

A comparison of the clinical manifestations of feeding whole and hydrolysed chicken to dogs with hypersensitivity to the native protein

Rebecca Ricci*, Bruce Hammerberg†, Judy Paps‡, Barbara Contiero* and Hilary Jackson‡

*Department of Animal Science, University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy

†Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh, NC 27606, USA

‡Clinical Sciences, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh, NC 27606, USA
Correspondence: Hilary Jackson, Veterinary Dermatology Referral Services, 528 Paisley Road West, Glasgow G51 1RN, UK.

Email: pinerail@mac.com

Sources of Funding

This study was funded by a grant from Nestle Purina Petcare Company, St. Louis, MO, USA

Conflict of Interest

At the time of this study, Hilary Jackson was a member of the Nestle Purina advisory council.

Abstract

Twenty-six dogs with known adverse food reactions were fed whole chicken for 14 days. From this group, 12 dogs with cutaneous manifestations following exposure to chicken meat were selected and randomly divided into two groups ($n = 6$). Each group was then fed hydrolysed chicken or hydrolysed soy for 14 days in a blinded crossover design with a 17-day washout period between each diet. Assessments of a CADESI (Canine Atopic Dermatitis Extent and Severity Index) score and pruritus were performed throughout the entire study, and combined in a global score (GS). Serum was collected weekly for the measurement of chicken- and soy-specific IgG and IgE. Dogs displayed the most severe clinical response when eating whole chicken compared to baseline ($P < 0.001$). The GS was significantly reduced in 11 of the 12 dogs when fed hydrolysed chicken were compared to those fed whole chicken (3.58 ± 2.81 versus 20.38 ± 14.65 , $P < 0.01$). Serum immunoglobulin G and E responses were variable and did not show relationship with specific dietary exposure.

Accepted 10 August 2009

Introduction

Hydrolysed diets have been widely used in small animal practice in the past decade for the diagnosis and

management of presumptive adverse food reactions. Potentially allergenic proteins must be large enough to bridge at least two IgE molecules on the surface of mast cells to elicit degranulation and consequent release of inflammatory mediators.¹ Protein epitopes are often conformational and polyvalent, although sensitization to linear epitopes can occur.² The aim of hydrolysis is to break the peptide bonds in the amino acid chains to generate smaller peptide fragments from the protein molecule in order to reduce allergenicity and increase digestibility.³ There are still controversies about the minimum molecular weight of a potentially allergenic protein. Generally, major food allergen proteins in humans have a molecular weight ranging from 10 to 60 kDa, but there is evidence that in some cases this threshold can be lower than 1.4 kDa.⁴ The molecular weight, however, is not the sole determinant of allergenicity.

Several studies have been carried out to validate the efficacy of different hydrolysed products in the presumptive diagnosis of canine adverse food reactions.^{3,5–11} Although hydrolysed diets have been demonstrated to be useful in the diagnosis of dogs with suspected adverse food reactions, the majority of these studies do not address the issue of whether hydrolysed diets control clinical signs in dogs hypersensitive to the parent protein, nor is it known whether adverse food reactions in dogs are necessarily mediated by IgE. Cave and Guildford⁸ demonstrated the *in vitro* suitability of hydrolysed chicken proteins for the development of hydrolysed diets using sera of dogs sensitized to chicken proteins. Beale and Laflamme⁵ measured changes in pruritus after feeding a soy hydrolysate diet containing cornstarch (Nestle Purina Veterinary diet HA-formula, Nestle Purina, St Louis, MO, USA) for 2 weeks to client-owned dogs with sensitivity to corn or soy. They reported that pruritus was reduced by 50% in soy allergic dogs ($n = 4$) and 80% in corn allergic dogs ($n = 6$). Jackson *et al.*⁷ demonstrated that 3/11 (21%) of Maltese $\times$ beagle dogs with demonstrated sensitivity to corn and soy exhibited worsening of clinical signs when fed the same hydrolysed soy diet, although the remainder of the dogs seemed to tolerate it well. Finally, six dogs with induced soy sensitivity were shown to tolerate ingestion of increasing amounts of hydrolysed soy with no ill effects.¹⁰ These studies represent a small number of dogs and further investigations are required to better understand the efficacy of using hydrolysed diets for the diagnosis and long-term management of dogs with adverse food reactions that may involve sensitivity to the corresponding native protein.

Therefore, the primary purpose of this study was to verify if dogs with spontaneous adverse reactions to chicken can tolerate dietary exposure to hydrolysed chicken. Both clinical and immunological parameters were monitored.

Material and methods

Animals

This study was conducted using 26 Maltese × beagle dogs with a genetic predisposition to developing cutaneous and/or gastrointestinal adverse food reactions. Dogs were housed in the laboratory research facility at the College of Veterinary Medicine, North Carolina State University, and were under the care of the L.A.R. (Laboratory Animal Research unit). The experimental protocol adopted for the present research was approved by the Institutional Animal Care and Use Committee.

At the time of the study (spring–summer 2005), the colony consisted of six neutered females, 10 intact females and 10 intact males. Their mean age was 61 months (range 24–93 months) and mean average weight 5.1 kg.

The animals were kept in purpose-designed kennelling in groups of two or three individuals. They were usually maintained on a limited antigen diet consisting of salmon and rice (Hill d/d salmon and rice; Hills Pet Nutrition Inc., Topeka, KA, USA) fed twice a day and fresh water was constantly available. This diet was continued throughout the study. Ten of the 12 selected dogs had historical oral exposure to duck meat 3 years prior to this study. Five of the selected dogs had taken part in the previously described study⁷ and had known prior exposure to intact and hydrolysed soy without adverse reaction. All dogs were bathed monthly in a benzoyl peroxide shampoo and received monthly heartworm prophylaxis with ivermectin 6 µg/kg orally.

This investigation was divided into two phases. Phase 1 (PH1) involved all 26 dogs in the colony and its purpose was to screen the colony for clinical reactivity to whole chicken meat. Phase 2 (PH2) was designed to assess whether a dog sensitive to chicken meat would tolerate hydrolysed chicken.

Phase 1

In this phase, 5 g/kg (equal to 1.4 g/kg of protein content, calculated by BCA protein assay kit; Pierce, BCA Protein Assay, Rockford, IL, USA) of boneless, skinless chicken breast, baked without dressing (180 °C for 30 min), was added to their regular salmon and rice diet once daily for 14 days (days 0–13). Afterwards, a 3-week washout period with the maintenance diet was observed in order to allow any clinical signs to resolve. Twelve dogs that demonstrated a cutaneous clinical hypersensitivity to chicken meat as assessed by increased Canine Atopic Dermatitis Extent and Severity Index (CADESI) scores were selected for entry into PH2 of the study. Dogs were divided into five different classes based on a numeric score for each individual calculated by subtracting the baseline CADESI score from the highest CADESI score achieved after challenge:

- Class 0: CADESI score 0 (no clinical reaction)
- Class 1: CADESI score between 1 and 4 (slight clinical reaction)
- Class 2: CADESI score between 5 and 9 (mild clinical reaction)
- Class 3: CADESI score between 10 and 19 (mild-to-severe clinical reaction)
- Class 4: CADESI score ≥20 (severe reaction)

Phase 2

The dogs selected to enter PH2 were divided into two equal groups by computerized randomization (groups 1 and 2), and each

group was challenged by including 1.4 g/kg protein in their diet; either 5.4 g/kg of hydrolysed chicken or 1.8 g/kg of hydrolysed soy. The amount of each hydrolysed diet were estimated according to the BCA protein assay kit equal to 1.4 g/kg of protein content, the same amount delivered with the chicken meat during PH1. Hydrolysed soy was chosen as the control as it was known that either the selected dogs had no prior exposure, or had not developed any adverse reaction when previously exposed to this product.⁷

PH2 of the study was divided into two periods: phase 2-period 1 (PH2.1) and phase 2-period 2 (PH2.2). PH2 began at day 78 and lasted until day 133; new baseline values were registered at day 78 for PH2.1 and at day 110 for PH2.2. During PH2.1 (from days 80 to 93), group 1 was fed hydrolysed chicken whereas group 2 was given hydrolysed soy; during PH2.2 (from days 110 to 123) group 1 received hydrolysed soy and group 2 hydrolysed chicken, resulting in a blinded crossover design. A washout period of 17 days was allowed between PH2.1 and PH2.2 to allow clinical signs to resolve in all subjects. Historically, clinical signs in these dogs resolved within 14 days. A timeline of procedures carried out during the entire study is shown in Figure 1.

Clinical assessments

In order to establish the clinical reactivity of the dogs to the chicken meat and to the hydrolysed diets, clinical and pruritus assessments were performed before starting the trial (baseline) and once and three times a week respectively, throughout the study.

For the clinical assessments, the same investigator (HAJ) assigned a cutaneous clinical score to each dog, using the criterion previously adopted.⁷ Briefly, a modification of the CADESI scoring system was used to grade cutaneous lesions (erythema, papules, macules and excoriations) in 40 different parts of the body giving a maximum score of 480. An otologic examination, followed by a cytologic exam when necessary, was also performed at each assessment. Pruritus was scored from 0 to 5 (0 represented no pruritus; 5 constant pruritus) by a single investigator (JP) to whose presence the dogs were habituated. Each dog was observed over 30-min periods at the same time on each day that pruritus was assessed. The use of any anti-inflammatory treatment was strictly banned during the period of the study. Both the CADESI and the pruritus assessors were blinded to the identity of the dogs assigned to the groups and to the order of administration of the hydrolysed diets to each group during PH2.

The highest CADESI and pruritus scores achieved with the whole and hydrolysed chicken intake were calculated for each dog by sub-

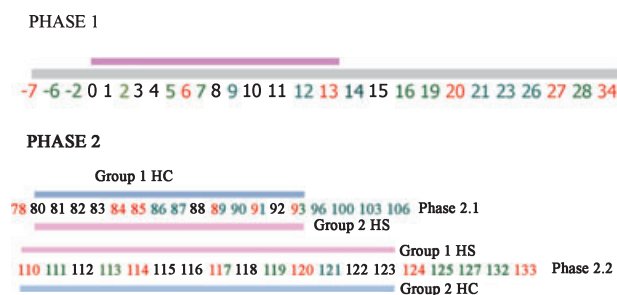


Figure 1. Phase 1: The grey bar represents the duration of the first phase. Numbers are days of the study. The purple bar represents the days when chicken was administered to all dogs. Baseline data was collected between days –7 and –2. Phase 2.1: Group 1 (blue) received hydrolysed chicken (HC) whereas Group 2 (pink) hydrolysed soy (HS). Phase 2.2: the sequence of administration of the diets was reversed. Green numbers: pruritus scores. Red numbers: CADESI scores and blood collection. Where numbers are red and green, all procedures were performed. Black indicates observation only. Where periods of observation were prolonged, some numbers have been missed.

tracting the baseline value from the highest value registered during the period when each food was fed. Additionally, a global score (GS = CADESI $\times$ pruritus/100) was calculated before and after whole and hydrolysed chicken administration as described elsewhere,¹² in order to combine all the information obtained from the clinical assessments.

Immunological investigation

A blood sample from all the dogs, at baseline and weekly thereafter, was collected for the measurement of serum chicken- and soy-specific IgE and IgG, as reported in Figure 1.

To date, no immunoglobulin values for IgG or IgE have been recognized as a 'threshold' above which a canine immunological adverse food reaction can be diagnosed. Nevertheless, for every sensitive dog, the increments of both chicken- and soy-specific IgE and IgG from the baseline were calculated at each phase by dividing the highest score achieved within PH1, PH2.1 and PH2.2 by the related baseline. The chicken-specific IgE and IgG increment from the baseline was also calculated for the chicken-unresponsive dogs during PH1.

Preparation of the allergens for the enzyme-linked immunosorbent assay

To prepare the chicken allergen for the enzyme-linked immunosorbent assay (ELISA), a sample of boneless skinless chicken breast, belonging to the same batch used to feed the dogs, was homogenized in a blender and then stirred at 10% w/v concentration in 0.9% NaCl overnight at 4 °C. The sample was centrifuged at 12 000 *g* for 30 min at 4 °C and the supernatant dialysed versus distilled water into 1 kDa cutoff membrane tubings to remove the excess salt. Finally, 10 $\times$ phosphate-buffered saline (PBS) at pH 7.4 was added to the sample in order to achieve a 1 $\times$ PBS solution. The solution was then centrifuged at 860 *g* for 30 min at 4 °C. 2-Mercaptoethanol 5% (v/v) was then added, and the solution heated for 10 min at 100 °C and dialysed versus 1 $\times$ PBS. After centrifuging the sample at 2500 rpm for 30 min at 4 °C, the supernatant was separated and stored at 4 °C for the ELISA experiments. The concentration was adjusted to 0.5 mg/mL (BCA protein concentration assay) for the experiments.

For the soy allergen, samples previously stored at -80 °C were used. They were obtained with an analogous procedure and their initial concentration (3.5 mg/mL) was adjusted to 0.5 mg/mL.

Measurement of serum allergen-specific IgE

Enzyme-linked immunosorbent assays were used to measure the serum chicken- and soy-specific IgE. The 96-well microtitre styrene U-bottom plates (Dyrex, Chantilly, VA, USA) were coated overnight at 4 °C with 50 μ L per well of a solution of the chicken or soy allergen diluted to 50 μ g/mL in 0.05 M sodium carbonate-bicarbonate buffer, pH 9.6. The coated plates were washed three times with PBS containing 0.05% Tween 20 (PBST) and then blocked with 200 μ L per well of 0.5% gelatin (Sigma, St. Louis, MO, USA) in PBST for 2 h at room temperature (RT). Blocked plates were washed once with PBST prior to adding 50 μ L per well of dog sera diluted 1:5 in PBST in duplicate. A reference serum with known high allergen-specific IgE diluted 1:5 was used in each plate in duplicate. After an incubation for 2 h at RT, plates were washed five times with PBST and then 50 μ L per well of 1 μ g/mL biotinylated mouse monoclonal 5.91 anti-canine IgE in PBST, containing 10% mouse serum and 10 μ g/mL of dog IgG (ChromPure Dog IgG, whole molecule; Jackson Labs, West Grove, PA, USA), was added. After incubation for 2 h at RT, plates were washed again five times and then incubated for 30 min at RT with 50 μ L per well of Zymax streptavidin-horseradish peroxidase conjugate (Zymed Laboratories, San Francisco, CA, USA) diluted 1:1000 in PBST with 1% bovine serum albumin. Finally, plates were washed five times with PBST before the addition of 50 μ L per well of ABTS (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA). The optical density of each well was read at 450 nm on a Tecan Sunrise microtitre plate reader (Tecan group, Durham, NC, USA) after an overnight incubation to obtain adequate colour development.

Measurement of serum allergen-specific IgG

The ELISA was used to measure the serum chicken- and soy-specific IgG following a procedure similar to that used for IgE. Briefly, the differences were that the canine serum samples and reference serum were diluted 1:1000 in PBST. Bound IgG was detected using 50 μ L per well of canine anti-IgG (peroxidase-conjugated affinity purified rabbit anti-dog IgG, Fc fragment-specific, 0.8 mg/mL; Jackson Labs) diluted 1:10 000 in PBST, containing 10% rabbit serum and 1 μ g/mL of dog IgE. The plates were then incubated at RT with 50 μ L per well of ABTS (Kirkegaard & Perry Laboratories) for 1 h before the optical density was read at 450 nm on a Tecan Sunrise microtitre plate reader (Tecan).

Statistical analysis

Clinical and serological data were analysed using PROC MIXED (SAS, 2003)¹³ of analysis of variance (ANOVA) in order to take into account the random effect of the animals, and *P*-values <0.05 were considered significant. A SPLIT-PLOT model was used because of the nested effect due to dogs within treatment groups. Phases, days of the study, group and the interactions between 'group' and 'phases', and between 'group' and 'days of the study' were considered fixed effects, whereas dog was considered a random effect. Least squares means (LSMEANS) have been calculated for the fixed effects and standard error has been provided as uncertainty index of the estimation.

To compare the average GS observed before and after whole chicken ingestion in the entire colony, before and after hydrolysed chicken ingestion in the 12 selected dogs, and to compare the increment of GS measured in the 12 dogs during whole and hydrolysed chicken intake, a *t*-test for two-paired samples with different standard deviations was used and *P*-values <0.05 were considered significant.

To compare the IgG increment from the baseline between the group of chicken-responsive and chicken-unresponsive dogs during PH1 of the study, a PROC GLM (SAS, 2003) general linear model ANOVA was used, whereas PROC MIXED (SAS, 2003) was used to compare the increments observed after whole and hydrolysed chicken in the same group of 12 chicken sensitive dogs.

Results

Clinical CADESI assessment

During PH1, 25 of 26 dogs showed an increased CADESI score after chicken meat once daily for 2 weeks. Twelve subjects, falling into classes 3 and 4, were selected for PH2 of the study. Prior to starting PH2, one dog developed unrelated problems and was substituted with a class 2 dog. Gastrointestinal disturbances were not recorded during PH1 or PH2.

The average CADESI score (LSMEANS) calculated during the entire study was not influenced by the group. Dogs displayed the most prominent clinical response while eating whole chicken, whereas no significant differences were detected between PH2.1 and PH2.2 (PH1:10.32 versus PH2.1:4.30 versus PH2.2:2.69, *P* < 0.001; Figure 2).

CADESI LSMEANS calculated for the 12 chicken-sensitive dogs analysed as a group therefore yielded the highest scores between days 6 and 20 of chicken meat administration, and scores were almost seven times higher than the baseline (*P* < 0.001). The principal clinical signs consisted of otitis, erythema, papules and excoriations (Figure 3). Significant differences were seen among days of the experimental period (*P* < 0.001), but when the two groups were compared, the CADESI scores were only significantly affected by

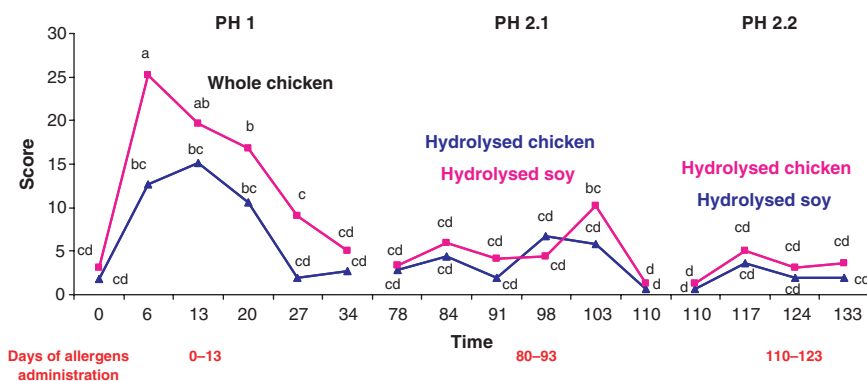


Figure 2. Least squares means (LSMEANS) of the clinical CADESI scores calculated at each time point in group 1 (blue line) and group 2 (pink line) during PH1, PH2.1 and PH2.2. On the top of the lines is the name of the allergen that each group was challenged with during the corresponding phase; on the bottom is the time interval in which the dogs were fed the allergens inclusion. Different letters mean differences at $P < 0.05$ both within groups (all along the entire experimental period) and between groups (at the same time). SE for all LSMEANS = 3.19.



Figure 3. Erythema and excoriations on the ventral abdomen of a participating dog after ingesting whole chicken.

phases ($P < 0.05$) but not by the days of the study ($P = 0.79$).

The clinical responses that two groups of dogs displayed towards each dietary exposure (whole chicken, hydrolysed chicken or hydrolysed soy) are compared in Figure 4. Both groups displayed the highest cutaneous response when eating whole chicken during PH1 (whole chicken-group 1 versus whole chicken-group 2, 7.5 versus 13.1, $P = 1.00$), and for group 2 this was significantly stronger than that registered during hydrolysed chicken administration in PH2.2 (whole chicken-group 1 versus hydrolysed chicken-group 1: 7.5 versus 3.7, $P = 0.13$; whole chicken-group 2 versus hydrolysed chicken-group 2: 13.1 versus 3.3, $P < 0.001$). Administering hydrolysed chicken or hydrolysed soy to the dogs did not result in a significant different clinical score either within group (hydrolysed chicken-group 1 versus hydrolysed soy-group 1: 3.7 versus 2.1, ns; hydrolysed chicken-group 2 versus hydrolysed soy-group 2: 3.3 versus 4.8, ns), or between groups.

The reaction to hydrolysed chicken was reduced compared to that with whole chicken in 11 of the 12 dogs when the CADESI score was considered. Eight of 12 dogs showed a reduction of 80% or greater. However, when compared with baseline values prior to entering

Table 1. Highest clinical CADESI score registered for the 12 chicken-sensitive dogs after eating whole and hydrolysed chicken and percentage of clinical improvement obtained switching from the native to the processed protein ingestion

Dog	CADESI		% of improvement
	After chicken intake	After hydrolysed chicken intake	
1	23	2	91.30
2	6	17	-183.33
3	29	8	72.41
4	20	4	80
5	10	2	80
6	12	0	100
7	46	8	82.61
8	21	14	33.33
9	21	0	100
10	36	6	83.33
11	17	7	58.82
12	17	2	88.24

CADESI, Canine Atopic Dermatitis Extent and Severity Index.

PH2, the CADESI score increased by five or more increments in six (50%) of the dogs eating hydrolysed chicken and four chicken-sensitive dogs (33%) eating hydrolysed soy (Table 1). Interestingly, two of these latter dogs had a prior history of soy exposure. Clinical signs worsened in only one dog when moving from intact to hydrolysed protein.

Pruritus assessment

The pruritus score at baseline on the maintenance diet alone was never more than 2 for all the dogs during all phases of the study. Among the 12 chicken-sensitive dogs, selected on the basis of most marked clinically evident lesions, pruritus increased by two or more grades (of five) in three dogs during PH1, and five dogs when hydrolysed chicken was fed. Only one dog showed the same pruritus reaction towards both whole and hydrolysed chicken. Three dogs increased pruritus by two or more grades when hydrolysed soy was fed, and all of them had previous soy exposure⁷. No statistically significant differences were detected in pruritus scores either between groups, phases or days of the experimental

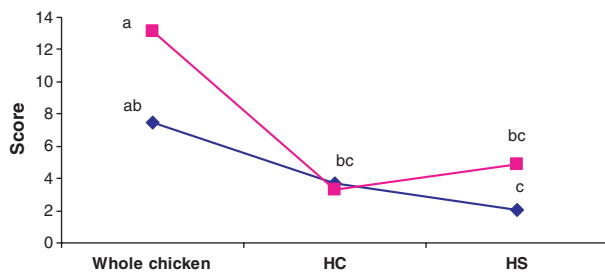


Figure 4. Contrast analysis of least squares means (LSMEANS) of the clinical CADESI scores obtained from group 1 (blue line) and group 2 (pink line) while dogs were on chicken, hydrolysed chicken (HC) and hydrolysed soy (HS). Different letters mean differences at $P < 0.05$ both within groups and between groups. SE for all LSMEANS = 2.30.

study (Figure 5). In addition, when the groups were compared, pruritus scores registered at different phases or at different days of the study were not statistically different. There was no relationship between the pruritus score and CADESI for individual dogs.

Global score

When CADESI and pruritus scores were combined in the GS, there was a significant increase over baseline after feeding whole chicken in PH1 (average GS $\pm$ standard deviation: baseline 0.038 ± 0.051 versus whole chicken 0.40 ± 0.48 , $P < 0.001$), whereas feeding hydrolysed chicken in PH2 resulted in much less of a reaction (average GS $\pm$ standard deviation: baseline PH2 0.067 ± 0.054 versus hydrolysed chicken 0.233 ± 0.267 , $P = 0.056$). When the increment of GS after whole and hydrolysed chicken (calculated as GS after challenge/GS baseline) were compared, the hydrolysed diet was associated with a reduction in 11 of 12 dogs (average GS $\pm$ standard deviation: whole chicken 20.38 ± 14.65 versus hydrolysed chicken 3.58 ± 2.81 , $P < 0.01$).

Quantification of serum allergen-specific immunoglobulins

Allergen-specific IgE measurements were very variable and in many cases undetectable by the ELISA, whereas

IgG antibodies were within the assay detection range. Therefore, only IgG measurements have been statistically analysed and discussed.

Figure 6 shows the chicken-specific IgG trend observed during the experimental study. Chicken- but not soy-specific IgG levels were affected by the phases of the study, and with time there was an overall trend towards reduced chicken-specific IgG ($P = 0.04$). Chicken-specific serum IgG concentrations did not differ during PH1 between the dogs that reacted clinically to the protein and those that were unresponsive.

A wide variability in both chicken and soy-IgG increments was observed among the chicken-responsive dogs after the different dietary treatments. In particular, chicken-IgG increments ranging from <1 (which indicate a decrease from the baseline) to 7.18 times the baseline were registered during whole chicken intake and from <1 to 19.47 after hydrolysed chicken. Serum chicken-IgG increments were detected in some dogs even after hydrolysed soy challenge (data not shown). In few cases, an increment of serum soy-specific IgG was identified in dogs fed hydrolysed chicken (data not shown).

When the effect of dietary treatments on the chicken-specific IgG increments were compared within chicken responsive group, no statistically significant differences were detected (IgG increment of chicken responsive group after whole chicken challenge: 2.28 versus IgG increment of chicken responsive group after hydrolysed chicken challenge: 3.22, $P = 0.54$).

Discussion

In this double-blinded, placebo-controlled study, we demonstrated that feeding a hydrolysed chicken diet to dogs with a cutaneous clinical response to whole chicken results in significant amelioration of their clinical signs as measured by CADESI and a GS, which included the evaluation of pruritus.

Chicken protein was tested as it is a common ingredient in puppy and adult dog foods, and its constant ingestion may be responsible for an early food sensitization. Moreover, of the three pet food companies that manufacture hydrolysed diets for dogs, one uses chicken as a

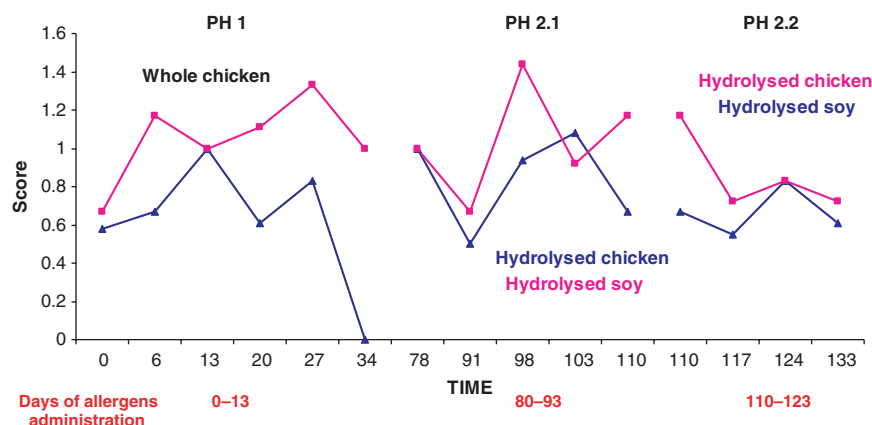


Figure 5. Least squares means (LSMEANS) of pruritus score calculated at each time point in group 1 (blue line) and group 2 (pink line) during PH1, PH2.1 and PH2.2. On the top of the lines is the name of the allergen that each group was challenged with during the corresponding phase; on the bottom is the time interval in which the dogs were fed the allergens inclusion. SE for all LSMEANS = 0.33.

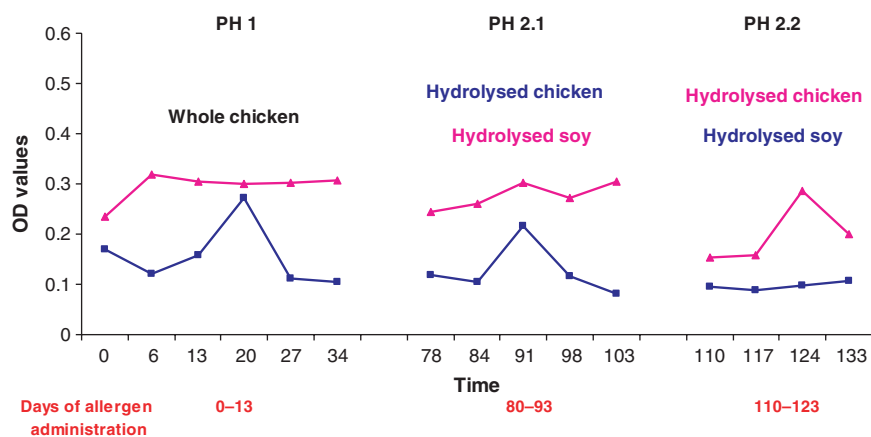


Figure 6. Least squares means (LSMEANS) of the chicken-specific IgG calculated at each time point in group 1 (blue line) and group 2 (pink line) during PH1, PH 2.1 and PH 2.2 of the study. On the top of the lines is the name of the allergen that each group was challenged with during the corresponding phase; on the bottom is the time interval in which the dogs were fed the allergens inclusion. SE for all LSMEANS = 0.12.

primary protein source, and one as a secondary source with soy being the primary hydrolysate. The first phase of the study aimed at detecting a subset of dogs sensitized to whole chicken. This was performed by feeding all dogs a known quantity of chicken meat once a day for 2 weeks and assessing the symptoms displayed by each individual using CADESI and pruritus scores. Twelve dogs showed evident cutaneous signs of adverse food reaction. Interestingly, ten of these twelve dogs had previous exposure to poultry containing dog food (d/d Duck and Rice; Hills Pet Nutrition, Topeka, KS, USA) 3 years prior to this study,⁷ which may account for their prior sensitization to poultry meat. Although there is no evidence to confirm this hypothesis in dogs, cross-reactivity between avian meat proteins has been reported in humans,^{14,15} and chicken serum albumin (also called *alpha-livetin*, m.w. 70 kDa) has been identified as the cross-reactive protein.^{16–20}

In the present study, hydrolysed chicken was well tolerated by 11/12 dogs, and eight of them experienced a highly satisfactory improvement (>80%) in the CADESI score compared with that seen when fed whole chicken. These findings are consistent with the previously cited studies, which demonstrate an improvement in clinical hypersensitivity when the hydrolysed protein is fed compared with the native protein in the majority of dogs.^{5,7,10} It is, however, important to highlight that Puigdemont *et al.*¹⁰ used dogs sensitized to soy by subcutaneous injections, whereas the studies performed by Jackson *et al.*,⁷ and Beale and LaFlamme⁵ employed dogs with naturally occurring hypersensitivity, which may be more relevant to clinicians and owners.²¹

In one dog, however, feeding hydrolysed chicken worsened the clinical signs compared with feeding whole chicken. This may be due to an insufficient degree of enzymatic proteolysis, which is an important variable for the successful outcome of a hydrolysed diet.¹ In this study, a sample of hydrolysed chicken had been submitted to a Western-blot analysis before being fed to the dogs, and the molecular weight was below 10 kDa (data not shown). Reactions to partial whey-hydrolysed formulas have been reported in cow's milk-allergic children, and occasional reactions to even the most extensively

hydrolysed casein or whey formulas have been demonstrated in highly sensitive children.^{22–24} Furthermore, it cannot be excluded that the reaction in this dog could have been triggered by a hapten effect or the reassembly of epitopes *in vivo* after the ingestion of the hydrolysed formula.²⁵ The possibility of an adverse reaction to a hydrolysed formula to dogs sensitized to the parent protein, even if rare, should always be considered and close monitoring during introduction of the diet is recommended.

Pruritus is a cardinal clinical sign in canine cutaneous dietary hypersensitivity.²⁶ It is present in the majority of cases, and its partial or complete resolution following a restriction diet and relapse on challenge with the normal diet are the generally adopted criteria to diagnose adverse food reactions.²⁷ In this study, however, challenging the dogs with the three diets (whole chicken, hydrolysed chicken and hydrolysed soy) provoked variable pruritus between individuals. In addition, pruritus did not generally correlate with the clinical lesion score, even though this included a measure of excoriation. It is nevertheless possible that pruritus was actually present but was not displayed by the dogs in the 30-min observation period.

A recent study using the same colony of dogs also found that pruritus and CADESI scores were not necessarily correlated.¹¹ Pruritus was a more prominent sign, but it was not always associated with an increase in lesion scores. This study, however, used a different allergen for oral challenge (corn) and a shorter duration of challenge (5 days), which might not have allowed obvious clinical signs to develop. In addition, the two-dimensional pruritus scale (PVAS) evaluated both the duration and intensity of pruritus. It has been shown that the design of the pruritus scale can significantly affect the final scores, particularly when assessed by dog owners^{28,29} or external observers.³⁰ Extrapolation from pruritus assessed by clients-owned dogs to a laboratory animal setting, however, may not be valid. An alternative method of pruritus assessment would be the use of an internal video-recording system as described by Plant.³¹

To compensate for the lack of correspondence between cutaneous lesions and pruritus in dogs with adverse reaction to foods, a GS (=CADESI × pruri-

tus/100) has been proposed to yield a unique score by merging all available clinical information.¹² Feeding hydrolysed chicken significantly ameliorated the GS in the majority of dogs sensitized to the parent protein.

In humans, there is a relationship between certain food (e.g. milk, egg and peanut) specific IgE concentrations and positive responses to oral challenge.³² It has therefore been proposed that food-specific IgE values could be used to predict the risk–benefit ratio of food challenges to reduce the likelihood of a significant allergic reaction. In our study, in contrast, no relationship was found between the clinical outcomes and the humoral-specific immune responses. Specifically, the IgE levels were very low to undetectable during the entire study. This might indicate that the adverse food reactions were not mediated by IgE. However, food-specific IgE has been detected previously in similar studies with this colony of dogs, so it is possible that there was a problem with the ELISA. If adverse food reactions in these dogs are at least partially mediated by IgE, then the low-serum IgE concentrations may reflect limited clinical severity, particularly in some of the mildly affected individuals, as noted in humans. As hydrolysed formulas were developed specifically for human infants with IgE-mediated milk allergy, it is interesting to speculate as to why we saw clinical improvement in these dogs in the absence of measurable allergen-specific IgE. Aside from problems with the IgE ELISA methodology, one possibility is that the hydrolysis procedure was sufficient to disrupt epitopes recognized by T-cells in the sensitized individuals. The administration of the different diets to dogs evoked no differences in the serum chicken- and soy-specific IgG concentrations both within group and between groups. This suggests that, in this population, feeding both an offending and a non-offending diet does not enhance a different allergen-specific IgG response. Interestingly, in some cases, an increment of chicken-specific IgG was observed following the hydrolysed soy challenge. This observation is similar to that described in a previous study using the same dogs,⁷ in which a group of 14 dogs sensitized to both corn and soy were challenged with corn, and some had a corn- and/or soy-specific IgE response. Such results do not support using serum allergen-specific IgE or IgG levels in the diagnosis or management of specific adverse food reactions in dogs, and as such is in agreement with previous reports.^{33,34}

The duration of dietary challenge in this study was different from that in previous reports,^{7,10,11,35} although the study by Muller and Tsohalis³⁴ did not mention the frequency of allergen administration. In both studies by Jackson *et al.*,^{7,35} the allergens were given orally on two occasions 24 h apart, whereas Puigdemont *et al.*¹⁰ administered six doses at increasing concentrations 20–30 min apart or until any adverse reaction was observed. Olivry *et al.*¹¹ provided corn-sensitive dogs with a low allergen diet containing corn starch twice daily for 5 days, but speculated that the lack of fluctuation of corn-specific IgE levels was due to the short duration of the challenge. Our study focused on the humoral immune response in response to persistent allergen ingestion (once daily for 14 days), which more closely resembles natural exposure to food allergens. Despite this, neither allergen-specific IgE nor IgG predicted the dogs' clinical

response. The trend towards reduced allergen-specific IgG levels during the study, however, may reflect decreased immunogenicity of the hydrolysed proteins.

In conclusion, 14-day administration of hydrolysed chicken was well tolerated by the majority of dogs with a confirmed reactivity to chicken meat. It therefore appears that hydrolysed chicken results in a satisfactory clinical improvement in the majority of cases with spontaneous adverse food reactions to chicken. Nevertheless, dogs sensitized to the native protein may still react to the hydrolysed protein, and treated dogs should be carefully monitored. Interestingly, previous exposure to duck may predict hypersensitivity to other closely avian meats. Allergen-specific IgE and IgG levels, in contrast, were not diagnostic and did not predict the response to the hydrolysed food. One weakness of the study was the relatively small number of dogs and the single protein employed, and further studies with larger cohorts of dogs and additional, potentially cross-reacting proteins, should be performed.

References

1. Taylor SL, Lehrer SB. Principles and characteristics of food allergens. *Critical Reviews in Food Science and Nutrition* 1996; 36(Suppl.): S91–118.
2. Untersmayr E, Jensen-Jarolim E. The role of protein digestibility and antacids on food allergy outcomes. *Journal of Allergy and Clinical Immunology* 2008; 121: 1301–8.
3. Biourge VC, Fontaine J, Vroom MW. Diagnosis of adverse reactions to food in dogs: efficacy of a soy-isolate hydrolysate based diet. *Journal of Nutrition* 2004; 134: 2062S–4S.
4. Van Hoeyveld EM, Escalona-Monge M, de Swert LF *et al.* Allergenic and antigenic activity of peptide fragments in a whey hydrolysate formula. *Clinical and Experimental Allergy* 1998; 28: 1131–7.
5. Beale KM, Laflamme DP. Comparison of a hydrolyzed soy protein diet containing corn starch with a positive and negative control diet in corn- or soy-sensitive dogs (abstract). *Veterinary Dermatology* 2001; 12: 237.
6. Rosser ER. Evaluation of a novel carbohydrate and hydrolyzed protein containing diet in previously confirmed food allergic dogs (abstract). *Veterinary Dermatology* 2001; 12: 230.
7. Jackson HA, Jackson MW, Coblenz L *et al.* Evaluation of the clinical and allergen specific serum immunoglobulin E responses to oral challenge with cornstarch, corn, soy and a soy hydrolysate diet in dogs with spontaneous food allergy. *Veterinary Dermatology* 2003; 14: 181–7.
8. Cave NJ, Guildford WG. A method for in vitro evaluation of protein hydrolysates for potential inclusion in veterinary diets. *Research in Veterinary Science* 2004; 77: 231–8.
9. Loeffler A, Lloyd DH, Bond R *et al.* Dietary trials with a commercial chicken hydrolysate diet in 63 pruritic dogs. *Veterinary Record* 2004; 154: 519–22.
10. Puigdemont A, Brazis P, Serra M *et al.* Immunologic response against hydrolysed soy protein in dogs with experimentally induced soy hypersensitivity. *American Journal of Veterinary Research* 2006; 67: 484–8.
11. Olivry T, Kurata K, Paps JS *et al.* A blinded randomized controlled trial evaluation the usefulness of a novel diet (Aminoprotect Care) in dogs with spontaneous food allergy. *Journal of Veterinary Medical Science* 2007; 69: 1025–31.
12. Olivry T, Paps JS, Bizikova P *et al.* A pilot open trial evaluating the efficacy of low-dose aminopterin in the canine homologue of human atopic dermatitis. *British Journal of Dermatology* 2007; 157: 1040–2.
13. SAS. Statistical Analysis Systems Institute. User's Guide: Statistics, Version 6. Cary, NC: SAS Institute Inc., 2003.

14. Cahen YD, Fritsch R, Wüthrich B. Food allergy with monovalent sensitivity to poultry meat. *Clinical and Experimental Allergy* 1998; 28: 1026–30.
15. Kelso JM, Cockrell GE, Helm RM *et al.* Common allergens in avian meats. *Journal of Allergy and Clinical Immunology* 1999; 104: 202–4.
16. de Blay F, Hoyet C, Candolfi E *et al.* Identification of alpha livetin as a cross reacting allergen in a bird-egg syndrome. *Allergy Proceedings* 1994; 15: 77–8.
17. Szepefalusi Z, Ebner C, Pandjaitan R *et al.* Egg yolk alpha livetin (chicken serum albumin) is a cross reactive allergen in the bird-egg syndrome. *Journal of Allergy and Clinical Immunology* 1994; 93: 932–42.
18. Bausela BA, Garcia-Ara MC, Martin Esteban M *et al.* Peculiarities of egg allergy in children with bird protein sensitization. *Annals of Allergy and Asthma Immunology* 1997; 78: 213–6.
19. Quirce S, Diez-Gomez ML, Eiras P *et al.* Inhalant allergy to egg yolk and egg white proteins. *Clinical and Experimental Allergy* 1998; 28: 478–85.
20. Quirce S, Maranon F, Umpierrez A *et al.* Chicken serum albumin (Gal d5) is a partially heat-labile inhalant and food allergen implicated in the bird-egg syndrome. *Allergy* 2001; 56: 754–62.
21. Kennis RA. Use of atopic dogs to investigate adverse reactions to food. *Journal of the American Veterinary Medical Association* 2002; 221: 638–40.
22. Saylor JD, Bahna SL. Anaphylaxis to casein hydrolysate formula. *The Journal of Pediatrics* 1991; 118: 71–4.
23. Giampietro PG, Kjellman N-IM, Oldaeus G *et al.* Hypoallergenicity of an extensively hydrolyzed whey formula. *Pediatric Allergy and Immunology* 2001; 12: 83–6.
24. Caffarelli C, Plebani A, Poesi C *et al.* Determination of allergenicity to three cow's milk hydrolysates and an amino acid-derived formula in children with cow's milk allergen. *Clinical and Experimental Allergy* 2002; 32: 74–7.
25. Cave NJ. Hydrolyzed protein diets for dogs and cats. *Veterinary Clinics of North America: Small Animal Practice* 2006; 36: 1251–68.
26. Verlinden A, Hesta M, Millet S *et al.* Food allergy in dogs and cats: a review. *Critical Reviews in Food Science and Nutrition* 2006; 46: 259–73.
27. Chesney CJ. Systematic review of evidence for the prevalence of food sensitivity in dogs. *Veterinary Record* 2001; 148: 445–8.
28. Hill PB, Lau P, Rybníček J. Development of an owner assessed scale to measure the severity of pruritus in dogs. *Veterinary Dermatology* 2007; 18: 301–8.
29. Rybníček J, Lau-Gillard PJ, Harvey R *et al.* Further validation of a pruritus severity scale for use in dogs. *Veterinary Dermatology* 2009; 20: 115–22.
30. Plant JD. Repeatability and reproducibility of numerical rating scales and visual analogue scales for canine pruritus severity scoring. *Veterinary Dermatology* 2007; 18: 294–300.
31. Plant JD. Correlation of observed nocturnal pruritus and actigraphy in dogs. *Veterinary Record* 2008; 162: 624–5.
32. Perry TT, Matsui EC, Conover-Walker M *et al.* The relationship of allergen-specific IgE levels and oral food challenge outcome. *Journal of Allergy and Clinical Immunology* 2004; 114: 144–9.
33. Jeffers JG, Shanley KJ, Meyer EK. Diagnostic testing of dogs for food hypersensitivity. *Journal of the American Veterinary Medical Association* 1991; 198: 245–50.
34. Muller R, Tsohalis J. Evaluation of serum allergen specific IgE for the diagnosis of food adverse reactions in the dog. *Veterinary Dermatology* 1998; 9: 167–71.
35. Jackson HA, Hammerberg B. Evaluation of a spontaneous canine model of immunoglobulin E-mediated food hypersensitivity: dynamic changes in serum and fecal allergen-specific immunoglobulin E values relative to dietary change. *Comparative Medicine* 2002; 52: 316–21.

Resume Vingt-six chiens atteints de réactions d'intolérance alimentaire ont été nourris avec du poulet pendant 14 jours. Douze chiens de ce groupe montrant des lésions cutanées suite à l'exposition à la viande de poulet ont été sélectionnés et répartis au hasard dans deux groupes ($n = 6$). Chaque groupe a ensuite été nourri avec du poulet hydrolysé ou du soja hydrolysé pendant 14 jours en aveugle et croisé avec une période de transition de 17 jours entre chaque régime. L'évaluation de l'extension de la dermatite atopique canine et du score de sévérité (CADESI) et de prurit ont été réalisés tout au long de l'étude et associés en un score global (GS). Du sérum a été prélevé chaque semaine pour mesurer les IgG et IgE spécifiques du poulet et du soja. Les chiens présentant la réponse clinique la plus sévère étaient ceux qui mangeaient le poulet complet ($P < 0.001$). Le GS était significativement plus bas pour 11 des 12 chiens quand ils étaient nourris avec du poulet hydrolysé en comparaison à ceux nourris avec le poulet complet (3.58 ± 2.81 vs 20.38 ± 14.65 , $P < 0.01$). Les réponses des immunoglobulines G et E étaient variables et ne présentaient pas de relation avec la nature du régime.

Resumen Veintiséis perros con diagnóstico de reacción adversa alimentaria fueron alimentados con pollo durante 14 días. Dentro de este grupo doce perros con manifestaciones cutáneas tras la exposición a carne de pollo fueron seleccionados y divididos al azar en dos grupos ($n = 6$). Cada grupo fue alimentado con pollo hidrolizado o soja hidrolizada durante 14 días en un diseño ciego cruzado con un intervalo de 17 días de limpieza entre cada dieta. Se estimaron los valores de extensión y severidad de dermatitis atópica (CADESI) y prurito a lo largo del estudio y se combinaron en un valor global (GS). Se tomó suero cada semana para medir las IgG e IgE específicas frente a pollo y soja. Los perros presentaron el valor clínico mas severo cuando fueron alimentados con pollo entero en comparación con el valor basal ($P < 0,001$). El GS se redujo de forma significativa en 11 de los 12 perros cuando fueron alimentados proteína hidrolizada de pollo en comparación con perros alimentados pollo entero ($3,58 \pm 2,81$ vs $20,38 \pm 14,65$, $P < 0,01$). Las respuestas de IgG e IgE fueron variables y no presentaron relación con la exposición a la dieta.

Zusammenfassung Sechszwanzig Hunden, bei denen eine Futterunverträglichkeit bekannt war, wurde 14 Tage lang ganzes Huhn gefüttert. Aus dieser Gruppe wurden zwölf Hunde, die eine kutane Manifestation auf die Hühnerfütterung zeigten, ausgewählt und nach dem Zufallsprinzip in zwei Gruppen aufgeteilt ($n = 6$). Jeder Gruppe wurde daraufhin nach einem geblindeten Crossover Design entweder hydrolysiertes Hühnchen oder hydrolysiertes Soja 14 Tage lang gefüttert, mit einer Wash-Out Periode von 17 Tagen zwischen den beiden Diäten. Beurteilungen nach dem „Canine Atopic Dermatitis Extent and

Severity Score'' (CADESI) und Bewertungen des Pruritus wurden während der ganzen Studie durchgeführt und in einem „Global Score'' (GS) zusammengefasst. Es wurde wöchentlich Serum gewonnen, um Huhn- und Soja-spezifische IgG und IgE zu messen. Im Vergleich zum Ausgangspunkt zeigten die Hunde eine hochgradige klinische Reaktion, wenn ihnen ganzes Hühnchen gefüttert wurde ($P < 0,001$). Der GS war bei 11 der 12 Hunde, wenn ihnen hydrolysiertes Hühnchen gefüttert wurde, im Vergleich zu denen, die ganzes Huhn bekamen signifikant reduziert ($3,58 \pm 2,81$ vs $20,38 \pm 14,64$, $P < 0,01$). Die Immunglobulin G und E Antworten im Serum waren variabel und zeigten keine Beziehung zu spezifischer Fütterung.