Clinical Efficacy of a Novel Elimination Diet Composed of a Mixture of Amino Acids and Potatoes in Dogs with Non-Seasonal Pruritic Dermatitis

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ABSTRACT. There has been a need for improvement of the elimination diet used for diagnosis of adverse food reaction (AFR) in dogs. Recently, a novel elimination diet composed of a mixture of amino acids and potatoes was developed. We evaluated the efficacy of the elimination diet for diagnosis of AFR in dogs. Twenty dogs that were suspected to have allergic dermatitis were enrolled in a 2-month food elimination trial using the diet. Before and after the trial, the clinical symptoms were evaluated based on the change in canine atopic dermatitis extent and severity index (CADESI), pruritus score and medication score. Of the 20 dogs, 15 completed the food elimination trial. The remaining 5 dogs were removed from the trial because of diet unpalatability, skin disease progression or diarrhea. On the basis of evaluation of the clinical scores, we observed that the clinical symptoms improved in 11 of the 15 dogs that completed the food elimination trial. Provocative challenge was performed in 10 of the 11 dogs that showed improvement in their clinical symptoms. Of the 10 dogs, 7 were diagnosed as having AFR against food ingredients such as pork, beef, chicken and wheat because their skin symptoms reappeared after intake of these ingredients. The results of the food elimination trial and the provocative challenge indicated the usefulness of the novel elimination diet for diagnosis of AFR.

KEY WORDS: adverse food reaction, canine, elimination diet.

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Adverse food reaction (AFR) in dogs is associated with intake of various food ingredients or food additives [20, 30, 35]. Elicitation of type I hypersensitivity responses to food allergens has been implicated at least in part in the pathogenesis of AFR [11–13]. It has been reported that 10–30% of dogs with non-seasonal chronic pruritic dermatitis are diagnosed as having AFR [5, 6, 8, 17, 18, 25, 27]. In order to diagnose AFR, it is necessary to eliminate possible food allergens from the original diet [20, 30, 35]. Intradermal skin testing (IDST) and allergen-specific serum IgE tests are often used for detection of environmental allergens; however, it is known that the sensitivity and specificity of these tests to food allergens are low [10, 15, 16, 21].

A dietary test combined with a food elimination trial and a provocative challenge has been considered the gold standard for diagnosis of AFR [20, 30, 35]. The food elimination trial is carried out to convert the original diet into a hypoallergenic elimination diet. Within 10–12 weeks of the food elimination trial, when improvements in clinical symptoms are observed, a provocative challenge is performed using the original diet or a series of single food ingredients.

The diagnosis of AFR is made when the provocative challenge leads to recurrence of clinical symptoms.

To make an accurate diagnosis of AFR, home-cooked diets [6, 10–16, 18, 19, 21, 25–27], novel protein diets [6, 10, 12, 16, 19, 25] and hydrolyzed protein diets [2, 3, 10, 11, 17, 18, 26] have been used for the food elimination trial. These diets generally contain a single source of protein and carbohydrate. Since these ingredients are not included in the original diets in most instances, it is unlikely that either of these food components would elicit allergenic responses [35]. However, long-term management with home-cooked diets is not adequate for the nutritional requirements of dogs [28]. Moreover, a single protein source present in home-cooked and novel protein diets may show immunologic cross-reactivity with the underlying food allergen present in the previously fed diet [19, 35].

Food allergens with a molecular mass of more than 10 kDa can bind to IgE antibodies on the surfaces of mast cells, resulting in the cross-linking of IgE and Fcε receptors, as well as subsequent degranulation and activation of mast cells [3, 30, 35]. The cross-linking of Fcε receptors with food allergens does not occur when the molecular weight of the food allergens is less than 10 kDa. Hydrolysis of proteins into smaller peptides can reduce the molecular weights of the original proteins to less than 3 kDa [3, 4]. Thus, hydrolysis of proteins possibly prevents the cross-linking of

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Fcε receptors and subsequent development of IgE-mediated type I hypersensitivity [2, 12, 13, 17, 18]. On the other hand, lymphocyte responses occur via recognition of T-cell epitopes comprising 8–10 amino acids [34]. Since the molecular weights of hydrolyzed proteins are considerably larger than those of T-cell epitopes, feeding hydrolyzed diets cannot totally avoid food hypersensitivity (FH) mediated by lymphocyte responses. We previously reported induction of lymphocyte blastogenic responses to offending food allergens in most dogs diagnosed with FH [10]. Recently, the *in vitro* proliferation responses of CD4⁺CD25⁺ helper T-cells to a culprit food antigen were revealed in dogs with FH [24]. These findings suggested that T-cell mediated immune responses were associated with the pathogenesis of dogs with FH [10, 24]. Use of amino acids for the elimination diet conceivably avoids both the IgE and T-cell mediated immune responses in dogs with FH.

Recently, a novel elimination diet (Aminoprotect Care®; Nosan Corporation, Kanagawa, Japan) composed of a mixture of amino acids and potatoes was developed as a collaborative project between the University of Tokyo and a pet food company (Nosan Corporation). Using a colony of Maltese x Beagle dogs having FH against corn, Olivry *et al.* [24] reported that the dogs did not exhibit dermatological symptoms and allergic immune responses to the novel elimination diet, although the diet contained corn starch. To extend the experimental findings demonstrated by the report of Olivry *et al.* [24], clinical studies on dogs are required to prove the usefulness of the novel diet for diagnosis and long-term management of dogs with AFR against various food allergens. We hypothesized that the diet could prevent the clinical manifestation of AFR in dogs, except in cases of potato allergy. The objective of this study was to evaluate the clinical usefulness of the newly developed elimination diet for diagnosis of AFR in dogs.

MATERIAL AND METHODS

Dogs: Twenty dogs (Cases 1–20) with possible allergic dermatitis were selected for the present study. These dogs were referred to the Veterinary Medical Center of the University of Tokyo between April 2004 and September 2005. The inclusion criteria were the presence of non-seasonal chronic pruritic dermatitis and early onset of the clinical manifestations (usually younger than 3 years old). Flea bite hypersensitivity, contact hypersensitivity and primary infectious skin diseases such as superficial pyoderma, mycotic skin diseases and ectoparasite infestations were ruled out. Prior to enrollment in this study, concurrent superficial skin infections with *Staphylococcus* spp. were treated with 0.5% chlorhexidine shampoo (Nolvasan® shampoo; Fort Dodge Animal Health, Fort Dodge, IA, U.S.A.) and/or oral antibiotics selected according to bacterial culture and sensitivity testing. Overgrowth of *Malassezia pachydermatis* was controlled with 2% chlorhexidine / 2% miconazole shampoo (Malaseb®; Leo Animal Health, Princes Risborough, UK).

Intradermal skin test (IDST): The procedures and aller-

gen extracts used for the IDST in this study were previously described [22]. The following 8 groups of allergen extracts were used: house dust mites (HDM; *Dermatophagoides farinae* and *D. pteronyssinus*), arthropods (fleas, 4 insect mix), cat epithelia, nutritional food ingredients (beef, cow's milk, hen's eggs, chicken, corn, rice, wheat, tuna and cod), molds (*Aspergillus* mix, *Mucor* mix and *Penicillium* mix), grasses (7 grass mix), weeds (National weed mix) and trees (Eastern 10 tree mix). These allergen extracts were purchased from Greer (Nuway Circle, NC, U.S.A.). Japanese cedar pollen allergen extract was purchased from a company in Japan (Cedar Pollen Extract-Cj; LSL, Tokyo, Japan). All the allergen extracts were used at a concentration of 1,000 protein nitrogen units (PNUs)/mL, except for the HDM (1:5,000 w/v), 4 insect mix (250 PNUs/mL) and Japanese cedar pollen extract (200 ng/mL). Histamine phosphate solution (0.01 mg/mL) and 0.9% sodium chloride were used as the positive and negative controls, respectively. The diameters of the wheal and flare were measured 15–20 min after injecting the extracts. When a flare reaction was observed at the injection site with a wheal diameter larger than that of the negative control, the reaction was judged as positive.

Allergen-specific serum IgE test: The serum allergen-specific IgE levels were measured with a human FcεRIα-based enzyme-linked immunosorbent assay (ELISA) (Allercept®; Heska Corporation, Ft Collins, CO, U.S.A.) [7] by a commercial laboratory in Japan (Hitachi Chemical, Ibaraki, Japan). The test was performed for the following 7 groups of allergen extracts: HDM mix (*D. farinae* and *D. pteronyssinus*), arthropods (flea saliva and cockroach), dog and cat epithelia, nutritional food ingredients (beef, chicken, pork, ram, fish mix consisting of cod, tuna and halibut, hen's eggs, cow's milk, wheat, corn, soy, rice and potato), molds (*Malassezia*, *Fusarium* and a mold mix consisting of *Alternaria*, *Rhodotorula*, *Aspergillus* and *Cladosporium*), ragweed, grass mix (ryegrass, timothy, meadow fescue, Kentucky bluegrass and Yorkshire fog) and trees (silver birch and Japanese cedar). Serum allergen-specific IgE levels >350 laboratory units (LUs) were interpreted as positive [7].

Assessment of lesional score: A modified version of the canine atopic dermatitis extent and severity index (CADESI) [23] was used for evaluation of skin lesions in the enrolled dogs. All the dogs were evaluated before, during and after the food elimination trial. The modified version of the CADESI was used to assess the severity (0, none; 1, mild; 2, moderate; and 3, severe) of the 4 cardinal signs of AD (pruritus, erythema, alopecia and lichenification) at 7 sites (face, pinna, thorax, axilla, inguina, feet and body trunk). Pruritus was assessed on the basis of the owner's report and objective signs indicative of pruritus such as excoriation. Therefore, the maximum CADESI that could be achieved was 84 (3 × 4 × 7). The CADESI was evaluated by one of the authors (S.K.) and validated by another author (J.I.). The accuracy of scores was verified using photographs of the skin lesions taken at each assessment period.

Table 1. Pruritic scoring system reported by Paterson [25]

Score	Severity of pruritus
1.	Dog not pruritic at all or scratches occasionally like a normal dog.
2.	Dog scratches/bites occasionally and is generally comfortable.
3.	Dog scratches and bites frequently, but not excessively.
4.	Dog scratches and bites frequently and often seems uncomfortable.
5.	Dog scratches and bites almost constantly and is considerably uncomfortable.

Decrease in the score by 5 points or more was judged as improvement of the skin lesions.

Assessment of the pruritus score: For assessing the level of pruritus, we applied a criterion reported by Paterson [25] (Table 1). The severity of the pruritus was categorized into 5 levels. Written information on the pruritus score was provided to the owners, and they recorded the pruritus scores daily during the study period. To evaluate the change of the pruritus score before and after the food elimination trials, average score for 3 weeks just before the food elimination trials were compared to those at the end of the trials. A decrease in the score by 0.7 points or more was judged as improvement of pruritus.

Assessment of medication score: Oral prednisolone (Predonine; Shionogi, Osaka, Japan) (0.5 mg/kg/day) was administered to the dogs when the pruritus score was 3 points or more or if it increased during the food elimination trial. The frequency of oral prednisolone administration was recorded by the owner as the medication score (times/week).

Elimination diet: A newly developed elimination diet (Aminoprotect Care®; Nosan Corporation) composed of potatoes and a balanced mixture of amino acids, corn starch, cellulose, soybean oil, fructooligosaccharides, vitamins, minerals and rosemary extract (Table 2) was used in this study. Potatoes were selected as an ingredient of the novel elimination diet because potato allergy has been rarely observed in dogs [6, 10, 11, 14–16, 19, 25, 30, 35], and it can reduce the high osmolality of the diet attributable to the free amino acid content. The metabolizable energy of the diet was 400 kcal/100 g dry matter. The nutrient contents of the diet met the minimal and maximal levels of the dog food nutrient profiles established by the Association of American Feed Officials (AAFCO) in 2009 [1].

Food elimination trial: The food elimination trial was performed using the novel elimination diet (Aminoprotect Care®) after informed consent was obtained from the owners who had agreed to use the novel elimination diet for their dogs. After at least 2 months of the food elimination trial, the dogs that showed improvement in the clinical symptoms, as determined on the basis of the changes in the 3 clinical scores (CADESI, pruritus score and medication score), were subjected to provocative challenge. Improvement in the dermatological symptoms was defined by cases that showed a decrease in the CADESI by 5 points or more or a decrease in the pruritus score by 0.7 points or more with an unchanged or decreased medication score. During the trial, the following parameters were assessed: body weight,

serum albumin concentration, gastrointestinal signs including the condition of stools, and palatability.

Provocative challenge: The procedures for the provocative challenge used in this study were previously described [11]. The following foods were used for the provocative challenge: meat (beef, pork, lamb and chicken), hen's eggs, milk products (cow's milk and cheese), fish (cod, tuna, salmon, mackerel and sea bream), grains (rice, bread and corn), vegetables (carrot, cucumber, broccoli, lettuce, cabbage, potherb-mustard, soybean, sweet-potato and potato) and fruits (apple, persimmon and banana). The offending foods were selected by the owners on the basis of the possibility of using them as diets for their dogs in the future and the practitioner's recommendation. The amounts of the offending foods for each ingredient were gradually increased for 1 week. Between the different provocative challenges, a 1-week washout period was set. Dogs that showed apparent recurrence of skin symptoms after the provocative challenge were diagnosed as having AFR to that particular food ingredient.

Supportive treatments: To control the overgrowth of *Staphylococcus* spp. and *Malassezia pachydermatis*, the dogs were shampooed with chlorhexidine shampoo (Nolvasan®) or chlorhexidine/miconazole shampoo (Malaseb®) 1–2 times a week.

Statistical analysis: The Wilcoxon rank-sum test was used for comparison of CADESI, pruritus score and medication score between groups before and after the food elimination trial. *P*-values less than 0.05 were considered significant. All statistical analyses were performed using the JMP version 8 software (SAS Institute, Cary, NC, U.S.A.).

RESULTS

Dogs: The breeds of the 20 dogs (Cases 1–20) enrolled in the present study were golden retriever (2 dogs), papillon (2), West Highland white terrier (2), Boston terrier (1), cavalier King Charles spaniel (1), English setter (1), French bulldog (1), German shepherd (1), Irish setter (1), Japanese terrier (1), Labrador retriever (1), miniature dachshund (1), Shiba dog (1), shih tzu (1), Siberian husky (1), Chihuahua (1) and a mixed breed (1). The ages of the dogs ranged from 1 year and 7 months to 11 years and 6 months (mean, 4 years and 9 months). The age at onset of clinical symptoms ranged from 2 months to 7 years and 3 months (mean, 2 years and 2 months). Of the enrolled dogs, 8 were males (1 castrated) and 12 were females (5 spayed).

Table 2. Nutrient profiles of the newly developed potato and amino acid based elimination diet (Aminoprotect Care[®], Nosan Corporation)

Nutrient	Units (dry matter basis)	Amino- protect Care [®]	AAFCO ^{a)}		
			Growth and reproduction minimum	Adult maintenance minimum	Maximum
Crude protein	%	24.8	22.0	18.0	
Arginine	%	1.7	0.62	0.51	
Histidine	%	0.5	0.22	0.18	
Isoleucine	%	1.1	0.45	0.37	
Leucine	%	2.1	0.72	0.59	
Lysine	%	1.6	0.77	0.63	
Methionine-Cystine	%	0.8	0.53	0.43	
Phenylalanine-Tyrosine	%	2.2	0.89	0.73	
Threonine	%	1.2	0.58	0.48	
Tryptophan	%	0.4	0.2	0.16	
Valine	%	1.5	0.48	0.39	
Crude fat	%	17.6	8.0	5.0	
Linoleic acid	%	8.8	1.0	1.0	
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Minerals					
Calcium	%	1.4	1.0	0.6	2.5
Phosphorus	%	0.9	0.8	0.5	1.6
Ca:P ratio		1.5:1	1:1	1:1	2:1
Potassium	%	0.8	0.6	0.6	
Sodium	%	0.5	0.3	0.06	
Chloride	%	0.8	0.45	0.09	
Magnesium	%	0.1	0.04	0.04	0.3
Iron ^{b)}	mg/kg	382.1	80.0	80.0	3,000.0
Copper ^{c)}	mg/kg	23.8	7.3	7.3	250.0
Manganese	mg/kg	33.3	5.0	5.0	
Zinc	mg/kg	267.2	120.0	120.0	1,000.0
Iodine	mg/kg	6.4	1.5	1.5	50.0
Selenium	mg/kg	0.2	0.11	0.11	2.0
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Vitamins					
Vitamin A	IU/kg	26,734.6	5,000.0	5,000.0	250,000.0
Vitamin D	IU/kg	3,849.0	500.0	500.0	5,000.0
Vitamin E	IU/kg	408.1	50.0	50.0	1,000.0
Thiamine ^{d)}	mg/kg	2.8	1.0	1.0	
Riboflavin	mg/kg	5.5	2.2	2.2	
Pantothenic acid	mg/kg	39.4	10.0	10.0	
Niacin	mg/kg	39.5	11.4	11.4	
Pyridoxine	mg/kg	2.3	1.0	1.0	
Folic acid	mg/kg	1.0	0.18	0.18	
Vitamin B12	mg/kg	0.1	0.022	0.022	
Choline	mg/kg	2,991.5	1,200.0	1,200.0	

a) Dog food nutrient profiles defined by AAFCO (2009) [1].

b) The amount of iron from carbonate and oxide source was excluded from the total amount of iron.

c) The amount of copper from oxide source was excluded from the total amount of copper.

d) The amount of thiamine was estimated in the dog food after processing.

Of the 20 dogs, 17 had already been fed elimination diets, including hydrolyzed diet (Cases 2, 8, 12, 13, 14 and 17), hydrolyzed and novel protein diets (Cases 11 and 20), novel protein diet (Cases 4, 5, 7, 9, 10, 15 and 16) and home-cooked diets (Cases 1 and 6) before initiation of the study.

The skin lesions were generally widespread and observed on the face (11 dogs), ears (9), thorax (6), legs (16), axilla (10), inguina (15), body trunk (5) and perianal area (5). The skin lesions included erythema (12), excoriation (3), scaling (16), hyperpigmentation (9), lichenification (5) and alopecia

(16).

Serum allergen-specific IgE levels were evaluated in 16 dogs. Of the 16 dogs, 10 were shown to be sensitive to allergens such as HDM (6 dogs), flea saliva (3), dog/cat epithelia (1), *Fusarium* (2), grass mix (1), silver birch (1) and potato (1) (Table 3).

Intradermal skin tests were performed in 17 dogs. Of the 17 dogs, 10 showed positive responses to allergen extracts including *D. farinae* (7 dogs), *D. pteronyssinus* (5), Japanese cedar pollen (2), 7 grass mix (1), molds (mixture of

Table 3. Results of allergen tests for the 20 dogs enrolled in the food elimination trial

Case No.	Positive results in the serum allergen-specific IgE test	Positive results in the intradermal skin test
1	Flea saliva, dog/cat epithelia	–
2	Silver birch	–
3	ND	ND
4	HDM, <i>Fusarium</i>	Der p, Der f, fleas, 4 insect mix, beef, Japanese cedar pollen, cow's milk, chickens
5	–	–
6	–	–
7	HDM	Der p, Der f, chickens, fish, grains
8	ND	Der f
9	–	–
10	HDM, flea saliva, <i>Fusarium</i>	Der f
11	–	Beef, cow's milk
12	–	Beef, Japanese cedar pollen, cow's milk, chickens, fish, grains
13	HDM	Der p, Der f
14	ND	Beef, cow's milk, chickens, grains
15	ND	–
16	–	ND
17	–	ND
18	HDM, potato, grass mix	Der p, Der f, fleas, 7 grass mix, molds
19	Flea saliva	–
20	HDM	Der p, Der f, 4 insect mix

HDM: House dust mite. ND: not done. –: not identified in the test carried out in this study. Der f: *D. farinae*. Der p: *D. pteronyssinus*. Chickens: mixture of chicken and hen's egg. Fish: mixture of tuna and cod. Grains: mixture of corn, rice and wheat.

Aspergillus mix, *Mucor* mix and *penicillium* mix extracts) (1), 4 insect mix (2), fleas (2), beef (4), cow's milk (4), chickens (mixture of chicken and hen's eggs extracts) (4), grains (mixture of corn, rice and wheat extracts) (3) and fish (mixture of tuna and cod extracts) (2) (Table 3).

Food elimination trial: Of the 20 dogs, 15 (Cases 1–15) completed the food elimination trial, whereas the remaining 5 could not because of skin disease progression (Case 18), diarrhea (Case 20) or diet unpalatability (Cases 16, 17 and 19) (Fig. 1). The changes in clinical scores of the 15 dogs before and after the food elimination trial are shown in Table 4. Improvement in the clinical scores was observed in 11 (Cases 1–11) of the 15 dogs that showed a decrease in the CADESI by 5 points or more or a decrease in the pruritus score by 0.7 points or more with an unchanged or decreased medication score. CADESI, pruritus score and medication score after the food elimination trial were significantly ($P<0.05$) lower than those before the trial in the improvement group (Cases 1–11). Improvement in the dermatological symptoms of the dogs that showed a decrease in the CADESI was observed predominantly on the face, legs, axilla and inguina (Fig. 2).

Provocative challenge: Of the 11 dogs that showed improvement in their clinical scores, 10 (Cases 1–10) could be subjected to the provocative challenge; this was because 1 owner refused to conduct the trial on her dog. The provocative challenge was carried out for 7–25 weeks using 5–13 food ingredients. Of the 10 dogs, 7 (Cases 1–7) showed recurrence of the clinical symptoms after exposure to offending food ingredients such as beef (2 dogs), pork (3), lamb (1), chicken (3), cow's milk (cheese) (2), salmon (1),

soybean (1), wheat (bread) (2), potato (1) and banana (1) (Table 5). Of these 7 dogs, 5 (Cases 1, 2, 3, 5 and 6) were shown to have AFR to at least 2 food ingredients. During the food elimination trials and the provocative challenge, none of the dogs showed nutritional insufficiency as indicated by body weight loss and/or decrease of serum albumin concentrations or gastrointestinal signs such as vomiting and/or diarrhea.

DISCUSSION

In the present study, 7 of the 14 dogs (50%) that completed both the food elimination trial and provocative challenges were shown to have AFR by using a newly developed potato and amino acid-based elimination diet (Aminoprotect Care®). The prevalence of AFR in dogs is not fully known; however, previous prospective and retrospective studies have indicated that approximately one-third of dogs with non-seasonal pruritus have AFR either alone or concurrent with other allergies [5, 6, 9, 16–18, 26, 27]. The frequency of AFR in dogs with non-seasonal pruritus observed in this study was higher than that reported in previous studies [5, 6, 9, 16–18, 26, 27], except for one (78%) [19]. Further studies should be conducted on a larger number of dogs to accurately determine the frequency of AFR in dogs.

For evaluation of the response to the food elimination trials, pruritus scores graded on 4- to 6-point scales have often been used [2, 5, 6, 17, 18, 25]. In these previous reports, a 50% decrease in the pruritus scores of enrolled dogs was considered to be improvement [2, 5, 6, 14, 15, 17, 18, 25]. In our study, the mean of the initial pruritus scores in the

Fig. 1. A schema for the results of the food elimination trial and provocative challenge. a) Dropout from the trial due to skin disease progression (1 dog), diarrhea (1) and diet unpalatability (3). b) Dropout due to owner's refusal.

Fig. 2. Changes of the skin lesions in 2 dogs that showed apparent improvement in dermatological symptoms after the food elimination trial. Lesions on the forelimbs in Case 2 before (a) and after (b) the food elimination trial. Erythema, lichenification and alopecia were markedly improved after the trial. Lesions extending from the abdominal to inguinal areas in Case 8 before (c) and after (d) the food elimination trial. Lichenification, hyperpigmentation and alopecia were markedly improved after the trial.

Table 4. Changes in the clinical scores of the 15 dogs that completed the food elimination trial

Case No.	Pruritus score (Points)		CADESI (Points)		Medication score (Times per week)		Change in the dermatological symptoms ^{e)}
	Before ^{a)}	After ^{b)}	Before ^{c)}	After ^{d)}	Before ^{a)}	After ^{b)}	
1	1.6	1.5	21	7	1.0	0.0	Improvement
2	2.5	1.7	32	10	0.0	0.0	Improvement
3	2.5	1.5	5	2	0.0	0.0	Improvement
4	1.9	1.0	31	18	1.3	0.0	Improvement
5	2.7	2.3	25	20	2.0	0.0	Improvement
6	1.4	1.8	12	2	1.0	0.0	Improvement
7	3.0	3.0	48	35	0.0	0.0	Improvement
8	3.7	1.7	51	29	1.3	0.0	Improvement
9	2.7	1.5	15	12	0.0	0.0	Improvement
10	2.0	2.0	35	8	4.7	2.3	Improvement
11	2.2	1.3	23	9	1.0	0.0	Improvement
12	2.3	2.8	12	10	0.0	3.7	No improvement
13	1.9	2.5	27	11	8.3	1.0	No improvement
14	3.0	3.5	8	8	0.0	0.0	No improvement
15	2.2	3.0	11	15	0.7	0.3	No improvement

a) Average score for 3 weeks just before the food elimination trial.

b) Average score for 3 weeks at the end of the food elimination trial.

c) Points at admission just before the food elimination trial.

d) Points at admission at the end of the food elimination trial.

e) Improvement in the dermatological symptoms was defined in cases that showed a decrease in the CADESI by 5 points or more or decrease in the pruritus score by 0.7 points or more with an unchanged or decreased medication score.

Table 5. Results of the provocative challenges in 10 dog

Case No.	Offending food ingredients used for the provocative challenges	
	Food ingredients that induced recurrence of dermatological symptoms	Food ingredients that did not induce recurrence of dermatological symptoms
1	Beef, pork, lamb, chicken, soybean, potato, banana	Cod, corn, apple, sweet potato, persimmon, carrot
2	Beef, wheat (bread)	Pork, chicken, hen's egg, rice, apple
3	Pork, chicken	Beef, cow's milk (cheese), wheat (bread)
4	Cow's milk	Beef, pork, lamb, chicken, hen's egg, rice, bread
5	Pork, cow's milk (cheese)	Beef, chicken, tuna, sea bream, mackerel, rice
6	Chicken, salmon	Rice, apple, potherb mustard
7	Wheat (bread)	Hen's egg, cow's milk, cabbage, carrot, cucumber, apple
8	—	Lamb, chicken, rice, sweet potato, broccoli
9	—	Beef, chicken, hen's egg, cod, bread, corn, cabbage, lettuce, carrot
10	—	Beef, pork, chicken, hen's egg, cow's milk, cod, rice, wheat (bread), cabbage, apple

—: Not identified in a series of provocative challenges in the present study.

enrolled cases was 2.4 points, and the basal pruritus score was 1 point. Since the mean of the 50% decrease of the pruritus score corresponded to 0.7 points [$0.7 \text{ points} = 1/2 \times (2.4 \text{ points} - 1.0 \text{ point})$], we tentatively defined improvement as a 0.7-point decrease or more. In contrast to the pruritus score, there have been a few studies using the lesional score for the outcome measure of food elimination trials in dogs [12, 13]. In these studies, the maximum decrease in CADESI after 1- to 2-month food-allergen avoidance by feeding of hydrolyzed diets was 6% (20/315 points) [12, 13]. In our study, a 6% change would correspond to a 5-point decrease in the CADESI (5/84 points). Using these criteria for improvement, the present study showed a significant decrease of clinical scores in 11 dogs (Cases 1–11) at the end of the trial.

Although further studies are required to assess the reliability and validity of the values used in the present study, clinical improvement after the food elimination trial was defined by the criteria as described in this study.

Of the dogs (Cases 1–10) subjected to provocative challenge, seven (Cases 1–7) showed recurrence of dermatological symptoms after intake of the offending food ingredients, which included beef, pork, chicken, cow's milk and wheat (Table 5). The common allergens reported to cause canine AFR are beef, pork, lamb, chicken, hen's eggs, cow's milk, rice, wheat and soybeans [6, 14–16, 21, 25, 30, 35]. Our previous studies on AFR in dogs in Japan [10, 11] also indicated similar results. Our findings in the present study were generally in agreement with the findings of pre-

vious studies on the allergenic food ingredients that induce AFR in dogs [6, 10–16, 21, 25, 30, 35].

In the present study, 4 (Cases 12–15) of the 15 dogs that had completed the food elimination trial showed no improvement of the dermatological symptoms. In these 4 cases, an increase in the pruritus score (0.5–0.8 points) was observed after the food elimination trial. The reasons why there was no improvement of the clinical scores at the end of the food elimination trial could not be precisely defined; however, it is possible that these dogs might have had AFR against potatoes or other ingredients included in the elimination diet. The potato and amino acid-based elimination diet developed in this study shows promise as an elimination diet, but further effort is needed to develop an ideal diet that does not elicit pruritus.

In the present study, Case 1 showed improvement in the clinical score following the food elimination trial and thereafter experienced recurrence of symptoms after provocation with boiled potatoes. Loss of allergenicity of processed food ingredients has been reported in cases of human food allergy [29]. It is possible that the potato proteins in the elimination diet might have lost their allergenicity during food processing. It has been reported that there are at least 4 major allergens in the potatoes that cause allergic reactions in humans [32, 33]. Further studies on the IgE binding epitopes in potato allergens in the elimination diet could improve the availability of a potato-based elimination diet for both diagnosis and long-term management of dogs with AFR.

Eleven (Cases 1–11) of the 15 dogs that completed the food elimination trial showed improvement in dermatological symptoms; however, even after completion of the food elimination trial, these dogs continued to show mild to moderate dermatological symptoms. It is possible that the dogs had a mild response to potatoes and other ingredients included in the elimination diet. Alternatively, the dermatological symptoms may be attributable to canine atopic dermatitis (cAD), which often occurs concurrently with AFR [2, 9, 18, 26]. We found that all of the dogs enrolled in this study fulfilled Willemse's criteria for cAD [36], and 6 of the 11 dogs (Cases 1, 2, 4, 7, 8 and 10) reacted positively to environmental allergens including HDM in the allergen-specific IgE test and/or IDST (Table 3). Alternatively, some of these dogs might have other skin diseases such as primary seborrhea, to which many of the breeds represented in this study are predisposed [31].

The elimination diet used in the present study was shown to be useful for diagnosis of AFR in dogs. Diagnosis of FH in dogs is known to be difficult because both IgE and T-cell mediated immune responses are conceivably involved in its pathogenesis [10–13, 24]. Elimination diets consisting of hydrolyzed protein can prevent cross-linking of Fcε receptors with food allergen-specific IgE; however, they are conceivably insufficient to prevent T-cell mediated immune responses because hydrolysis of the protein cannot necessarily break down the T-cell epitopes of 8–10 amino acids. The elimination diet consisting of amino acids and potato

protein is thought to avoid both IgE and T-cell mediated immune responses in FH.

In conclusion, the results of the food elimination trial and provocative challenge indicated the usefulness of the newly developed potato and amino acid-based diet as an elimination diet for diagnosis of AFR in dogs.

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