitis has included a control for the dogs' regular diet.

It has been estimated that approximately 20% of dogs with allergic pruritus can be adequately controlled by essential fatty acid supplementation alone, with no other treatment necessary.[13] On the other hand, essential fatty acids appear to have an additive or a synergistic effect in decreasing clinical signs in canine atopic dermatitis, when used in combination with antihistamines and corticosteroids.[14–16] However, only one of the studies reporting this finding was designed as a blind and placebo-controlled trial,[16] and none of the studies included a control for the dogs' regular diet.

Systemic glucocorticoids are used most widely for symptomatic treatment of canine atopic dermatitis. Glucocorticoids exert a wide range of anti-inflammatory and immuno-suppressive effects. In addition to having a direct inhibitory action on many cells involved in inflammation, glucocorticoids may increase transcription of genes coding for anti-inflammatory proteins, and inhibit the expression of multiple inflammatory genes. Of particular importance is the suppression of many inflammatory cytokines and chemokines that amplify and perpetuate allergic inflammation (reviewed by Barnes[17]). Unwanted side effects, however, are common in both long and short-term treatment and are often dose related.[18] Conversely, therapeutic use of essential fatty acids seldom leads to undesirable side effects.[19,20] In cases of canine atopic dermatitis where fatty acids alone can not alleviate clinical signs, it is therefore of great interest to evaluate the potential steroid sparing effect of dietary supplementation with essential fatty acids.

The aim of the present study was to examine whether a fatty acid supplement consisting of omega-6 fatty acid from borage seed oil and omega-3 fatty acid from fish oil would have a steroid sparing effect in the treatment of canine atopic dermatitis when given in addition to a standardized basal diet.

## MATERIALS AND METHODS

### *Study design*

The present study was performed as a randomized, double blind, multicentre and multi-investigator trial with parallel group design of 12 weeks' duration. A total of six investigators from Denmark (1), Finland (1), Norway (1) and Sweden (3) participated. The dogs were allocated to receive either a fatty acid supplement (Viacutan®, Boehringer Ingelheim Danmark AS, Copenhagen, Denmark) ($n$ = 28) or a placebo ($n$ = 32) in accordance with a prerandomization list performed by simple randomization[21] within each participating centre. Medium Chain Triglycerides (MCT) oil was used as placebo because it contains no n-6 or n-3 fatty acids. The fatty acid supplement and the placebo were provided in identical airless pump dispensers. From 3 weeks before entering the study and throughout the test period, all dogs were fed the same commercial complete and balanced dry food for adult dogs containing grounded maize and chicken and turkey meal (Table 1). The owners and the investigators were unaware of the treatment given until the trial was closed and the codes were broken.

**Table 1.** The essential fatty acid composition of the basal diet reported as percentage of dry matter (DM)

| Fatty acid | | % DM |
|---|---|---|
| C18 : 2n-6 | Linoleic acid | 3.497 |
| C18 : 3n-6 | Gamma-linolenic acid | 0.034 |
| C18 : 3n-3 | Alpha-linolenic acid | 0.246 |
| C18 : 4n-3 | Octadecatetraenoic acid | 0.028 |
| C20 : 3n-6 | Dihomo-gamma-linolenic acid | 0.005 |
| C20 : 4n-6 | Arachidonic acid | 0.037 |
| C20 : 5n-3 | Eicosapentaenoic acid | 0.005 |
| C22 : 4n-6 | Adrenic acid | 0.007 |
| C22 : 5n-3 | Docosapentaenoic acid | 0.005 |
| C22 : 6n-3 | Docosahexaenoic acid | 0.013 |
| Total n-6 fatty acids | | 3.591 |
| Total n-3 fatty acids | | 0.320 |

### *Patient enrolment*

Privately owned dogs with atopic dermatitis were eligible for inclusion in the present study. Diagnosis was based on compatible history and clinical signs, and exclusion of other causes of pruritic dermatitis.[13,22] Dogs included had nonseasonal clinical signs, and each dog had visible signs of pruritus when entering the study. Additionally, all dogs had IgE antibodies against environmental allergens as assessed by intradermal testing and/or allergen-specific IgE testing.

Exclusion criteria were systemic and dermatological diseases other than atopic dermatitis. Ectoparasite infestations were excluded as a cause of the pruritus by vacuum cleaning,[23] skin-scraping, tape impression, hair-plucking, serological evaluation for *Sarcoptes scabiei* antibodies[24,25] and/or lack of response to trial therapy. None of the dogs had a history of flea-infestation and all dogs were negative for fleas at examination. An elimination diet with a novel protein source was fed as either a home-cooked recipe or a commercially available diet for a minimum of 6 weeks prior to the study to rule out adverse food reactions, and dogs with a partial or complete reduction in clinical signs while being fed the diet were excluded. Dermatophytosis was ruled out by fungal cultures. When considered appropriate, a standard biochemistry profile, complete blood count and histopathological examination were performed to rule out systemic diseases and skin diseases incompatible with canine atopic dermatitis, respectively.

Secondary skin infections (pyoderma and/or *Malassezia* dermatitis) were treated with antibiotics/antimycotics and had to be cleared prior to enrolment in the study. Dogs with generalized secondary seborrhea were excluded. None of the dogs had received any fatty acid supplements or any other systemically administered

anti-inflammatory medications for at least 12 weeks prior to the investigation. Dogs treated with long-acting glucocorticoids were excluded. The owners provided written informed consent and were allowed to withdraw their dog at any time during the investigation period.

*Study protocol*

The owners recorded the severity of pruritus daily (every evening) by drawing a mark on a 10-cm visual analog scale (VAS).[26] The VAS had six divisions and the leftmost edge was labelled 'not itchy'; the rightmost edge 'extremely itchy'. The dosage of prednisolone (number of 5-mg tablets) to be given the next day was established on the basis of the pruritus score, according to written instructions: with a pruritus score of less than 30 mm, the prednisolone dose was to be reduced by one step; with a pruritus score between 30 and 50 mm, the same prednisolone dose was to be given the next day. In cases where the pruritus score was between 51 and 90 mm the prednisolone dose was to be increased one step. With a pruritus score exceeding 90 mm the owner was instructed to call the investigator in charge. The aim of this treatment regimen was to lower the prednisolone dosage to the minimum required by that particular patient at any given time. The initial prednisolone dosage given was determined by consultation with the investigator.

The owners were instructed to administer the supplement (essential fatty acids or placebo) orally, once daily, according to the manufacturer's guidelines: one pump stroke for dogs weighing less than 10 kg; two pump strokes for dogs weighing between 10 and 25 kg, and three pump strokes for dogs weighing more than 25 kg. One pump stroke of the fatty acid supplement (0.6 mL) consisted of 190 mg *cis*-LA, 105 mg gamma-linolenic acid (GLA), 9.9 mg eicosapentaenoic acid (EPA) and 6.6 mg docosahexaenoic acid (DHA).

No treatment other than an antibacterial shampoo containing ethyl lactate and benzalkonium chloride (Etiderm®, Virbac, Carros Cedex, France) and an ear-cleanser containing lactic and salicylic acid (Epi-Otic®, Virbac) were allowed. The owners were supplied with shampoo and ear-cleanser at the start of the study.

The pruritus score, dosage of prednisolone, and the use of shampoo and ear-cleanser were recorded on a daily basis in a diary supplied by the investigator when the dog entered the study. Throughout the test period, the dogs in both groups received the basal diet as their sole diet. The owners were instructed to feed the diet according to the feeding ranges suggested by the manufacturer. The daily amount of dry food given was determined according to the dogs' body weight: 50–65 g for dogs weighing 2.5 kg; 85–110 g for dogs weighing 5 kg; 145–190 g for dogs weighing 10 kg; 240–315 g for dogs weighing 20 kg; 325–430 g for dogs weighing 30 kg, 405–535 g for dogs weighing 40 kg, and 475–630 g for dogs weighing 50 kg. For dogs over 50 kg, 40 g extra was added per 5 kg body weight. If necessary, the daily amount of dry food given was adjusted to maintain optimal body weight. The owners were furthermore instructed not to feed the dogs any other food or treats.

The same investigator examined a given dog on days 0, 42 and 84 and assessed the pruritus and dermatitis by evaluation of primary and secondary skin lesions. Scores from 0 (no lesion) to 4 (severe lesion) were assigned each for erythema, alopecia, excoriations, scales, crusts, lichenification and hyperpigmentation in 15 different regions spanning the entire body surface (face, periorbital regions, ear pinnae, ear canals, front legs, front paws, ventral neck, back, lumbar region, abdomen, groin, rear legs, rear paws, perianal region, tail). The maximum score achievable was 420 ($4 \times 7 \times 15$).

The primary outcome variable of efficacy in the study was the dosage of prednisolone used. The owner-assigned pruritus scores and the investigator-assessed clinical scores were defined as secondary outcome variables of efficacy. The use of concurrent treatment (shampoo and/or ear-cleanser) was also recorded.

*Withdrawal and drop-out*

The discontinuation of the trial prematurely due to worsening of clinical signs, development of adverse effects or occurrence of diseases interfering in some manner with the study protocol was classified as withdrawal.

Serious protocol deviations and owner desire to withdraw were classified as drop-outs.

*Statistical analysis*

The results are given as either mean values or median values with 95% confidence intervals and/or total ranges. The confidence intervals for the mean and the median were calculated using the Student's procedure[27] and the Bernoulli–Wilcoxon procedure,[28] respectively. In the case of skewness in the empirical distribution of a given variable, the nonparametric expression was used. The use of prednisolone is reported as the area under the curve (AUC) and was calculated using the Trapezium rule.[29] All comparisons were performed two-tailed and differences were considered significant with $P$-values $\leq 5\%$.

Comparison of the active and placebo groups for variables assumed to be continuously and symmetrically distributed was performed using analysis of variance (ANOVA) with repeated measurements.[30] For comparison of data within the groups the ANOVA model for paired data was used.[31] Non-normally distributed variables were compared using the Wilcoxon rank-sum test[28] and analyses of changes within the groups were performed using the Wilcoxon signed rank test.[32] Categorical data were compared using the Fisher's exact test.[33]

Statistical analyses were performed as an intention-to-treat analysis. When patients did not complete the study, the missing data were replaced with the last observed values (the last-observation-carry-forward-rule).

As it was anticipated that there would be a time lag before any beneficial effect of the dietary fatty acid

| Variables | Active group | 95% CI | Placebo group | 95% CI |
|---|---|---|---|---|
| Age (y) | 4.5 | (3.7–5.3) | 3.9 | (3.1–4.6) |
| Duration of symptoms (mo) | 34.8 | (27.0–42.6) | 30.9 | (22.7–39.1) |
| Body weight (kg) | 28.1 | (22.5–33.7) | 28.1 | (24.6–31.6) |
| Sex | | | | |
| Female | 14 | | 14 | |
| Male | 14 | | 18 | |
| Previous feeding | | | | |
| Commercial dry | 19 | | 23 | |
| Commercial wet | 1 | | 0 | |
| Home-cooked | 4 | | 4 | |
| Combination | 4 | | 4 | |
| Previous pyoderma | 23 | | 27 | |
| Previous *Malassezia* dermatitis | 8 | | 7 | |
| Previous otitis externa | 11 | | 17 | |

**Table 2.** Comparison of selected variables between the active group ($n = 28$) and the placebo group ($n = 32$). Continuous data are given as mean with 95% CI. Categorical data are given as number of dogs

supplementation,[34] the test period was sequentially divided into 43–84, 50–84, 57–84, 64–84, 71–84 and 78–84 days. The amount of prednisolone used in the two groups was calculated for the whole test period ($AUC_{1-84}$) as well as for the periods from day 43 to day 84 ($AUC_{43-84}$); day 50 to day 84 ($AUC_{50-84}$); day 57 to day 84 ($AUC_{57-84}$); day 64 to day 84 ($AUC_{64-84}$); day 71 to day 84 ($AUC_{71-84}$), day 78 to day 84 ($AUC_{78-84}$), and the statistical procedure reiterated.

To investigate a possible dose–response effect of fatty acid supplementation, based on the amount of GLA received from the supplement, the use of prednisolone within the different quartiles was compared.

## RESULTS

### *Patients*

Sixty dogs were included in the study. Of these, 28 were females and 32 were males. Mean age was 4.1 years, with ages ranging from 1 to 9 years. Twenty-eight different breeds and mixed breeds were represented; mean body weight was 28.1 kg, with body weights ranging from 8 to 82 kg. Due to the dose instructions, the exact dosage of essential fatty acids received varied between dogs with different body weights. The mean dose of n-6 and n-3 fatty acids received per kilogram of body weight was 32 mg (range: 10.8–53.6 mg) and 1.8 mg (range: 0.6–3 mg), respectively.

Originally, 42 dogs were fed mainly commercial dry-food, one dog was fed mainly canned food and eight dogs were fed mainly home-cooked food. Eight dogs received some combination of these alternatives. This information was not available for one dog. Prior to the study, 50 and 28 dogs had been treated for pyoderma and otitis externa, respectively. No significant differences were identified between the active and placebo group with respect to age, gender, body weight, duration of symptoms, previous feeding, previous pyoderma, *Malassezia* dermatitis and otitis externa (Table 2).

### *Withdrawals and drop-outs*

A total of six dogs, all belonging to the placebo group, discontinued the trial prematurely or did not comply properly with the study protocol. Two of these dogs dropped out due to serious protocol deviations and one dog dropped out because the owner withdrew consent. In addition, three dogs developed acute folliculitis, *Malassezia* dermatitis, and acute gastro-enteritis, respectively. These dogs were withdrawn from the study after examination at day 42, but were included in the statistical analyses.

**Table 3.** The pruritus scores in the active group ($n = 28$) and the placebo group ($n = 29$) on days 0, 41 and 83. Data are given as mean with 95% CI

| | | Mean | 95% CI |
|---|---|---|---|
| Day 0 | Active group | 52.0 | (46.3–57.8) |
| | Placebo group | 51.2 | (44.5–57.9) |
| Day 41 | Active group | 26.0 | (19.2–32.7) |
| | Placebo group | 25.2 | (19.8–30.6) |
| Day 83 | Active group | 19.6 | (14.0–25.2) |
| | Placebo group | 27.8 | (22.3–33.2) |

### *Owner-assigned pruritus scores and use of prednisolone*

On days 0 and 41, the pruritus scores were almost equal in the active and placebo group, whereas on day 83 the pruritus score was significantly lower in the active group ($P = 0.04$) (Table 3). A statistically significant reduction in the pruritus scores from day 0 to day 41 was apparent in both groups ($P < 0.0001$). From day 41 to 83, a further reduction was seen in the active group; however, this reduction was not statistically significant ($P = 0.06$). In the placebo group there was an increase in the pruritus score in the same time period, although not statistically significant. The mean percentage reduction in the pruritus score in the active and placebo group was 57 (95% CI: 43.3–70.5) and 38 (95% CI: 19.1–56.9), respectively.

There was a distinct difference in the use of prednisolone between the active and placebo group in the test period, and the dogs in the active group received considerably less prednisolone in the last period of the study (Fig. 1). The difference between the groups was not statistically significant for the whole test period. However, for the last 3 weeks of the study (from day 64 to 84) the difference between the active and placebo group was statistically significant ($P = 0.04$). The

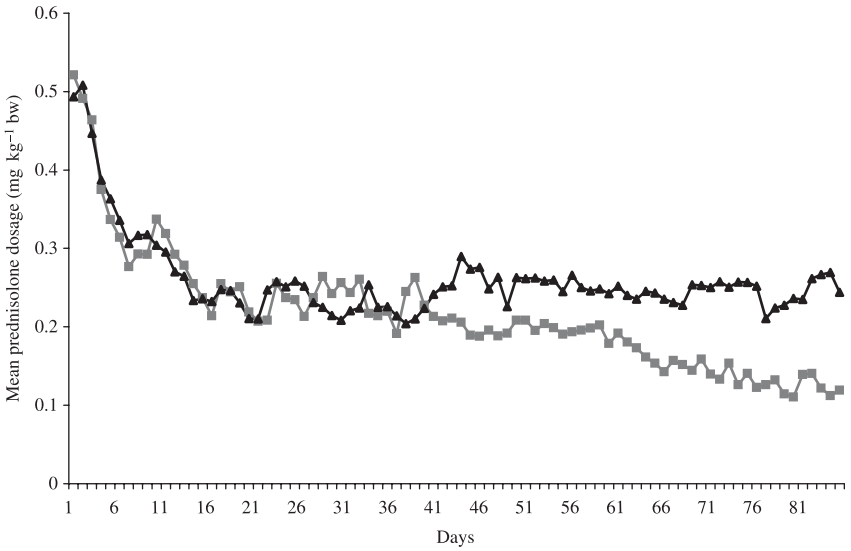


**Figure 1.** The mean dose of prednisolone in mg per kg body weight received in the active group ($n$ = 28) (■) and the placebo group ($n$ = 29) (▲) in the test period (day 1 to 84).

**Table 4.** The use of prednisolone (mg kg$^{-1}$) expressed as the area under the curve (AUC) for different time periods in the active ($n$ = 28) and placebo group ($n$ = 29). Data are given as mean with 95% CI

| | Active group | | Placebo group | | |
|---|---|---|---|---|---|
| | Mean | 95% CI | Mean | 95% CI | $P$-values |
| $AUC_{1-84}$ | 17.9 | (13.2–20.1) | 21.5 | (15.2–27.8) | 0.324 |
| $AUC_{43-84}$ | 6.7 | (4.9–8.5) | 10.2 | (6.3–14.1) | 0.094 |
| $AUC_{50-84}$ | 5.4 | (3.9–6.8) | 8.4 | (5.1–11.7) | 0.081 |
| $AUC_{57-84}$ | 4.0 | (2.8–5.1) | 6.6 | (4.1–9.7) | 0.059 |
| $AUC_{64-84}$ | 2.7 | (1.9–3.5) | 4.9 | (3.1–6.8) | 0.037 |
| $AUC_{71-84}$ | 1.7 | (1.1–2.2) | 3.2 | (2.0–4.4) | 0.024 |
| $AUC_{78-84}$ | 0.7 | (0.5–1.1) | 1.5 | (0.9–2.1) | 0.018 |

**Table 5.** The use of prednisolone (mg kg$^{-1}$) expressed as the area under the curve (AUC) for different time periods in quartiles in the active group ($n$ = 28). The quartiles (1st quartile–4th quartile) are based on the amount of gamma-linolenic acid (GLA) received from the supplement in mg per kg body weight. Data are given as mean with total range (the amount of GLA and the body weight) or mean with 95% CI (AUC)

| | 1st quartile | 2nd quartile | 3rd quartile | 4th quartile |
|---|---|---|---|---|
| GLA (mg/kg) | 7.6 (3.8–9.0) | 9.8 (9.1–10.3) | 11.3 (10.3–13.1) | 16.9 (14.0–19.1) |
| Body weight (kg) | 44.9 (34.9–82.0) | 29.1 (22.2–33.0) | 25.8 (8.0–30.5) | 12.6 (11.0–15.0) |
| $AUC_{1-84}$ | 20.7 | 11.7 | 25.7 | 13.5 |
| 95%CI | (9.4–32.0) | (7.1–16.4) | (17.0–34.4) | (8.6–18.4) |
| $AUC_{64-84}$ | 4.0 | 1.6 | 3.6 | 1.6 |
| 95%CI | (1.4–6.6) | (0.5–2.6) | (1.6–5.7) | (0.4–2.9) |
| $AUC_{71-84}$ | 2.4 | 0.9 | 2.3 | 1.0 |
| 95%CI | (0.8–3.9) | (0.2–1.7) | (0.9–3.8) | (0.2–1.8) |
| $AUC_{78-84}$ | 1.0 | 0.5 | 1.1 | 0.4 |
| 95%CI | (0.3–1.6) | (0.02–1.0) | (0.4–1.9) | (0.03–0.7) |

difference between the groups increased towards the end of the trial (Table 4).

In the active group, the amount of GLA received from the supplement was highest in the 4th quartile and lowest in the 1st quartile. If a dose–response effect was present, the use of prednisolone (AUC) should be lowest in the quartile receiving the highest dose of GLA (the 4th quartile). The AUC should thereafter increase in the third and second quartiles, and be highest in the 1st quartile. This was not the case and, on the contrary, the AUC fluctuated from quartile to quartile (Table 5).

### *Investigator-evaluated clinical scores*

On days 0 and 42, the total clinical scores were higher in the active group, whereas on day 84 the total clinical score was lowest in the active group. The differences were not statistically significant. A statistically significant reduction in total clinical scores from day 0 to 42 was apparent in both groups ($P < 0.0001$). From day 42 to 84, a further reduction was seen in both groups; however, this reduction was less pronounced and not statistically significant (Table 6). The median percentage reduction in the total clinical score in the active and placebo group was 83.1 (95% CI: 66.7–93.6) and 76.5

**Table 6.** The total clinical scores in the active group ($n$ = 28) and placebo group ($n$ = 29) on days 0, 42 and 84. Data are given as median with 95% CI and total range

| | | Median | 95% CI | Range |
|---|---|---|---|---|
| Day 0 | Active group | 33.5 | (16.0–49.0) | (7.0–101.0) |
| | Placebo group | 24.0 | (17.5–38.0) | (9.0–93.0) |
| Day 42 | Active group | 7.5 | (4.0–18.0) | (0.0–58.0) |
| | Placebo group | 7.0 | (3.0–12.0) | (0.0–80.0) |
| Day 84 | Active group | 4.0 | (3.0–12.0) | (0.0–65.0) |
| | Placebo group | 6.0 | (2.5–10.5) | (0.0–80.0) |

**Table 7.** The use of shampoo and ear-cleanser (expressed as number of times used during the test period) in the active group ($n$ = 28) and placebo group ($n$ = 29). Data are given as median with 95% CI and total range

| | | Median | 95% CI | Range |
|---|---|---|---|---|
| Active group | Shampoo | 4.5 | (1.0–8.0) | (0.0–15.0) |
| | Ear-cleanser | 4.0 | (0.0–6.0) | (0.0–24.0) |
| Placebo group | Shampoo | 3.0 | (0.5–5.5) | (0.0–35.0) |
| | Ear-cleanser | 2.0 | (0.0–3.5) | (0.0–25.0) |

(95% CI: 39.9–90.9), respectively. Regarding the individual clinical parameters, there was a statistically significant reduction in all skin lesions, except crusts, in both groups.

### *Use of shampoo and ear-cleanser*

The use of shampoo and the ear-cleanser did not differ significantly between the active and placebo groups, although both were used more frequently in the former (Table 7).

## DISCUSSION

The results found in the present study indicate that essential fatty acid supplementation has a steroid sparing effect in dogs with atopic dermatitis. This finding is in accordance with a previous open study, which reported that supplementation with omega-6 and omega-3 dietary fatty acids reduced the prednisolone requirements in eight of 11 dogs with atopic dermatitis.[15] The beneficial effect of borage seed oil and fish oil supplementation in the treatment of canine atopic dermatitis has recently been reported by Harvey.[35] Harvey gave the fatty acid supplement as the sole anti-inflammatory treatment, and in considerably higher concentrations than those used in the present study.[35] Neither of these earlier studies included a control for the dogs' regular diet. According to Watson,[12] the variability in background fatty acid intake could be at least as great as the level of supplemental fatty acids provided. Hence, in the present study, by feeding both groups of dogs the same basal diet the influence of diet would not bias the outcome of the study.

Most of the previously published controlled studies reporting the efficacy of omega-6 and omega-3 fatty acid supplementation in canine atopic dermatitis have investigated the effect of omega-6 fatty acids at dosages higher than 100 mg $kg^{-1}$ $day^{-1}$.[1] Thus, the dosage of omega-6 fatty acids provided by the supplement in the present study was relatively low and, due to the dose instructions, also varied between dogs with different body weights. However, the dosage of GLA provided by the supplement is comparable to that provided by the essential fatty acid supplement in an open study evaluating the steroid sparing effect of essential fatty acid supplementation.[15] Conversely, the dosages of omega-3 fatty acids provided by omega-6/omega-3 essential fatty acid supplements in previous controlled studies have generally been low,[1] at least when compared with the dosage of omega-3 fatty acids provided (66 mg $kg^{-1}$ $day^{-1}$) in a controlled cross-over study, which reported that marine oil supplementation was effective in the treatment of canine allergic pruritus.[36] Both the effect of the low-dosage omega-3 supplementation and the importance of the omega-6 : omega-3 ratio in the present study need to be investigated in future controlled studies.

The dry food used as the basal diet in the present study is a complete food marketed as 'a nutritional aid for recurrent struvite urolithiasis'. Its protein content is slightly reduced, and calcium, phosphorus and magnesium are reduced while fat and carbohydrate are increased compared with a 'maintenance' food produced by the same manufacturer. In a 20-kg dog fed according to the manufacturer's suggestions, the estimated daily intakes of LA, GLA, EPA and DHA (based on per cent of dry matter) would be 11 g $day^{-1}$, 107.1 mg $day^{-1}$, 15.8 mg $day^{-1}$ and 41 mg $day^{-1}$, respectively. Apart from GLA, the daily intake of essential fatty acids is comparable to the daily intake of a 20-kg dog fed a 'typical dry food'.[12] The daily intake of GLA is approximately 3.5 times higher in the diet used in the present study than in the 'typical dry food'. Hence, the effect of a basal diet rich in GLA should not be underestimated. The high amount of GLA in borage seed oil[37] could be further strengthened by the high GLA content in the basal diet, although other factors in addition to the absolute concentration could be important for the availability of fatty acids.[38,39]

Results from a previously performed study have indicated a dose–response effect of borage seed oil and fish oil supplementation in the treatment of canine atopic dermatitis.[35] Accordingly, the lack of a demonstrable dose–response effect in the present study was somewhat unexpected. However, in the study performed by Harvey,[35] the dosages of GLA given were considerably higher (21 mg GLA $kg^{-1}$ and 42 mg GLA $kg^{-1}$) than the dosages used in the present study. Moreover, the number of dogs in each quartile was low and the influence of a certain individual's lack of response to the fatty acid supplementation[11,15] or lack of owner-compliance could heavily influence the results. Both owner-compliance and the individual response to fatty acid supplementation could have been more closely assessed in the present study by measurement of fatty acid patterns in sera, blood cells and/or the skin. If these parameters had been monitored, subgroup analysis could have been performed in

dogs that had shown changes in fatty acid patterns, after exclusion of dogs that failed to show changes in the pattern. Moreover, we did not quantify the exact amount of dry food consumed daily by each patient, and hence we were unable to report the total amount of GLA received (from both the supplement and the basal diet) within each quartile. However, there was an inverse relationship between increasing body weight and the amount of dry food received per kilogram. Hence, the amount of GLA received from the basal diet was lowest in the 1st quartile and highest in the 4th quartile. Likewise, the amount of GLA received from the supplement was highest in the 4th quartile and lowest in the 1st quartile. The results reported in the present study, however, do not suggest the optimal dose for a steroid sparing effect of fatty acid supplementation in the treatment of canine atopic dermatitis. A higher dose of the supplement could have lead to a further reduction in the steroid dosage. Conversely, a lower dose could have provided the same outcome.

Although there is general agreement that the effect of dietary fatty acid supplementation does not have an immediate effect, the length of the required treatment before maximum benefit from fatty acid supplementation is attained is still debated. According to Bond and Lloyd,[15] Harvey[35] and Logas and Kunkle,[36] dogs treated with various fatty acid supplements required between 4 to 12 weeks before improvement was maximized. Conversely, Scott *et al.*[11] reported that maximal response was achieved within 1 to 3 weeks. Results from a study by Bond and Lloyd[15] in which combined treatment with essential fatty acids and prednisolone was used suggest that 8 or more weeks of dietary supplementation are necessary before the full therapeutic effect develops. The data found in the present study support the results of Bond and Lloyd.[15] Moreover, since the difference in the use of prednisolone between the active and placebo group increased towards the end of the study, there is a possibility that maximum benefit from the treatment was not reached at the close of the trial.

In this study we have confirmed that most dogs with atopic dermatitis respond very well to prednisolone treatment. Adverse events during glucocorticoid administration, however, are common, particularly with long-term treatment.[20] To avoid some of the undesirable effects, alternate day treatment with oral use of short-acting glucocorticoids such as prednisone/prednisolone is advocated. In the present study we hypothesized that the use of dietary supplementation with essential fatty acids would reduce or eliminate the need for prednisolone treatment. Relatively few dogs were managed without prednisolone for longer periods of time. However, the use of prednisolone in both the active group and the placebo group was low at the end of the study (0.12 mg $kg^{-1}$ $day^{-1}$ and 0.24 mg $kg^{-1}$ $day^{-1}$, respectively, at the close of the trial). Results from a study by Chastain and Graham[40] strongly suggested that replacement doses of short-acting glucocorticoids (doses less than 0.3 mg of prednisone $kg^{-1}$ $day^{-1}$) might be given daily for at least several weeks without causing extra-adrenal adverse effects. None of the owners reported unacceptable side effects of the treatment in the present study. However, some owners reported increased appetite and thirst, which faded when the dose of prednisolone was lowered. The occurrence of skin infections in two dogs in the placebo group could be due to prednisolone treatment[20] or because of the predisposition of most dogs with atopic dermatitis to contract secondary skin infections.[22,41]

In the present study, the use of concurrent therapy was limited to a shampoo and an ear-cleanser. To avoid any confounding effect on the outcome of the study, their uses were recorded daily in a diary. Recurrent pyoderma and otitis externa are common in dogs with atopic dermatitis.[22,41] Indeed, the majority of the dogs enrolled in the present study had suffered from pyoderma (83.3%) and/or otitis externa (46.7%) once or several times previously. The use of shampoo and ear-cleanser was a measure to prevent recurrence of skin infections and ear infections in both groups of dogs, and hence keep the withdrawal rate as low as possible. However, it should be noted that the regular use of both shampoo and ear-cleanser *per se* could contribute to a decrease in the use of steroids in both groups. Adjunctive antimicrobial shampoos and ear-cleansers are indicated in most dogs with atopic dermatitis to reduce the degree of pruritus resulting from microbial infection of the skin.

In summary, the results of the present study indicate that essential fatty acid supplementation has a steroid sparing effect in the treatment of canine atopic dermatitis and, furthermore, that it may take 12 weeks or more before the full therapeutic effect develops. As no permanent curative therapy is available for canine atopic dermatitis at present, these findings support the multifaceted approach to the canine patient with atopic dermatitis. Our results support concurrent use of fatty acid supplementation as well as regular use of shampoo and ear-cleanser in dogs with atopic dermatitis receiving long-term glucocorticoid treatment. Further studies are needed to investigate the optimal dose for a steroid sparing effect of essential fatty acid supplementation in canine atopic dermatitis.

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## REFERENCES

1. Olivry T, Marsella R, Hillier A. The ACVD task force on canine atopic dermatitis (XXIII): are essential fatty acids effective? Veterinary Immunology and Immunopathology 2001; 81: 347–62.
2. Hill PB, Olivry T. The ACVD task force on canine atopic dermatitis (V): biology and role of inflammatory cells in

cutaneous allergic reactions. Veterinary Immunology and Immunopathology 2001; 81: 187–98.
3. Sinke JD, Rutten VPMG, Willemse T. Immune dysregulation in atopic dermatitis. Veterinary Immunology and Immunopathology 2002; 87: 351–6.
4. Miller CC, McCreedy CA, Jones AD et al. Oxidative metabolism of dihomogammalinolenic acid by guinea pig epidermis: evidence of generation of anti-inflammatory products. Prostaglandins 1988; 35: 917–38.
5. Vaughn DM, Reinhart GA, Swaim SF et al. Evaluation of effects of dietary n-6 to n-3 fatty acid ratios on leukotriene B synthesis in dog skin and neutrophils. Veterinary Dermatology 1994; 5: 163–73.
6. Ziboh VA, Miller CC, Cho Y. Metabolism of polyunsaturated fatty acids by skin epidermal enzymes: generation of antiinflammatory and antiproliferative metabolites. American Journal of Clinical Nutrition 2000; 71: 361S–6S.
7. Endres S, Ghorbani R, Kelley VE et al. The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. New England Journal of Medicine 1989; 320: 265–71.
8. Søyland E, Nenseter MS, Braathen L et al. Very long chain n-3 and n-6 polyunsaturated fatty acids inhibit proliferation of human T-lymphocytes *in vitro*. European Journal of Clinical Investigation 1993; 23: 112–21.
9. Carrick JB, Schnellmann RG, Moore JN. Dietary source of Ω-3 fatty acids affects endotoxin-induced peritoneal macrophage tumor necrosis factor and eicosanoid synthesis. Shock 1994; 2: 421–6.
10. Marsh KA, Ruedisueli FL, Coe SL et al. Effects of zinc and linoleic acid supplementation on the skin and coat quality of dogs receiving a complete and balanced diet. Veterinary Dermatology 2000; 11: 277–84.
11. Scott DW, Miller WH, Reinhart GA et al. Effect of an omega-3/omega-6 fatty acid-containing commercial lamb and rice diet on pruritus in atopic dogs: Results of a single-blinded study. Canadian Journal of Veterinary Research 1997; 61: 145–53.
12. Watson TGD. Diet and skin disease in dogs and cats. Journal of Nutrition 1998; 128: 2783S–9S.
13. Scott DW, Miller WH, Griffin CE. Muller and Kirk's Small Animal Dermatology, 6th edn. Philadelphia: W.B. Saunders Co., 2001: 543–666.
14. Paradis M, Lemay S, Scott DW. The efficacy of clemastine (Tavist), a fatty acid-containing product (Derm Caps), and the combination of both products in the management of canine pruritus. Veterinary Dermatology 1991; 2: 17–20.
15. Bond R, Lloyd DH. Combined treatment with concentrated essential fatty acids and prednisolone in the management of canine atopy. Veterinary Record 1994; 134: 30–2.
16. Paterson S. Additive benefits of EFAs in dogs with atopic dermatitis after partial response to antihistamine therapy. Journal of Small Animal Practice 1995; 36: 389–94.
17. Barnes PJ. Anti-inflammatory actions of glucocorticoids: molecular mechanisms. Clinical Science 1998; 94: 557–72.
18. Behrend EN, Kemppainen RJ. Glucocorticoid therapy pharmacology, indications, and complications. Veterinary Clinics of North America: Small Animal Practice 1997; 27: 187–213.
19. Reedy LM, Miller WH, Willemse T. Allergic Skin Diseases of Dogs and Cats, 2nd edn. London: W.B. Saunders Co., 1997: 150–72.
20. Scott DW, Miller WH, Griffin CE. Muller and Krik's Small Animal Dermatology, 6th edn. Philadelphia: W.B. Saunders Co., 2001: 207–73.
21. Pocock SJ. Clinical Trials. A Practical Approach. Chichester: John Wiley & Sons, 1983: 66–89.
22. Willemse T. Atopic skin disease: a review and a reconsideration of diagnostic criteria. Journal of Small Animal Practice 1986; 27: 771–8.
23. Klayman E, Schillhorn van Veen TW. Vacuum cleaner method for diagnosis of ectoparasitism. Modern Veterinary Practice 1981; 62: 767–71.
24. Bornstein S, Thebo P, Zakrisson G. Evaluation of an enzyme-linked immunosorbent assay (ELISA) for the serological diagnosis of canine sarcoptic mange. Veterinary Dermatology 1996; 7: 21–8.
25. Curtis CF. Evaluation of a commercially available enzyme-linked immunosorbent assay for the diagnosis of canine sarcoptic mange. Veterinary Record 2001; 148: 238–9.
26. Larsen S, Aabakken L, Lillevold PE et al. Assessing soft data in clinical trials. Pharmaceutical Medicine 1991; 5: 29–36.
27. Altman DG. Practical Statistics for Medical Research. London: Chapman & Hall, 1991: 179–228.
28. Hollander M, Wolfe DA. Nonparametric Statistical Methods. New York: John Wiley & Sons, 1973: 67–82.
29. Altman DG. Practical Statistics for Medical Research. London: Chapman & Hall, 1991: 396–439.
30. Kleinbaum DG, Kupper LL, Muller KE et al. Applied Regression Analysis and Other Multivariable Methods, 3rd edn. Pacific Grove, CA: Duxbury Press, 1998: 589–638.
31. Kleinbaum DG, Kupper LL, Muller KE et al. Applied Regression Analysis and Other Multivariable Methods, 3rd edn. Pacific Grove, CA: Duxbury Press, 1998: 484–515.
32. Hollander M, Wolfe DA. Nonparametric Statistical Methods. New York: John Wiley & Sons, 1973: 26–66.
33. Altman DG. Practical Statistics for Medical Research. London: Chapman & Hall, 1991: 229–76.
34. Campbell KL, Czarnecki-Maulden GL, Schaeffer DJ. Effects of animal and soy fats and proteins in the diet on fatty acid concentrations in the serum and skin of dogs. American Journal of Veterinary Research 1995; 56: 1465–9.
35. Harvey RG. A blinded, placebo-controlled study of the efficacy of borage seed oil and fish oil in the management of canine atopy. Veterinary Record 1999; 144: 405–7.
36. Logas D, Kunkle GA. Double-blinded crossover study with marine oil supplementation containing high-dose eicosapentaenoic acid for the treatment of canine pruritic skin disease. Veterinary Dermatology 1994; 5: 99–104.
37. Kämmerer W. Essentielle fettsäuren zur therapie der atopischen dermatitis. Pharmazeutische Zeitung 1994; 139: 9–15.
38. Lawson LD, Huges BG. Triacylglycerol structure of plant and fungal oils containing γ-linolenic acid. Lipids 1988; 23: 313–7.
39. Gunstone FD. Gamma linolenic acid-occurrence and physical and chemical properties. Progress in Lipid Research 1992; 31: 145–61.
40. Chastain CB, Graham CL. Adrenocortical suppression in dogs on daily and alternate-day prednisone administration. American Journal of Veterinary Research 1979; 40: 936–41.
41. Reedy LM, Miller WH, Willemse T. Allergic Skin Diseases of Dogs and Cats, 2nd edn. London: W.B. Saunders Co., 1997: 25–49.

**Résumé** 60 chiens présentant une dermatite atopique ont été enrolés dans une étude randomisée, en double aveugle, contre placebo, pour évaluer l'effet d'épargne corticoïdes d'une supplémentation en acides gras essentiels. Les chiens ont reçu soit une combinaison d'huile de bourrache et d'huile de poisson, soit un placebo, en plus de comprimés de prednisolone. Tous les chiens recevaient une alimentation de base standardisée. Les propriétaires déterminaient le prurit en utilisant une échelle visuelle de 10 centimètres, et la dose de prednisolone était adaptée en fonction du prurit, selon les instructions de la notice du produit. Les doses de corticoïdes et l'utilisation de tout traitement associé (shampooing et/ou nettoyant auriculaire) étaient notés par les propriétaires tous les jours. Les investigateurs gradaient les lésions cliniques à J0, J42 et J84. L'utilisation de prednisolone était plus faible dans le groupe traité que dans le groupe placebo, mais la différence n'était pas significative ($P = 0.32$). La période de traitement a été divisée en 43, 50, 57, 64, 71 et 78 jours. A J64, la différence entre le groupe traité et le groupe placebo était significative ($P = 0.04$), avec une augmentation de l'effet vers la fin de l'essai. Une diminution statistiquement significative des scores de prurit et clinique entre J0 et J84 était notée dans les deux groupes ($P < 0.0001$). A la fin de l'étude, le prurit et les lésions étaient plus faibles dans le groupe traité. Nos résultats indiquent un effet d'épargne corticoïde d'une supplémentation en acides gras essentiels chez le chien souffrant de dermatite atopique, mais il existe une période de latence avant d'obtenir cet effet.

**Resumen** Se llevó a cabo un estudio multicéntrico doble ciego, al azar, con control placebo, durante 12 semanas, en 60 perros con dermatitis atópica para evaluar el cómo la suplementación con ácidos grasos esenciales permite limitar el uso de los esteroides. Se asignó, al azar, una combinación de aceite de semilla de borraja y aceite de pescado, o un placebo, además de comprimidos de prednisolona. Todos los perros recibieron una dieta básica estandarizada. Los propietarios de los perros registraron diariamente el prurito utilizando una Escala Análoga Visual de 10 cm y la dosis de prednisolona fue establecida en base a la puntuación del prurito, de acuerdo con instrucciones escritas. El propietario registró diariamente la dosis de prednisolona y el uso de cualquier tratamiento concomitante (champú y/o limpiadores óticos). Los investigadores gradaron las lesiones cutáneas los días 0, 42 y 84. El uso de prednisolona durante el período de prueba fue menor en el grupo activo, pero la diferencia no fue estadísticamente significativa ($P = 0.32$). El período de prueba fue posteriormente fragmentado en 43, 50, 57, 64, 71 y 78 días. El día 64, la diferencia entre el grupo activo y el grupo placebo alcanzó significación estadística ($P = 0.04$) con una diferencia creciente hacia finales del estudio. En ambos grupos se produjo una reducción estadísticamente significativa del grado de prurito y de las puntuaciones clínicas totales entre el día 0 y el 84 ($P < 0.0001$). Al final del estudio, tanto la puntuación del prurito como la puntuación clínica total fueron inferiores en el grupo activo. Nuestros hallazgos indican que la suplementación con ácidos grasos esenciales en la dermatitis atópica canina permite una reducción en el uso de esteroides y que, además, existe un lapso de tiempo antes de lograr el efecto.

**Zusammenfassung** Um den steroid-sparenden Effekt einer Supplementierung mit essentiellen Fettsäuren zu beurteilen, wurde bei 60 Hunden mit atopischer Dermatitis eine randomisierte, doppelt verblindete, placebokontrollierte, multizentrische klinische Studie mit einer Dauer von 12 Wochen durchgeführt. Die Hunde wurden nach dem Zufallsprinzip eingeteilt, entweder eine Kombination von Borretschsamen- und Fischöl oder einen Placebo zusätzlich zu Prednisolon Tabletten zu bekommen. Alle Hunde erhielten eine standardisierte Grunddiät. Die Besitzer der Hunde beurteilten täglich den Juckreiz anhand einer 10 cm langen visuellen Analog-Skala und die Dosierung von Prednisolon wurde basierend auf der Juckreizauswertung entsprechend schriftlichen Anweisungen eingesetzt. Die Dosierung von Prednisolon und der Einsatz von jeglicher gleichzeitiger Behandlung (Shampoo und/oder Ohrreiniger) wurden durch den Besitzer täglich protokolliert. Die Untersucher beurteilten die Hautveränderungen an den Tagen 0, 42 und 84. Der Einsatz von Prednisolon während der Testphase war in der aktiven Gruppe geringer, aber der Unterschied war nicht statistisch signifikant ($P = 0.32$). Die Testphase wurde folglich unterteilt in 43, 50, 57, 64, 71 und 78 Tage. Am Tag 64 erreichte der Unterschied zwischen der aktiven und der Placebo-Gruppe statistische Signifikanz ($P = 0.04$) mit zunehmender Differenz gegen Ende der Studie. Eine statistisch signifikante Reduktion der Juckreiz-Testergebnisses und des klinischen Testergebnisses von Tag 0 zu Tag 84 waren in beiden Gruppen vorhanden ($P < 0.0001$). Am Ende der Studie waren sowohl das Juckreiz-Testergebnis als auch das klinische Gesamt- Testergebnis in der aktiven Gruppe niedriger. Unsere Ergebnisse weisen auf einen steroid-sparenden Effekt einer Supplementierung mit essentiellen Fettsäuren bei der caninen atopischen Dermatitis und darüber hinaus darauf hin, dass es eine Zeitverzögerung gibt, bevor dieser Effekt erzielt wird.