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Application of a black soldier fly larvae (*Hermetia illucens*) based diet in the diagnosis and treatment of food allergy in dogs and cats: pilot study

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Summary

Food allergy is among the more common problems found in dogs and cats. The disease is the cause of severe non-seasonal pruritus in these animals. The diagnosis is based on following a hypoallergenic diet for several weeks (from 2 to 8 weeks). The aim of the study was to assess the efficacy of a novel protein-based diet based on insect (black soldier fly larvae) protein in the treatment of food allergy in dogs and cats. The diet was used in diagnosis and treatment of 10 dogs and 10 cats for 10 weeks with food allergy. The diagnosis was confirmed by a provocation trial using the previously fed food. The Pruritus Visual Analog Scale (PVAS; dogs and cats), Canine Atopic Dermatitis Extent and Severity Index (CADESI 04; dogs) and the Scoring Feline Allergic Dermatitis (SCORFAD; cats) were used for efficacy evaluation. In dogs, a significant decrease was reported in both CADESI 04 (from 18.8 to 6.2, p = 0.037) after six weeks of treatment and PVAS (from 5.7 to 3.4, p = 0.007) after four weeks of treatment. In cats, both the PVAS (from 6.3 to 3.1, p = 0.004) after six weeks and SCORFAD (from 4.6 to 3, p = 0.029) after four weeks also decreased significantly. The evaluated diet was useful in the diagnosis and treatment of food allergy in dogs and cats.

Keywords: food allergy, dogs, cats, insects, black fly larvae

Allergy skin diseases are among the most common and important dermatological problems found in companion animals. Food allergy is considered one of the more common dermatological problems, and its cases are believed to account for at least 5% of all skin diseases and about 25% of allergy diseases in dogs (29). In contrast, for cats, it is estimated that food allergy is less common and accounts for approximately 1% of all diseases in this species, less than 5% of skin diseases, and approximately 10% of allergy skin diseases (29).

Food allergy and food intolerance are collectively referred to as adverse food reactions (AFR). These problems can have both immunological (in case of food allergy) and non-immunological cause (in case of food intolerance). As regards immunological mechanisms, types I, III, and IV hypersensitivity play important roles (24, 39).

The allergens responsible for the development of allergies are diverse. For dogs, these most commonly include beef and dairy proteins, poultry as well as proteins contained in cereals (wheat, maize) (23, 24, 28, 34). In turn, for cats, it was found that beef, chicken, eggs, pork, lamb and rabbit are most commonly responsible for food allergies (10).

Clinical signs of food allergy in dogs appear similar to atopic dermatitis (AD), with severe, usually non-seasonal pruritus being typical. Pruritus affects the head, auricles, axillae, groin and distant parts of extremities. In dogs with food allergies, gastrointestinal symptoms may also be present (in 10-15% of cases) (39). The disease usually develops before six years of age (12, 32, 33).

In cats, the clinical signs of food allergy and feline atopic skin syndrome are also identical. The disease

symptoms in this species include pruritus affecting the head and neck (this is the most common clinical manifestation of the disease), eosinophilic syndrome, miliary dermatitis and extensive alopecia (42). While uncommon, concurrent dermatologic and gastrointestinal signs have been reported in about 10-15% of food-allergic cats (27).

The differentiation between AFR and cAD relies on the administration of an hypoallergenic elimination diet (ED) for at least eight weeks (30). The diagnosis of food allergy is made when, following the diet, there is a reduction in skin lesions by at least 50% or a reduction in pruritus by 50-80% (22, 30, 38). In order to confirm a suspected allergy, a provocation (challenge) test is performed with ingredients of the previous diet. Provocation testing is necessary for the identification of the causative food components (39).

To date, serological methods have not been recommended for diagnosis due to their low sensitivity (2, 3, 13, 43).

The elimination diet can be self-prepared by the animal owner based on products that the animal has not been exposed to. Alternatively, in diagnostics, commercial monoprotein diets or ready-made hypoallergenic diets based on hydrolysed (or partially hydrolysed) protein can also be used (1, 5, 32, 37).

Recently, insect protein-based diets have become increasingly common (7). The protein sources used in feeds and diets of this type include the black soldier fly larvae, housefly larvae, tropical house crickets, mealworm larvae (4, 8, 9, 20). Insect-based diets are characterized by high digestibility (depending on the species of insect used, it ranges from 80 to over 90%) and contain protein that has a high level of essential amino acids (16). In addition, insects contain large amounts of fatty acids and are a good source of micronutrients, such as iron, zinc, copper, magnesium and selenium (19). What is also important is that producing a kilogram of insect-based protein is associated with many times lower greenhouse gas emissions (around 12-13 kg, compared to between 50 and over 300 for poultry and beef) (4). Studies have shown that diets based on this protein are effective in cases of food allergy in dogs (6, 21). Moreover, insect protein is highly palatable and can also have a positive effect on the intestinal microbiome (7, 19).

The aim of the study was to demonstrate the efficacy of the diet, based on insect (black soldier fly, *Hermetia illucens*) protein and applied to dogs and cats with food allergies. To date, there have been no studies on the efficacy of such diets in cats, whereas studies on dogs are very scarce (6, 20).

Material and methods

Animals. For the experiment, 20 dogs with non-seasonal pruritus and symptoms of atopic dermatitis and 19 cats with symptoms of feline atopic skin syndrome (miliary dermatitis, pruritus of the head and neck) were selected. In dogs, the clinical criteria according to Favrot et al. (15) were used in the diagnosis (set 2). Similarly, for cats, the diagnostic criteria according to Favrot et al. (2011) were used, which is a version for cats in which flea allergies are excluded (the cats met at least six criteria) (14). The qualifications were performed on patients of the Dermatology Clinic at the Faculty of Veterinary Medicine, the University of Life Sciences in Lublin. The owners of the animals gave their consent to the participation in the study. The animals participating in the study had not previously been fed diets containing insect proteins.

Before the animals were included in the study, other diseases involving pruritus (parasitic, bacterial and fungal infections) were excluded on the basis of Wood's lamp examination, microscopic examination of the hair, and scraping and cytological examinations. The animals that qualified for the study had undergone regular flea prophylaxis for a period of no less than two months prior to the study and during the experiment. In case of complications in patients, e.g., bacterial infections or seborrhoea, they were treated prior to the study according to the applicable rules. While receiving the diet, the animals were not treated using anti-inflammatory or anti-pruritic drugs.

The animals that exhibited no clinical improvement (a reduction in pruritus and skin lesions by more than 50% over a period of eight weeks (ten dogs and nine cats) were subsequently fed another hypoallergenic diet with no clinical improvement. These animals were diagnosed with atopic dermatitis (dogs) or feline atopic skin syndrome (cats). These animals were excluded from further evaluation of the efficacy of the diet. These individuals were then successfully treated for feline atopic skin syndrome or atopic dermatitis with symptomatic anti-pruritic treatment.

The animals included in the statistical evaluation showed improvement after the diet was applied, confirming the presence of a food allergy. The diagnosis was confirmed by a provocation trial using the previously fed food (return of pruritus symptoms 1 to 10 days after the diet change). Because the animal was fed commercial diets with a varied composition, the specific factor responsible for the development of the allergy was not diagnosed; only the recurrence of pruritus symptoms was confirmed. The group of animals subjected to the evaluation of the efficacy of the novel protein-based diet comprised ten dogs (American bulldog, Labrador, dachshund hybrid n = 2, French bulldog, West Highland White Terrier, American Stafford Terrier, Yorkshire terrier, German pointer), and ten cats (European Shorthair). The dogs participating in the assessment were aged between 20 and 120 months (a median of 42 months) and weighed between 8 and 35 kg (a median of 17.5 kg). This group comprised four males and six females. The cats participating in the diet assessment were aged between 12 and 144 months. This group comprised six females and four males.

Clinical evaluation. The animals, both the dogs and cats, were exclusively fed an insect protein-based diet (Brit Veterinary Diet Ultra-Hypoallergenic) for 10 weeks. Composition of diets is listed in Table 1.

For the dogs, the evaluation of the diet efficacy was carried out based on the clinical indices PVAS (pruritus visual

Tab. 1. Composition of diets Brit Veterinary Diet Ultra-Hypoallergenic for dogs and cats

Composition Diet for dogs		
dehydrated insect (30%), yellow peas, dried apple pulp, coconut oil, pea protein, linseed (4%), calcium carbonate, dried algae (2.5%, *Schizochytrium limacinum*), pea flour, hydrolyzed yeasts (0.5% – a source of inositol and amino acids), yeast extract (source of mannan-oligosaccharides, 0.02%), β-glucans (0.02%), dried sea buckthorn (0.015%), fructo-oligosaccharides (0.013%), Mojave yucca (0.013%), *Lactobacillus helveticus* HA – 122 inactivated (15×10^9 cells/kg)		
Nutritional additives per 1 kg:		
vitamin A	26,000	IU
vitamin D3	1,800	IU
vitamin E	1,000	mg
vitamin C	600	mg
choline chloride	750	mg
biotin	3.5	mg
vitamin B1	2.5	mg
vitamin B2	9.5	mg
niacinamide	32	mg
calcium-D-panthothenate	25	mg
vitamin B6	2.5	mg
folic acid	1.3	mg
vitamin B12	0.12	mg
zinc	150	mg
iron	90	mg
manganese	45	mg
iodine	0.85	mg
copper	20	mg
selenium	0.2	mg
taurine	2,000	mg

Composition of Diet for cats		
dehydrated insect (30%), yellow peas, dried apple pulp, coconut oil, pea protein, linseed (4%), calcium carbonate, dried algae (2.5%, *Schizochytrium limacinum*), pea flour, hydrolyzed yeasts (0.5% – a source of inositol and amino acids), yeast extract (source of mannan-oligosaccharides, 0.02%), β-glucans (0.02%), dried sea buckthorn (0.015%), fructo-oligosaccharides (0.013%), Mojave yucca (0.013%), *Lactobacillus helveticus* HA – 122 inactivated (15×10^9 cells/kg)		
Nutritional additives per 1 kg:		
vitamin A	24,000	IU
vitamin D3	850	IU
vitamin E	1,000	mg
vitamin C	300	mg
choline chloride	2,300	mg
biotin	3	mg
vitamin B1	10	mg
vitamin B2	30	mg
niacinamide	60	mg
calcium-D-panthothenate	50	mg
vitamin B6	35	mg
folic acid	15	mg
vitamin B12	0.15	mg
zinc	110	mg
iron	55	mg
manganese	35	mg
iodine	3	mg
copper	3	mg
selenium	0.2	mg
taurine	2,300	mg
L-carnitine	600	mg
L-methionine	6.000	mg

analogue scale) and CADESI 04 (canine atopic dermatitis extent and severity index). The above clinical indices were evaluated before initiating the treatment (T 0) and five times at two-week intervals (after 2, 4, 6, 8 and 10 weeks).

For the determination of the CADESI, the CADESI 04 system was used (Olivry et al., 2014). The CADESI 04, a validated scoring system designed to assess the severity of AD in dogs, is derived by scoring three lesions (erythema, lichenification and alopecia/excoriation) across 20 body sites commonly affected in AD. Each area is scored for severity on a scale between 0 and 3 (0 – none; 1 – mild; 2 – moderate; 3 – severe) with a maximum score of 180. The severity of pruritus was evaluated according to the numerical PVAS scale (0-10) (35). The PVAS scores were evaluated in centimeters from 0 to 10 and classified as follows: normal animal: less than 2 cm; mild pruritus: 2-4 cm; moderate pruritus: > 4-6 cm; severe pruritus: > 6-8 cm; and very severe pruritus: > 8-10 cm. CADESI 04 was evaluated by the same veterinarian and PVAS scores were obtained by owner observation.

For cats, the evaluation of the clinical indices was carried out at the same time intervals as for dogs. The following were used to evaluate the efficacy of the diet: an evaluation of the pruritus severity PVAS according to the same methodology as for dogs and an evaluation of the severity of skin lesions according to the SCORFAD index (SCORing Feline Allergic Dermatitis). The SCORFAD was calculated according to the methodology provided by Steffan et al. (36). SCORFAD is a validated dermatologic scale to assess skin lesions associated with allergic dermatitis in cats. This scale allows for the assessment of the severity of 4 common skin lesions: excoriations, self-induced alopecia, eosinophilic plaques, and miliary dermatitis. Ten body regions are described, and lesions are assessed with a severity scale of 0-4 with 0 being none, and 4 being severe.

All procedures in this study were non-invasive and did not require institutional approval (the diet approved for sale was used).

Statistical evaluation. The normality of the distribution of the results obtained was tested using the Kolmogorov-Smirnov test. The differences in statistical significance between the results at individual time intervals were calculated based on the Mann-Whitney U test. Values of $p < 0.05$ were considered significant.

All calculations were made using JASP Team (2024). JASP (Version 0.19.2) [Computer software].

Results and discussion

Effects of the diet on dogs. The dogs in the study group on day T0 (before initiating the treatment) showed the severity of skin lesions, evaluated using the CADESI 04 index, at a level of 18.8, whereas pruritus was evaluated at a level of 5.7 using the PVAS scale. During the application of the diet, there was a gradual clinical improvement: a decrease in the severity of pruritus and a reduction in the CADESI 04 index. After two weeks of the application of the diet, there was a slight reduction in pruritus (PVAS = 4.9), with this value not being statistically significant ($p = 0.337$). After four weeks of the diet application, PVAS decreased to a value of 3.4, which was a significant decrease compare to T0 ($p = 0.007$). After six weeks, PVAS amounted to 2.1 ($p = 5.945 \times 10^{-4}$), after eight weeks to 1.6 ($p = 3.402 \times 10^{-4}$), and after 10 weeks to 1.1 ($p = 2.799 \times 10^{-4}$). After two weeks, the CADESI 04 index decreased to 12.9, which was not a significant decrease ($p = 0.24$). During further application of the diet, when evaluated after four weeks, the CADESI 04 continued to decrease, but this was still not a decrease of statistical significance (the CADESI 04 value was 9.5, $p = 0.12$). After six weeks, the CADESI 04 decreased further to a value of 6.2, which already was statistically significant ($p = 0.037$). After eight weeks, this parameter was 4.8 ($p = 0.011$), with its value being similar after 10 weeks ($p = 0.009$).

The results and the information on statistically significant differences concerning the CADESI 04 and PVAS values in dogs are provided in Figures 1 and 2.

To sum up the results, the positive effect of the diet on dogs was evident as early as week 4 of its application for the pruritus assessment and at week 6 for the CADESI 04 evaluation.

Effects of the diet on cats. At the start of the diet application (T0), the cats participating in the evaluation exhibited the following clinical parameter values: SCORFAD of 4.6 and PVAS of 6.3. During the application of the diet, as it was for the dogs, there was a gradual clinical improvement and a decrease in the clinical evaluation parameter values. For this species, after two weeks of the application of the diet, the reduction in pruritus was not yet statistically significant (its value was 5.9, $p = 0.725$). After four weeks, it amounted to 4.7, and this decrease continued not to be statistically significant ($p = 0.09$). After six weeks, the pruritus already decreased significantly: 3.1 ($p = 0.004$). Further observation found continued decreases in this parameter: after eight weeks, it amounted to 1.7 ($p = 2.938 \times 10^{-4}$), whereas after ten weeks it was only 1 ($p = 1.772 \times 10^{-4}$).

Similarly to pruritus, the SCORFAD gradually decreased, and after two weeks, it amounted to 3.6, which was not a statistically significant decrease ($p = 0.152$). After four weeks, this parameter amounted to 3, which

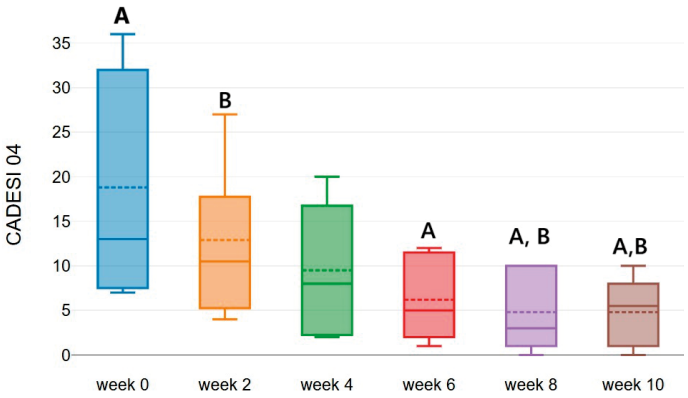

Fig. 1. CADESI 04 values in dogs over the 10-week period of the diet application. A, B statistically significant differences

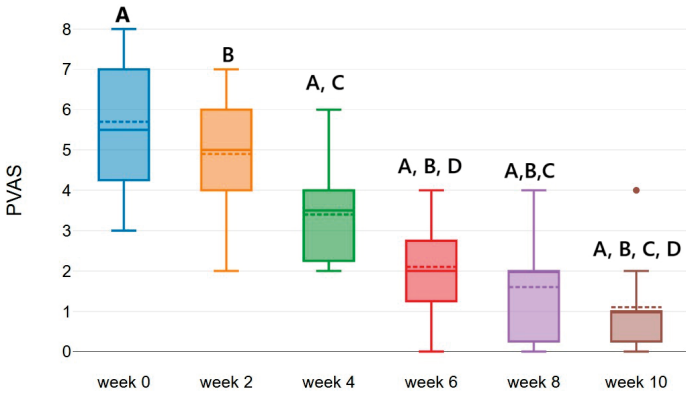

Fig. 2. PVAS values in dogs over the 10-week period of the diet application. A, B, C, D statistically significant differences

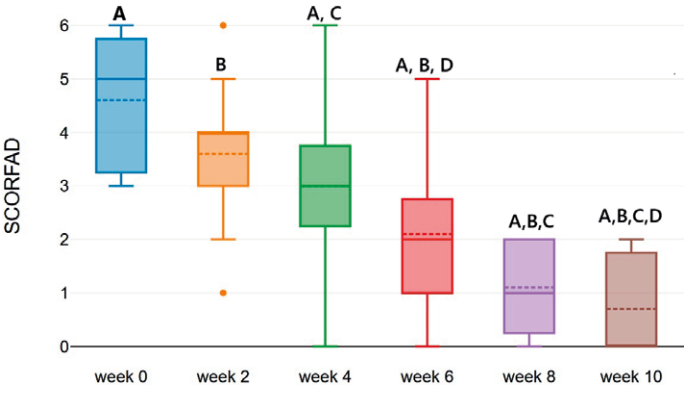

Fig. 3. SCORFAD values in cats over the 10-week period of the diet application. A, B, C, D statistically significant differences

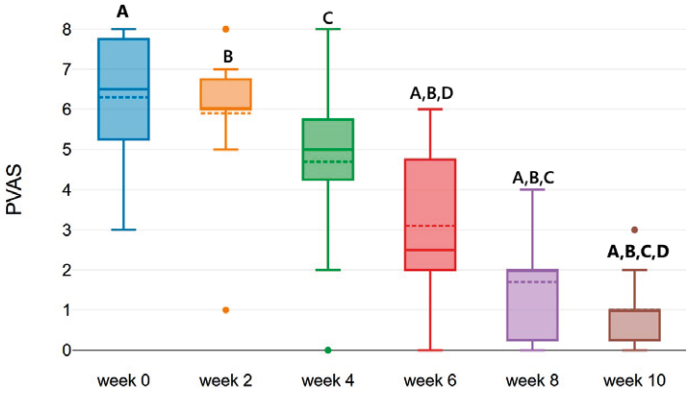

Fig. 4. PVAS values in cats over the 10-week period of the diet application. A, B, C, D statistically significant differences

already was statistically significant ($p = 0.029$). After six weeks, it was 2.1 ($p = 0.003$), whereas after 8 weeks, it was 1.1 ($p = 1.538 \times 10^{-4}$), and after 10 weeks, it amounted to 0.7 ($p = 1.358 \times 10^{-4}$).

The detailed results for the SCORFAD and PVAS in cats are provided in Figures 3 and 4.

To summarise the results, the positive effects of the diet in cats were already evident from week 6 of the pruritus assessment and from week 4 of its application for the SCORFAD score.

In patients participating in the evaluation of the insect protein-based diet a statistically significant improvement in terms of decrease in the pruritus value was observed after four weeks for the dogs and after six weeks for the cats. As regards the parameters evaluating the severity of lesions, i.e. the CADESI 04 for the dogs and the SCORFAD for the cats, an improvement was noted after six weeks for the dogs and after four weeks for the cats. These results show that the minimum period after which positive outcomes of the application of a novel protein based diet are noted is no less than 4 to 6 weeks. It should be noted that an improvement was evident in all the individuals under evaluation only after eight weeks.

The literature offers a variety of recommendations as to how long the elimination diet should be applied. Some authors report that an improvement can be observed after three weeks of treatment (18, 25, 26, 40, 41) and even after two weeks of the diet application (6). In many cases, however, such a short period is not sufficient to notice an improvement, and it may occur in only 25% of the dogs (32), whereas in cats, an improvement is only evident after six weeks (36, 40).

An earlier study conducted by the authors of the present study to evaluate the efficacy of a diet based on hydrolysed salmon protein noted an improvement involving a reduction in pruritus and skin lesions after four weeks of the application of the diet in both dogs and cats and observed a significant improvement in most animals after eight weeks (37). These results are similar to those obtained in the present study (a significant improvement after 4-6 weeks, depending on the clinical parameter under assessment).

To date, an evaluation of novel protein based diets based on insect proteins has not been the subject of extensive research, with only few publications on this subject available. A study by Böhm et al. (6) obtained results that were better than those of the present study. However, since the authors only carried out their study for two weeks, it is difficult to compare it fully with the current study. Those authors noted a reduction in pruritus as well as a decrease in the severity of skin lesions (their study used a different clinical index, i.e. the Canine Atopic Dermatitis Lesion Index (CADLI)) in the majority of the individuals under study after only two weeks of the diet application. This is a better result than that obtained in the current study, where such an improvement only occurred after six weeks. The difference may be due, among other things, to the different system of evaluation of clinical lesions adopted in the experiment conducted by the authors.

Another paper evaluating the efficacy of this type of diet for food allergy provides the results published by Kang-Il Lee et al. (21) which confirmed the efficacy of an insect protein-based diet in dogs with food allergy. The dogs under study showed a statistically significant decrease in the CADESI 04 index at week 8 of the application of the diet. Interestingly, the authors found no significant reduction in pruritus values during the observations. Therefore, the results obtained by the authors are inferior to those obtained in the current study, in which the CADESI 04 index decreased significantly after six weeks. The difference between the observations may be due to the fact that Kang-Il Lee et al. adopted different evaluation points and performed the evaluation at four-week intervals in contrast to the two-week intervals applied in the current study. It is difficult to explain why the reduction in pruritus, which was observed in the current study as early as after four weeks, was not observed in these authors' study. This may have been due to different initial values of the parameter being assessed, which were significantly lower on the day of initiating observations compared to the current study (less than 3, compared to 5.7 in the current study).

In addition, a clinical case of the successful application of a diet based on black soldier fly larval protein in a dog with food allergy, showing gastrointestinal symptoms, has recently been published. In the case described, an improvement occurred as early as 12 days after changing the diet to one based on insect proteins (11).

In the absence of studies on the clinical evaluation of insect protein-based diets in cats, the current results cannot be compared to studies by other authors.

The results obtained in the present study are consistent with those reported for other hypoallergenic diets, in which clinical improvement in dogs was observed after a comparable period (approximately 3-4 weeks) (17, 23, 25, 40, 41). Whereas in cats the improvement generally occurred slightly later, typically around the sixth week of dietary intervention (36, 40).

In conclusion, the insect (black soldier fly larvae) protein-based diet is a promising alternative both in the diagnosis and treatment of food allergy in dogs and cats, with the efficacy being similar to that of other hypoallergenic diets. An additional advantage of the diet is the use of insects as the source of proteins, as they are an excellent source of high-value protein, and their use in the production of the diet has a positive effect on a reduction in the carbon footprint linked to its production. The use of black soldier fly larval meal can be considered a sustainable source of protein, as it uses approximately 80% of its biomass compared to traditional sources of animal protein extraction such as beef and poultry, which only use approximately

40-60%, resulting in lower amounts of residual by-products (17). We can expect high effectiveness due to the lack of use of insect protein in dog and cat nutrition. A limitation of the study was the relatively small number of animals in the individual groups, which necessitates cautious interpretation of the final conclusions and indicates the need for further studies evaluating the efficacy of insect protein-based diets.

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