Dietary Beta-1,3/1,6-Glucans Reduce Clinical Signs of Canine Atopy

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Abstract: Problem statement: There was evidence that beta-1,3/1,6-glucans modulate inflammatory activity. In an open, non-controlled trial, purified beta-1,3/1,6-glucans were found to improve the clinical signs of dogs with undefined chronic skin disorders. Given the design of that study, further work was required on the efficacy of beta-1,3/1,6-glucans in the treatment of canine atopy. **Approach:** The influence of a purified preparation of beta-1,3/1,6-glucans (MacroGard®) on canine atopy was assessed in a double-blind, placebo-controlled trial. Privately owned dogs were used and the clinical signs of atopic dermatitis were evaluated by the owners. For a period of 8 weeks, the dogs daily received a complete dry food without (n = 16) or with 800 ppm beta-1,3/1,6-glucans (n = 15). During the trial, all dogs were treated three times with the use of a flea remedy in order to exclude any influence of flea-bite allergy. To assess the severity of atopic dermatitis, the clinical signs scored were itching, redness, scaling, thickening and stripping of skin. **Results:** For all five clinical signs, the group-mean improvement, expressed as change of severity score over time, was greater in the test group than in the controls. Within each group, the changes for the five clinical signs were added up to arrive at an overall index of improvement of atopic dermatitis. The extra improvement caused by the ingestion of beta-1,3/1,6-glucans was 63%. The difference between the pooled group-mean changes of the scores for the control and test dogs was statistically significant (P<0.001). To correct for the differences in baseline scores, dose equivalents required for the observed change between baseline and final scores were calculated. It was found that the dose equivalents for the combined placebo and treatment effects seen in the test group were much greater than those required for the placebo effect in the control group. **Conclusion:** Beta-1,3/1,6-glucans can be considered safe and it is put forward that a dose of 800 ppm in a dry food is beneficial for dogs with atopic dermatitis.

Key words: Atopic dermatitis, canine atopy, skin disorders, dietary treatment, functional ingredient, betaglucans, double-blind, placebo-controlled trial

INTRODUCTION

Skin disorders in dogs are an important part of small animal practice. After flea-bite hypersensitivity, atopy is the most commonly diagnosed allergic dermatitis in dogs. The clinical symptoms are pruritis, erythema, oedema and self-trauma. Atopy is generally caused by inhaled allergens that cannot be avoided. Clinical management of canine atopic dermatitis aims at suppression of the chronic inflammatory reactions in the skin. Drug treatment often involves the administration of glucocorticoids or antihistamines. For optimum management of canine atopy, further research remains necessary, including that on potential functional ingredients of the diet.

Berge (Nordberg Veterinary Clinic, Oslo) found that the intake of purified beta-1,3/1,6-glucans improved the clinical signs of dogs with undefined chronic skin disorders (Unpublished data, 2003). The study was open and lacked a placebo group so that the observation should be confirmed by a double-blind, placebo-controlled trial. The outcome of the study of Berge and the reported beneficial effects of beta-1,3/1,6-glucans on ova-albumin-induced allergic

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reactions in mice (Kimura *et al.*, 2007) and allergic rhinitis in human patients (Kirmaz *et al.*, 2005), may indicate that atopy in dogs may be managed by feeding a diet containing the functional ingredient.

This study addresses the efficacy of a purified preparation of beta-1,3/1,6-glucans (MacroGard®) in the treatment of canine atopy. In a double-blind, placebo-controlled trial, privately owned dogs were used and the clinical signs were evaluated by the owners. For a period of 8 weeks, the test dogs daily received a complete dry food without or with 800 ppm beta-1,3/1,6-glucans.

MATERIALS AND METHODS

Animals: Dogs with signs of atopic dermatitis were recruited through the websites of breed associations and dog fancier clubs and newsletters of veterinarians. The (potential) participants were informed about the purpose and design of the trial and had to sign a statement on informed consent. Fourty dogs were subjected to either the placebo or test group. Nine dogs did not finish the trial so that the data for 31 dogs (16 control and 15 test dogs) were available for analysis. The reasons for dropping out were as follows (control/test): poor acceptance of experimental food (2/1), unacceptable increase in severity of clinical signs (2/2) or non-defined sickness (1/1). Table 1 shows the characteristics of the dogs as based on the intake questionnaire that was completed by their owners.

There was a wide variety of dog breeds; there were West Highland white terriers (n = 3), Staffordshire Bullterriers (n = 3), German Shepherds (n = 4), Heidewachtels (Small Munsterlander Pointers) (n = 2), cross breeds (n = 4) and others (n = 15). The anti-inflammatory drugs used were as follows: prednisone (n = 3), Hydrocortiderm (n = 2) and Prednocutin Forte (n = 1). In one dog, Efavet 660 was used as supplement. Other drugs administered were Artuvetrin (n = 1), Atopica (n = 2) and antibiotics in the form of Surolan (n = 1), Aurizon (n = 2) and Cerferal (n = 2). The owners were instructed to continue as usual with the administration of drugs and/or supplement during the course of the trial. If no drug or supplement was used, this was maintained throughout the experiment.

Table 1: General characteristics of the dogs

Characteristic	Placebo group (n = 16)	Beta-1,3/1, 6-glucans (n = 15)
Atopy diagnosed by veterinarian, yes/no	10/6	13/2
Mean age, years (range)	5.0 (2 -8)	4.9 (1-10)
Mean body weight, kg (range)	23.9 (8-60)	33.5 (9-85)
Gender, female/male/castrated	7/7/2	2/4/9
Use of drugs, yes/no	5/11	5/10

Experimental design: Recruitment of the dogs, maintaining contact with the dog owners, supplying of food, data collection and general coordination of the trial was done by FvA, EAV, JM, LDJ and MS who were blinded to treatment modality. In the intake questionnaire, the owners indicated the severity of atopy as described below for the trial questionnaire. Main criteria for the entering of dogs into the trial were diagnosis of atopy by a veterinarian or reasonable ground for exclusion of flea-bite and food allergy. The eligible dogs were allocated to either the placebo or treatment group by DHJS, who kept the treatment code closed until statistical analysis of the data. Allocation was done so that the distribution of severity of atopy, as based on the intake questionnaire, would be similar among the two groups.

All dogs were fed on the same complete dry food (Carocroc Chicken and Rice 23/12, Vobra Special Petfoods BV, Veghel, The Netherlands), which was supplied in 15- or 20-kg, blank packaging. The test food contained 800 ppm of a beta-1,3/1,6-glucans preparation (MarcoGard®, Biorigin) which was added prior to extrusion. The foods were sent by courier to the dog owners. The trial lasted 10 weeks. The first week served as a baseline. During the second week the dogs were gradually transferred from their habitual diet to the food supplied. During the third week only the food supplied was fed, which was continued for another 8 weeks.

To exclude any influence of flea-bite allergy on the clinical signs, all dogs were treated with Advantix (Bayer). The flea remedy was sent to the dog owners together with the food. The original package insert was used as instruction. The accesory pipette of appropriate size had to be filled and emptied dropwise on the skin nearby the shoulderblades. The dose applied was related to the body weight of the dog. The flea remedy was applied on day 0 (start) and during weeks 5 and 9.

Trial questionnaire: The trial questionnaire was in the form of a booklet, which also provided instructions, including a completed example of a question in the format used. The severity of the signs of atopic dermatitis was scored by the owners by marking with a cross a 10-cm, horizontal line. The line was without any unit, but functioned as a scale in combination with the description. The signs to be scored by owners were: pruritis (itchiness), redness of skin, scaling of skin, thickening of skin, stripping of skin. Coat quality, body condition and feces consistency were also scored. The signs were scored on day 0 (start) and weekly afterwards.

To aid in scoring the signs, the following descriptions were given. Pruritis (itchiness). "Signs of itchiness are scratching, biting and licking on skin areas

that itch. It is also possible that your dog rubs against furniture or walls. How intensive are the signs of itching in your dog? Does your dog only scratch briefly or is it a long-lasting activity that can hardly be stopped?" The scale ran from "Often and violent" (extreme left) to "Not" (extreme right). Redness of skin. "Red spots on the skin of your dog can be caused by excessive scratching, but may also relate to inflammatory reactions in the skin. Your dog may have many red spots on its skin, these spots having a light red color to flaming red." The scale ran from "Many and severe" (extreme left) to "None" (extreme right). Scaling of skin. "Shedding scales by the skin may be caused by inflammation. Is the skin of your dog encrusted with scales or not at all? Is there little scaling or would you qualify it as severe" The scale ran from "Many and severe" (extreme left) to "None" (extreme right). Thickening of skin. "Through chronic inflammation and frequent scratching the skin can thicken. It is recognized by the skin being like leather and thick to the touch. Do you observe such skin changes on your dog? Is thickening of the skin minor or would you qualify it as severe". The scale ran from "Many and severe" (extreme left) to "None" (extreme right). Stripping of skin. "By scratching, biting and rubbing the skin, the top layer may be removed. This may lead to bleeding and crust formation. Do you observe this on your dog and if you do, is it on many areas of the body? Do you consider the stripping of the skin as minor or as severe?" The scale ran from "Many and severe" (extreme left) to "None" (extreme right). Coat quality. "How is the coat of your dog with regard to hairs linking up together, discoloration and shining? Sound implies that the hair coat is connected nicely, shines and is not discolored. Affected implies that the coat has broken hairs or bald areas and/or has discolored parts and/or is dull." The scale ran from "Affected" (extreme left) to "Sound" (extreme right). Body condition. "What is the body condition of your dog? In an obese dog, the ribs are not visible and are covered by a layer of fat tissue. In addition, the belly is not slimmer than the chest and thus shows no waist. A dog with normal body condition has ribs that are just visible and shows a waist. A skinny dog has pronounced ribs". The scale ran from "Very skinny" (extreme left) to "Very fat" (extreme right). Feces consistency. "As a rule, your dog should produce normal feces once or twice a day without any problems. If your dog has to strain for defecation, it may point at hard feces. Diarrhea may also occur: thin, watery feces." The scale ran from "Hard and dry" (extreme left) to "Thin and watery" (extreme right).

Data analysis: The marked horizontal lines in the booklets were transferred into values by using the distance, expressed in mm, of the crosses from the left side (= 0 mm). To calculate the baselines, the values for day 0 and week 1 were averaged per variable per dog. To calculate the final values, those for weeks 8, 9 and 10 were averaged. For each dog and each variable, the change over time was calculated. The data are presented without units. To identify treatment effects, the changes over time for the placebo and test group were subjected to the Student's t test or Mann-Whitney U test with two-tailed $P < 0.05$ as criterion of statistical significance.

The data were not only evaluated in the form of distances on the horizontal lines in the booklet, but were also converted to so-called dose equivalents with the use of a standard sigmoid dose-response function. The baseline scores for the clinical signs were generally higher for the test animals, indicating less severity of atopic dermatitis. This causes bias when comparing the changes over time within the two groups. The bias would be associated with the phenomenon of regression to the mean and baseline-dependent sensitivity to improvement. Animals with less severe signs of disease generally are less sensitive to treatment. Furthermore, the control dogs showed a placebo-effect. Thus, when comparing the control and test group, the effect of beta-1,3/1,6-glucans on atopy will be underestimated. To solve the problem of bias, the baseline and final values for each group were expressed as dose equivalent and then the dose equivalent required to induce the change was calculated. For the control group the dose equivalent was a measure of the placebo effect and for the test group it was the sum of the placebo and treatment effect.

Dose-response curves for anti-inflammatory drugs are generally sigmoid in shape, with a distinct threshold, linear slope in the midpart and maximum response (Woolcock *et al.*, 1984). This type of dose-response curve has as characteristic that at higher baseline y values more additional dose is required for a certain increase than for lower baseline y values. Here, the y values are the scores for the severity of atopic dermatitis. For the dose-response curve a standard equation was used. The y values range between 0 and 100 as do the scores for the clinical signs. It was assumed that at the x value (dose) of 100 a y value (response) of 50 is attained. The equation is then as follows: $y = 100/(1+10^2\text{-log}x)$. It is thus possible to calculate a dose equivalent for any y value with the following equation: $\log x = 2 - \log (100/y-1)$. For the group-mean baseline and final scores of each clinical sign the dose equivalents were calculated. The difference between the baseline and final dose equivalents is an index of either the placebo effect in

the control group or the combined placebo and treatment effect in the test group. This index corrects for the decreasing sensitivity to beta-1,3/1,6-glucans with increasing baseline values.

The pooled group-mean changes for the scores and dose equivalents for the five symptoms of atopic dermatitis were compared between control and test group with the use of the paired Student's t test and considering two-tailed P<0.05 as significant. In addition, the group-mean changes over time for the five clinical signs were added up to arrive at an overall index of improvement of atopic dermatitis. The overall index was calculated for both the control and test dogs and for both the scores and the dose equivalents.

RESULTS

Except for the frequency of atopy diagnosis and gender distribution, the general characteristics of the placebo and test group were similar (Table 1). The intake values for the clinical signs of atopic dermatitis (data not shown) changed erratically over time towards the beginning of the trial. As a result, the baseline values for the clinical signs were not comparable for the test and placebo group (Table 2). The test animals had higher group mean scores for itching, redness, thickening and stripping of skin, but none of the differences was statistically significant.

When compared to the baseline values, all five clinical signs showed a group-mean increase in score for both the control and test group (Table 2). The increases were systematically greater in the test group. The increase in score during the course of the trial reached statistical significance for itching (p = 0.029) and redness (p = 0.038) in the control group. In the test group, the increases in itching (p = 0.021) and redness (p = 0.012) scores were statistically significant, while the increases in the scores for scaling (p = 0.066), thickening (p = 0.100) and stripping (p = 0.080) approached significance. When the changes over time of the two groups were compared, there were no statistically significant differences (Table 2).

Figure 1 also illustrates that the group-mean improvement for all five clinical signs was greater in the dogs fed the diet with beta-1,3/1,6-glucans than in the control dogs. The changes over time for the five clinical signs were added up for each group to arrive at an overall index of improvement of atopic dermatitis.

The improvement index was 49.7 for the placebo group and 80.8 for the test group (Fig. 1). The extra improvement caused by the ingestion of beta-1,3/1,6-glucans was 63%. The difference between the pooled group-mean changes of the scores for the control and test dogs was highly statistically significant (P<0.001).

Table 2: Group-mean baseline values and changes over time in the signs of atopic dermatitis (improvement is indicated by a + sign)

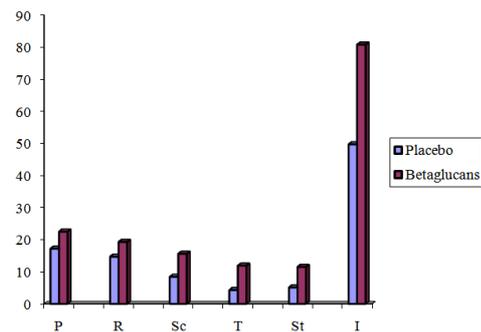| Variable	Placebo (n = 16)		Beta-1,3/1,6-glucans (n = 15)		P value for difference in change
	Baseline	Change	Baseline	Change	
Pruritis	32.2	+17.2	45.8	+22.5	0.640
Redness	46.1	+14.7	60.8	+19.3	0.623
Scaling	63.3	+8.4	56.3	+15.6	0.459
Thickening	64.2	+4.3	73.1	+11.9	0.345
Stripping	61.3	+5.1	78.0	+11.5	0.472

Fig. 1: Effect of beta-1,3/1,6-glucans on clinical signs of atopic dermatitis in control and test dogs. The bars represent the magnitude of improvement of clinical signs. The improvement was calculated as the difference between final and initial scores on a 0 (severe signs) to 100 (no signs) scale. P = pruritis (itching); R = redness of skin; Sc = scaling of skin; T = thickening of skin; St = stripping of skin; I (index) = improvement of the five clinical signs combined. The group-mean changes were pooled for the five clinical signs and the difference between control and test dogs was found to be highly statistically significant (p<0.001)

To correct for the differences in baseline scores between the control and test group, the dose equivalents required for the observed change between baseline and final scores were calculated. The dose equivalents for the changes seen in the test group were much greater than those in the control group (Fig. 2).

The sum of the dose equivalents for the changes over time for the five clinical signs was 276 for the control group and 1489 for the group fed the diet containing beta-1,3/1,6-glucans (Fig. 2). Thus, the summed dose equivalent corresponding with the changes in the test group was more than five-fold higher than that for the control group. The difference between the pooled group-mean dose equivalents required for the changes in clinical signs in test versus control dogs approached statistical significance (p = 0.090).
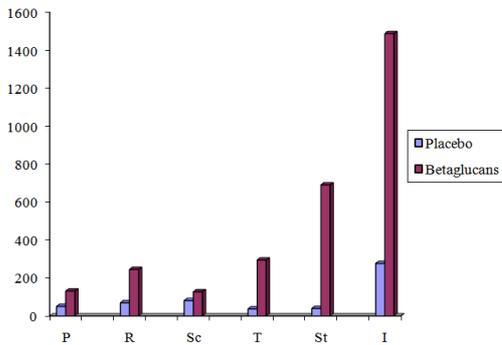
Fig. 2: Effect of beta-1,3/1,6-glucans on clinical signs of atopic dermatitis in control and test dogs. The bars represent the magnitude of improvement of clinical signs expressed as dose equivalent required for the effect. The improvement was calculated as the difference between the dose equivalents of the final and initial scores. P = pruritis (itching); R = redness of skin; Sc = scaling of skin; T = thickening of skin; St = stripping of skin; I (index) = improvement of the five clinical signs combined. The group-mean changes were pooled for the five clinical signs and the difference between control and test dogs tended to be statistically significant (p = 0.090)

Group-mean baseline and final scores for coat quality were 60.6 and 71.6 in the control dogs and 46.7 and 64.6 in the test animals. The P values for the changes over time were 0.123 and 0.016 in the control and test dogs. For body condition, the mean baseline and final scores were 48.3 and 45.4 for the controls and 45.2 and 44.8 for the test dogs. The changes over time were not statistically significant. Mean baseline and final scores for feces consistency were 47.0 and 46.3 for the control dogs and 40.4 and 44.8 for the test dogs; the changes in feces scores were not significant. When the changes over time of the two groups were compared, there were no statistically significant differences for coat quality, body condition and feces consistency.

DISCUSSION

For evidence-based application of beta-1,3/1,6-glucans in the treatment of canine atopic dermatitis there should be proven efficacy in double-blind, placebo-controlled clinical trials. The dogs fed the diet containing beta-1,3/1,6-glucans showed greater numerical improvement as to the scores of itching, redness, scaling, thickening and stripping of skin, but the differences between the control and test treatment did not reach statistical significance. The systematic,

positive effects of beta-1,3/1,6-glucans on the signs of atopic dermatitis resulted in an improvement of the atopy index by 63%. Thus, it appears that the lack of statistical significance of the beneficial effects of beta-1,3/1,6-glucans is caused by insufficient statistical power in combination with placebo effects rather than by an inefficacy of the functional ingredient. Through the double-blind nature of the trial any observer bias is excluded, but placebo effects are not ruled out. The placebo effects were taken into account by comparing the pooled group-mean changes of the five clinical scores for the control and test dogs. The positive effect of consumption of beta-1,3/1,6-glucans was found to be highly statistically significant.

Additional proof for the ameliorating influence of dietary beta-1,3/1,6-glucans on canine atopy was obtained after expressing the placebo and treatment effects as dose equivalents. At the beginning of the trial, the clinical signs of atopic dermatitis were generally less severe for the test animals. This would imply that the test versus control animals would be less sensitive to the positive effect of beta-1,3/1,6-glucans, leading to a biased comparison of the two groups. As described above, the dose equivalent required to induce the group-mean change from baseline to final values was calculated for each clinical symptom. The dose equivalent is a measure of the placebo effect in the control group and a measure of the sum of the placebo and treatment effect in the test group. The effect of administration of beta-1,3/1,6-glucans was evaluated by subjecting the pooled group-mean dose equivalents to statistical analysis. The difference between control and test dogs tended to be significant, substantiating the beneficial effect of dietary beta-1,3/1,6-glucans on canine atopic dermatitis.

In this study, 13 (87%) out of the 15 test dogs showed a decrease in the severity of atopic dermatitis as based on the change in the average score of the five clinical signs. In the control group, 11 (69%) out the 16 animals showed improvement. Thus, despite of the lower severity of baseline atopic dermatitis, there was a greater fraction in the test group showing improvement during the course of the trial. It also appears that some dogs may not have responded to the treatment with beta-1,3/1,6-glucans. The phenomenon of responders and non-responders is well-known in the management of canine atopic dermatitis (Badakky-Taugbol *et al.*, 2005). In the test group, the baseline average score of the five clinical signs was negatively correlated with the change (r = -0.80; n = 15). In other words, dogs with low initial scores and thus severe signs of atopic dermatitis, may be more sensitive to treatment with beta-1,3/1,6-glucans. This may be explained by both

true hyperresponsiveness and apparent hyperresponsiveness due to intra-individual variation of severity of atopic dermatitis.

Coat quality is not a specific indicator of canine atopic dermatitis, but it is relevant. By scratching, biting and rubbing the skin in response to itching, the hair condition may be affected and alopecia may develop. Many owners are anxious about the quality and appearance of their dog's coat. The consumption of beta-1,3/1,6-glucans significantly improved coat quality when baseline and final scores were compared. In the control dogs there was no significant change over time in coat quality. The initial score for hair quality was lower in the test dogs than in the controls while the dose equivalent required to induce the observed improvement was not higher. In the control dogs, the dose equivalent required for the placebo effect was 98, whereas it was 95 for mediating the improvement seen in the test animals. This attenuates the observation that dietary beta-1,3/1,6-glucans had positive influence on coat quality.

For the observed positive effect of beta-1,3/1,6-glucans in the treatment of canine atopic dermatitis there is a scientific basis. Research in pigs (Li *et al.*, 2006) has demonstrated that the feeding of beta-1,3/1,6-glucans reduced the plasma concentrations of the pro-inflammatory cytokines, IL-6 and TNFα and raised the concentration of the anti-inflammatory cytokine, IL-10. Thus, the intake of beta-1,3/1,6-glucans may have anti-inflammatory activity. Current dietary treatment of canine atopy consists of the feeding of fish oil and/or borage oil. Double blind, placebo-controlled experiments in dogs with atopic dermatitis have shown that supplements of both borage oil, rich in Gamma-Linolenic Acid (GLA) and fish oil, rich in Eicosapentaeonic Acid (EPA), lower the severity of clinical signs (Logas and Kunkle, 1994; Harvey, 1999). The beneficial effect of borage oil and fish oil can be explained by the formation of anti-inflammatory eicosanoids from di-homo-GLA and EPA. It is concluded that fish oil, borage oil and beta-1,3/1,6-glucans have a documented, positive effect on atopic dermatitis in dogs. Since the underlying mechanisms of the anti-inflammatory actions of the three ingredients are different, it may be anticipated that the combination acts synergistically.

In canine atopic dermatitis, pruritis-induced self-trauma of the skin is common. The anti-inflammatory action of beta-1,3/1,6-glucans may explain the observed reduction of itching followed by amelioration of the other clinical signs. It could be speculated that beta-1,3/1,6 glucans also have a direct effect on stripping of the skin and perhaps also on redness, scaling and thickening. Betaglucans have been shown to offer protection against skin damage by detergent challenge or UV-A irradiation in healthy volunteers (Zulli *et al.*, 1998), to be effective in the treatment of partial-thickness burns in children (Delatte *et al.*, 2001) and to improve wound healing in a mouse model of type 2 diabetes (Berdal *et al.*, 2007). It should be noted that the effects of betaglucans were seen after topical application rather than ingestion.

In an earlier, double-blind, placebo-controlled trial we have demonstrated the efficacy of a preparation of beta-1,3/1,6-glucans (MacroGard ®) in the treatment of canine osteoarthritis (Beynen and Legerstee, 2010). The dogs fed the diet containing beta-1,3/1,6-glucans showed greater numerical improvement as to the scores for activity, stiffness, swelling of joint, lameness and pain. When the changes over time for the five clinical signs were added up to arrive at an overall index of improvement of osteoarthritis, the extra improvement caused by the ingestion of beta-1,3/1,6-glucans was found to be 76%. It was suggested (Beynen and Legerstee, 2010) that the positive effect of beta-1,3/1,6-glucans on canine osteoarthritis is caused by inhibition of the degradation of collagen in the cartilage matrix associated with a reduction in inflammation and pain sensation.

CONCLUSION

This study provides suggestive evidence that consumption of beta-1,3/1,6-glucans diminishes the clinical signs in dogs with atopic dermatitis. Beta-1,3/1,6-glucans are safe (Lehne *et al.*, 2006) and heat stable and can be added to dog food prior to extrusion. This study indicates that a dose of 800 ppm in a dry food is beneficial for dogs with atopic dermatitis. The efficacy of beta-1,3/1,6-glucans may be mediated through its anti-inflammatory action.

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