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Clinical application of insect-based diet in canine allergic dermatitis

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The objective of this study was to evaluate the beneficial effects of the short-term application of insect-based diet in canine allergic dermatitis. Total 19 atopic dogs with concurrent cutaneous adverse food reactions were enrolled and classified into 3 groups. The treatment group (n = 7) was fed insect-based diet, the positive control group (n = 6) was fed salmon-based diet, and the negative control group (n = 6) was fed commercial or homemade diet for 12 weeks. The degree of skin lesions was evaluated based on canine atopic dermatitis extent and severity index (CADESI-4). Additionally, transepidermal water loss (TEWL) and pruritus visual analog scale were evaluated. All indices were evaluated every 4 weeks after the initial administration of hypoallergenic diets. In the treatment group, significant decrease in the CADESI-4 score was observed at 8 weeks compared to the baseline score ($p = 0.031$). There were significant differences in the CADESI-4 score between the groups at 8 weeks ($p = 0.008$), 12 weeks ($p = 0.012$), and TEWL at 12 weeks ($p = 0.022$). This preliminary result demonstrates the potential hypoallergenicity of an insect-based diet through features that diminish cutaneous lesions and skin barrier dysfunction.

Keywords: canine atopic dermatitis; cutaneous adverse food reaction; dogs; hypoallergenicity; insect-based diet

Introduction

Canine atopic dermatitis (CAD) is an allergic skin disease that causes pruritus in dogs with genetic predisposition [1]. Cutaneous adverse food reaction (CAFR) is caused by food allergens and is a common allergic dermatosis that makes CAD difficult to control by triggering the flare of CAD [2,3]. It is generally characterized by non-seasonal pruritus, secondary infection associated with ingestion of various kinds of food allergens such as beef, dairy products, chicken, and wheat in dogs, and exhibit a partial-to-poor response to glucocorticoids [4,5]. As allergic skin reactions to food allergens present CAD-like clinical signs, it is challenging to clearly distinguishing CAFR from CAD based on lesion distribution alone [6,7].

The gold standard for diagnosis in CAFR is based on a strict elimination diet trial, resulting in improved clinical signs, followed by a provocative test. If the clinical signs relapse within a few minutes to several hours or up to 2 weeks after feeding the test food, CAFR is diagnosed. Even though the trial duration of an elimination diet is not exactly defined, the recommended period has been suggested as 8 to 12 weeks to improve clinical signs in most cases [8,9]. An ideal elimination diet trial should be performed using a diet consisting of ingredients that have never been previously exposed to the patient. However, most commercial diets consist of a wide variety of ingredients, making the choice of an appropriate diet difficult [10]. In recent decades, various protein sources, especially chicken, soy, and fish, have been commercially utilized to alleviate and manage the clinical signs of CAFR. Although chicken or soy hydrolyzed diets have been used as alternatives, they may not be useful for dogs that are allergic to these foods. Unfortunately, a recent study suggested that these are no longer valid as novel protein sources, and that no diet is effective in all cases of CAFR [11,12].

Homemade diets for diagnostic purposes could be a fundamental alternative to commercial hypoallergenic diets. However, sustaining an elimination diet is difficult because of its high cost, low efficiency, inconvenience, and low compliance of owners. Moreover, it does not guarantee nutritional soundness or true hypoallergenicity [8,12-14].

With increasing demand for a novel protein, various food sources have been attempted to diagnose or manage CAFR. Studies have reported that insects, which are a novel food source, offer high-quality proteins as well as beneficial fatty acids [15-17]. Insect-derived products have been reported to have high digestibility and palatability, and they have great potential to serve as immunomodulators of microbiota as well as nutrient sources to animals [18]. In addition, insect proteins are not recognized by the animal's body; therefore, they less likely to cause irritation.

However, there has been only one clinical study on the complementary effects of insect-based diets in dogs with CAFR [19]. In that previous study, 20 dogs with CAFR were shown to have improved lesion scores and coat quality after being fed an insect-based diet for 2 weeks. Therefore, the present study was performed to evaluate the beneficial effects of an insect-based diet in dogs diagnosed with concurrent CAD and CAFR.

Materials and Methods

Animals

This study enrolled 19 client-owned dogs concurrently diagnosed with CAD and CAFR at the Chungbuk National University Veterinary Teaching Hospital (Cheongju, Korea). To diagnose CAD, we utilized diagnostic criteria based on the medical history, typical clinical signs proposed by the International Committee on Allergic Diseases of Animals guidelines, satisfaction with at least 5 criteria in Favrot's diagnostic criteria, and/or a positive intradermal skin test [10,20]. This study also ruled out other diseases that could trigger pruritus and associated skin lesions through proper dermatologic tests and prophylactic treatments. The inclusion criteria for CAFR were dogs with a definite dietary history and poorly responsive pruritus to glucocorticoids. In addition, to confirm the minimal improvement, dogs with a minimum score of 3 on the pruritus visual analog scale (PVAS) or 30 in the canine atopic dermatitis extent and severity index (CADESI-4) score were included in this study.

Among the 19 dogs, the insect-based diet group (n = 7) and salmon-based diet group (n = 6) were prescribed with insect-based diet and salmon-based diet as the experimental and positive control groups, respectively, while the negative control group (n = 6) received a non-prescribed commercial or homemade diet according to the owner's choice. There was no restriction of snacks or treats during the study in negative control group, whereas the experimental and positive control groups restricted their food excluding prescribed diet. All formerly prescribed drugs for managing CAD or secondary infections continued during the study period. Informed consent was obtained from all the owners before the experiment.

Administration of novel protein diet

The insect-based diet and salmon-based diet was obtained from the National Institute of Animal Science, Rural Development Administration (Wanju, Korea). The single protein and carbohydrate source of the insect-based diet is the fat-removed *Tenebrio molitor* larvae, commonly called yellow mealworms, and brown rice with mixing proportions of 30% and 35%, respectively. The salmon-based diet is hydrolyzed single protein and carbohydrate source and mixed with brown rice 30% and 50%, respectively. The insect-based and salmon-based diet was stored at room temperature and administered to the dogs for 12 consecutive weeks in the amount previously fed.

Clinical assessments

While assessment of PVAS was conducted by dog owners, the CADESI-4 score, transepidermal water loss (TEWL), and medication score were assessed by 3 trained veterinarians (K.I. L., T. Y., and Y. K) for each patient. This study surveyed all clinical indices every 4 weeks from baseline to 12 weeks.

PVAS

The PVAS score was used to grade the severity of pruritus based on history taking and evaluation by the owners. The PVAS consists of 10 scales, with 0 indicating normal status and

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10 indicating the most severe pruritus [21].

CADESI-4

CADESI-4 was developed to assess 20 body sites and to evaluate the extent and severity of skin lesions in this study. Three types of skin lesions, including lichenification, erythema, and excoriation/alopecia, were assessed based on a four-point severity scale. The scale indicated none (score 0), mild (score 1), moderate (score 2), or severe (score 3). We assessed 20 body sites, 3 types of lesions, and 4 grades of severity, thus generating a maximal score of $20 \times 3 \times 4 = 180$ [22].

TEWL

Before measuring TEWL, the dogs were given time intervals to acclimate to the environment of the test room. TEWL was measured using an unventilated closed-chamber device, VaporMeter SWL3 (Delfin Technologies Ltd., Finland) according to the manufacturer's instructions. In a constant environment, 5 consecutive measurements of the pinnae, axillae, and groin were performed for 10 seconds to minimize the variety of TEWL (room temperature, 24°C to 26°C; relative humidity, 44% to 66%). Subsequently, the maximum and minimum values were excluded, and the remaining results were averaged and presented in $g/m^2/h$.

Medication scores

Altered doses of the prescribed drugs for managing CAD, such as prednisolone, oclacitinib, and cyclosporine, were converted into a medication score. A medication score of 1.0 was assigned to the drug dose that was administered before the start of the elimination diet trial.

Evaluation for safety

The theoretical adverse effects of insect-based diets in dogs include hypersensitivity according to individual susceptibility, the occurrence of CAFR in these foods, and infections [23,24]. Adverse effects, such as gastrointestinal (e.g., vomiting, diarrhea, anorexia) and dermatologic signs (e.g., erythema, pruritus), were monitored at home by the owners or at every visit by the veterinarians.

Statistical analysis

Data analysis was performed using GraphPad Prism 7 software (GraphPad Software Inc., USA). The Wilcoxon test was conducted to analyze the PVAS, CADESI-4, TEWL, and medication scores in both groups. The difference values obtained between-groups every 4 weeks were also analyzed using the non-parametric Mann-Whitney U-test. All data are expressed as the mean ± standard deviation (SD). Differences were considered statistically significant at $p < 0.05$.

Results

Animal population

Patient information, such as signalments (age, sex, and breed), previously prescribed drugs, and their dosage, are listed in Table 1. The patients were grouped into the insect-based diet group (n = 7), salmon-based diet group (n = 6), and control group (n = 6). The major dog breeds in the insect-based diet and salmon-based diet groups were Maltese (n = 5), while those in the control group were Maltese (n = 2) and Shih Tzu (n = 2) dogs. The mean ages in the insect-based diet, salmon-based diet, and control group were 5.14 ± 2.67, 9.17 ± 2.32, and 7.5 ± 4.14 years (mean ± SD), respectively. In the insect-based diet group, one dog was administered cyclosporine (6 mg/kg, Cipol-N; Chong Kun Dang Pharmaceutical Corp., Korea) every alternate day to manage CAD before the start of the experiment, one dog was not prescribed any drug, and the others were administered oclacitinib (0.4 to 0.6 mg/kg, Apoquel; Zoetis Inc., USA) once daily. In the salmon-based diet group, one dog was administered prednisolone (0.5 mg/kg, Solondo; Yuhan Corp., Korea) every alternate day and the others were not prescribed any drugs. All dogs in the control group were managed using oclacitinib (0.4 to 0.6 mg/kg) once daily.

Clinical assessments

PVAS

The mean and SD of the control group's PVAS increased gradually from the onset of the experiment to 12 weeks, while those of the insect-based diet and salmon-based diet groups remained nearly the same throughout the experimental period. Nevertheless, no significant differences within-group were found in any of the groups (Fig. 1A). In addition, there were no significant differences between-groups at any time point (Fig. 1A).

CADESI-4

The CADESI-4 score of the insect-based diet group decreased significantly at 8 weeks compared with baseline (0 vs. 8 weeks, $p = 0.031$) (Fig. 1B) and that of the control group increased at 8 weeks, resulting in a significant difference between the 2 groups (8 weeks, $p = 0.008$). The CADESI-4 score of the insect-based diet group was slightly increased from 28 to 33.3, at 12 weeks when compared with 8 weeks; therefore, the significant differ-

Table 1. Signalments and previously prescribed drugs for treating 19 dogs with concurrent atopic dermatitis and cutaneous adverse food reactions

Patient no.	Age (y)	Sex	Breed	Previously prescribed drugs
I-1*	8	IF	Maltese	Cyclosporine 6 mg/kg eod
I-2	7	SF	Cocker Spaniel	None
I-3	4	IF	Maltese	Oclacitinib 0.4 mg/kg sid
				Amoxicillin-clavulanic acid 25 mg/kg bid
				itraconazole 5 mg/kg sid
I-4	3	SF	Maltese	Oclacitinib 0.6 mg/kg sid
I-5*	1	SF	Maltese	Oclacitinib 0.4 mg/kg sid
I-6	8	SF	Maltese	Oclacitinib 0.4 mg/kg sid
I-7*	5	CM	Bichon Frise	Oclacitinib 0.6 mg/kg sid
S-1	11	CM	Shih Tzu	None
S-2	12	CM	Shih Tzu	Prednisolone 0.5 mg/kg eod
S-3	9	SF	Maltese	None
S-4	7	CM	Maltese	None
S-5	10	SF	Chihuahua	None
S-6	6	SF	Maltese	None
C-1	6	SF	Maltese	Oclacitinib 0.4 mg/kg sid
				Itraconazole 5 mg/kg sid
C-2*	3	SF	Mixed breed	Oclacitinib 0.4 mg/kg sid
C-3	4	IF	Shih Tzu	Oclacitinib 0.4 mg/kg sid
C-4	7	SF	Pekingese	Oclacitinib 0.6 mg/kg sid
C-5*	13	IF	Maltese	Oclacitinib 0.6 mg/kg sid
				Amoxicilline-Clavulanic acid 25 mg/kg bid
				Itraconazole 5 mg/kg sid
C-6*	12	SF	Shih Tzu	Oclacitinib 0.6 mg/kg sid
				Itraconazole 5 mg/kg sid

I, insect-based diet group; S, salmon-based diet group; C, control group; IF, intact female; SF, spayed female; CM, castrated male; eod, every other day; sid, once daily; bid, twice daily.
*Diagnosis of cutaneous adverse food reactions after provocative challenge.

ence between baseline and 12 weeks disappeared. The between-group differences were maintained at 12 weeks (p = 0.012). Moreover, the CADESI-4 score of the salmon-based diet group was significantly lower than that of the insect-based diet group at 8 and 12 weeks (8 weeks, p = 0.002; 12 weeks, p = 0.002) and the control group throughout the 12 weeks (baseline, p = 0.007; 4 weeks, p = 0.004; 8 weeks, p = 0.002; 12 weeks, p = 0.002), respectively.

TEWL

There was no statistically significant difference in TEWL between the groups over time (Fig. 1C). However, the TEWL values of the salmon-based diet group were significantly lower than those of the control group at all time points (baseline, p = 0.002; 4 weeks, p = 0.046; 8 weeks, p = 0.004; 12 weeks, p = 0.015). In addition, the TEWL value of the insect-based diet group was significantly lower than that of the control group at the end of the experiment (12 weeks, p = 0.022).

Medication score

The medication scores of all groups remained nearly unchanged for 12 weeks. Therefore, there were no significant within-group and between-group differences at any time point (p > 0.99). The medication scores of all groups remained nearly unchanged for 12 weeks (Fig. 1D).

Evaluation for safety

Both the control and treatment groups had no adverse effects during or after the experimental period.

Discussion

Insects have been widely consumed as food in humans and animals for a long time. One of the most common edible in-

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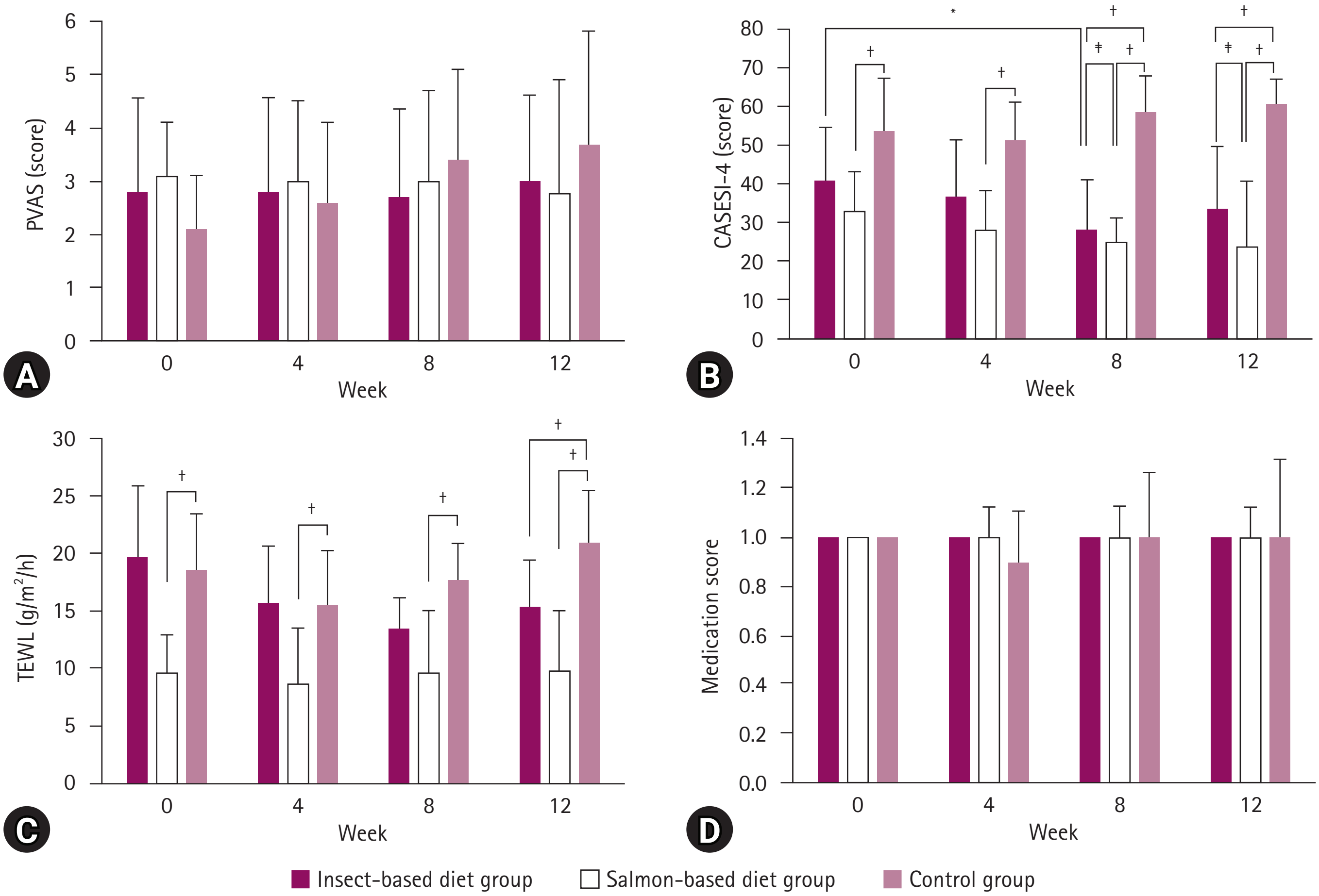

Fig. 1. Temporal changes in (A) pruritus visual analog scale (PVAS), (B) canine atopic dermatitis extent and severity index (CADESI-4), (C) transepidermal water loss (TEWL), and (D) medication score in the insect-based diet, salmon-based diet, and control groups. (A, D) In the PVAS and medication scores, there were no significant between- and within-group differences at all time points. (B) The CADESI-4 score of the insect-based diet group at 8 weeks was decreased significantly compared with baseline ($^*p = 0.031$); Moreover, the CADESI-4 score of the treatment group was significantly lower than that of the control group at 8 weeks ($^\dagger p = 0.008$), and this significant difference was maintained until 12 weeks ($^\dagger p = 0.012$). Significantly lower CADESI-4 scores were found in salmon-based diet group than in the control group at 12 weeks (baseline, $^\dagger p = 0.007$; 4 weeks, $^\dagger p = 0.004$; 8 weeks, $^\dagger p = 0.002$; 12 weeks, $^\dagger p = 0.002$) and insect-based diet group in 8 and 12 weeks (8 weeks, $^\ddagger p = 0.002$; 12 weeks, $^\ddagger p = 0.002$). (C) TEWL showed a similar tendency to CADESI-4 in all groups. There was no significant difference within each group, nevertheless, TEWL of the control group was significantly higher than that of the insect-based diet group at 12 weeks ($^\dagger p = 0.022$) and that of the salmon-based diet group at every time point (baseline, $^\dagger p = 0.002$; 4 weeks, $^\dagger p = 0.046$; 8 weeks, $^\dagger p = 0.004$; 12 weeks, $^\dagger p = 0.015$). Error bars indicate the mean ± SD.

sects—the yellow mealworm—is widely used as animal feed because of its short life cycle and ease of handling and breeding worldwide [17,25]. In 2014, the Ministry of Food and Drug Safety of Korea recognized yellow mealworm larvae as a new food ingredient and added it to the list of edible insects, which was temporarily accepted in 2016. In May 2018, it was adopted as a general food ingredient, making it suitable for universal consumption. In addition, the safety standards for heavy metal content in yellow mealworms were presented as follows: < 0.1 mg/kg of Pb, 0.05 mg/kg of Cd, and 0.1 mg/kg of As in dried insects.

In veterinary medicine, insect-based diets have been applied to increase the productivity of industrial animals such as pigs, poultry, fish, and shellfish, as well as to manage and diagnose CAFR in terms of a novel protein source [17-19]. In Europe, at least 12 types of insect-based pet food have been launched over the 4 years since 2015. Nonetheless, only one clinical study has been conducted on the complementary effects of insect-based diets in dogs [26]. This report applied a marketed diet (Insect Dog; Josera Petfood GmbH & Co., Germany), which utilized black soldier fly larvae as a single protein source in dogs with CAFR for 2 weeks [19]. The skin lesion and hair coat scores showed significant improvements, but the experimental period was much shorter than the 8 weeks, which is a minimally recommended period for an elimination diet trial. In contrast, the present study was performed using a yellow mealworm-based diet as a single protein source for 12 weeks in dogs already diagnosed or suspected with CAFR based on clear dietary history.

It was found that CADESI-4 scores of the insect-based diet group decreased significantly at 0 vs. 8 weeks; however, this was not found in the control group. In 3 dogs in the control group, clinical deterioration occurred between 8 and 12 weeks, resulting in a significantly higher CADESI-4 score than that of the insect-based diet group. This result is consistent with previous reports that approximately 95% of dogs showed improved skin lesions after 8 weeks of an elimination diet trial with an adequate hypoallergenic diet [9]. However, the CADESI-4 score and TEWL of the salmon-based diet group were significantly lower than those of the control group at all time points, and significantly lower at 8 and 12 weeks when compared to the insect-based diet group. In particular, the statistical significance of the CADESI-4 score between the salmon-based diet group and the control group consistently increased from 0.007 to 0.002 during the experimental period. In addition, the TEWL of the control group showed an increasing trend 8 weeks onward with a significantly high measurement at 12 weeks compared to the insect-based diet group. Therefore, it was suspected that the insect-based diet improved the clinical signs of CAFR as a hypoallergenic diet; however, the degree of lesion improvement demonstrated that the hypoallergenicity of the insect-based diet was higher than that of the salmon-based diet.

On the other hand, PVAS of the insect-based diet group was maintained without any deterioration during the 12 weeks, but there was no significant decrease between and within all groups. This experiment was performed on dogs with relatively well-controlled mild pruritus, which made it difficult to accurately determine the effectiveness of the insect-based diet since little change in PVAS was observed. As a result, there was no change in the dose of the applied CAD drug, which indicated the same medication score in the treatment group. Relapse of clinical signs occurred between 8 and 12 weeks in 3 dogs in the control group, increasing the pruritus and worsening the skin lesion. However, eliminating the allergen was a more fundamental treatment than increasing the dose of CAD drugs; therefore, the medication score of the control group was not altered.

The digestibility of an insect-based diet is affected by not only the insect species and the processes, such as drying and defatting but also the presence of chitin, which is a component of insect exoskeleton [18]. In general, chitin reduces protein digestibility. However, it has been shown that the ratio of *Escherichia coli* and *Salmonella* spp. is reduced and that of *Lactobacillus* spp. is increased to modulate intestinal microflora and improve skin health [27]. Since the beneficial effects of insect proteins on animals are rarely found in dogs, further studies are needed.

Possible theoretical adverse effects of an insect-based diet, such as excessive immune reaction by the susceptible individuals, CAFR outbreaks due to the insect-based diet, and systemic infection have been reported [23,24]. Hypersensitivity to insects can be due to inhalants and contact with body parts, waste products, or excreta [23]. Several studies have revealed that *E. coli*, *Salmonella* spp., and heavy metals such as Hg, Pb, As, and Cd were not detected in the larvae of *T. molitor* [16,28,29]. Short-term safety was also demonstrated when freeze-dried yellow mealworms were administered to rats for 90 days to assess toxicity [29].

These results support the suitability of introducing mealworms in animal feed as well as in human food. In this study, no adverse effects were detected by dog owners and veterinarians during and after the experiment. However, the long-term effects of ingesting insect-based diets should be consistently monitored and evaluated.

The limitation of this study is the relatively small number of enrolled dogs. Although a significant between-group difference was not confirmed in the PVAS and medication scores, additional large cohort studies are needed to evaluate the beneficial effects of an insect-based diet. In addition, studies involving only patients previously diagnosed with CAFR are needed. In future studies, medication for concurrent CAD management must be stopped to confirm the effects of an insect-based diet alone. It was nearly impossible for the dog owners to participate in this experiment without any prescription for CAD for their dogs. Therefore, the dogs enrolled in this study were maintained with a minimum dose of medication to manage CAD. Unlike main food allergens weighing more than 20 kDa, the analysis of yellow mealworm proteins that could have shown hypoallergenicity was not carried out [30]. In addition, fecal analysis to evaluate the effect of an insect-based diet on intestinal microflora was not conducted.

In conclusion, although our preliminary results indicated that administration of an insect-based diet had supplementary effects on improving skin lesions and skin barrier function in dogs with CAD and CAFR, it was not enough to alleviate pruritus and the dosage of prescribed drugs for controlling CAD. Further studies are needed to clarify these aspects.

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