J Vet Intern Med 2010;24:1350–1357

A Randomized, Open-Label, Positively-Controlled Field Trial of a Hydrolyzed Protein Diet in Dogs with Chronic Small Bowel Enteropathy

P.J.J. Mandigers, V. Biourge, T.S.G.A.M. van den Ingh, N. Ankringa, and A.J. German

Background: Hydrolyzed protein diets are commonly used to manage canine chronic enteropathies (CE), but their efficacy has not yet been critically evaluated.

Hypothesis: A hydrolyzed protein diet is superior to that of a highly digestible (control) diet in the management of CE in dogs.

Animals: Twenty-six dogs (18 test diet, 8 control diet) referred for investigation and management of naturally occurring chronic small intestinal disease.

Methods: Randomized, open-label, positively controlled trial. After a full diagnostic investigation, which included endoscopy, dogs were assigned either to the test diet or control diet on a 2 : 1 basis (test : control). Cases were re-evaluated 3 times (at approximately 3, 6–12 months, and 3 years). Outcome measures included response of clinical signs (complete, partial, none), change in severity of signs (based upon clinical disease activity index; canine inflammatory bowel disease activity index [CIBDAI]), change in body weight, and need for other therapy.

Results: There were no significant differences in baseline characteristics (eg, signalment, body weight, and duration of clinical signs), and histopathologic severity between test and control diet groups. However, despite randomization, CIBDAI was significantly higher in the test diet group ($P = .013$). Most dogs had responded by first evaluation, with no difference between groups ($P = .87$). However, significantly more dogs on the test diet remained asymptomatic at both the second ($P = .0012$) and third ($P < .001$) re-evaluation, and the decrease in CIBDAI was significantly greater ($P = .010$).

Conclusions and Clinical Importance: A hydrolyzed protein diet can be highly effective for long-term management of canine chronic small bowel enteropathy.

Key words: Canine; Gastrointestinal; Inflammatory bowel disease.

Adverse reactions to food (ARF) are a common cause of gastrointestinal signs and can be divided into 2 major groups: immunologic (eg, dietary hypersensitivity where an aberrant immune responses is involved) and nonimmunologic (including food intolerance and dietary indiscretion).[1,2] Although etiologies may vary, clinical signs are usually indistinguishable. Furthermore, it can also be difficult to distinguish ARF from other chronic enteropathies (CE) such as inflammatory bowel disease (IBD), even after endoscopy and histopathologic assessment of gastrointestinal biopsies.[2,3] There are also some suggestions that either IBD can be triggered by prior ARF, or ARF could arise secondary to the immune dysregulation that develops during IBD.[1] As a result, some recent studies have preferred to use the term CE, and to subdivide cases based upon response to therapy.[4,5]

Abbreviations:

ARF	adverse reaction to food
CE	chronic enteropathy
CIBDAI	canine inflammatory bowel disease activity index
IBD	inflammatory bowel disease
TLI	trypsin-like immunoreactivity.

Traditional exclusion diets incorporate a single novel protein, and a variety of formulations are available, but their use is limited by the fact that most commercial maintenance diets contain a mix of proteins, meaning that dogs are often already exposed to numerous potential allergens. It is also possible that widespread feeding of table scraps and treats exacerbates the problem by expanding the range of proteins to which dogs are exposed, such that it can sometimes be difficult to find a suitable exclusion diet.

Recently, several hydrolyzed protein diets have been developed for management of food allergy in humans,[6] and similar rations recently have become available for companion animals. These diets are suggested to be "hypoallergenic" because the hydrolytic process reduces the native protein into polypeptides of a size that are unlikely to stimulate the immune system.[7] Results from a recent experimental model of canine dietary allergy do indeed suggest that this may be the case.[8,9] However, such diets have not yet been critically evaluated in dogs with CE. Therefore, based upon the hypothesis that hydrolyzed protein diets would be superior in managing canine CE, the purpose of this randomized, positively controlled study was to compare the efficacy of such a diet with that of a highly digestible (control) diet.

From the Department of Clinical Sciences of Companion Animals, Utrecht University, The Netherlands (Mandigers); The Royal Canin Research Center, Aimargues, France (Biourge); TCCI Consultancy BV, Utrecht, The Netherlands (van den Ingh); the Department of Pathobiology, Faculty of Medicine, Utrecht University, The Netherlands (Ankringa); and the School of Veterinary Science, University of Liverpool, Neston, UK (German). All studies were performed at the Veterinary Specialist Centre "De Wagenrenk," BN Wageningen, The Netherlands.

Corresponding author: Alexander J. German, School of Veterinary Science, University of Liverpool, Leahurst Campus, Chester High Road, Neston CH64 7TE, UK; e-mail: ajgerman@liverpool.ac.uk.

Submitted May 23, 2010; Revised August 25, 2010; Accepted September 15, 2010.

10.1111/j.1939-1676.2010.0632.x

Materials and Methods

Study Objectives

The objective of the study was to determine whether a hydrolyzed protein diet was superior to a highly digestible diet in managing cases of naturally occurring canine CE, as judged by subjective improvement in clinical signs at 3 re-evaluation appointments. Study participants were defined as owners who gave informed consent for their dogs to be enrolled in the study, and study units were defined as the individual dogs that were enrolled.[10] In all cases, each participant was only responsible for a single study unit.

Participants and Study Units

The primary study observer (P.J.J.M.) was responsible for recruiting and enrolling all participants and their respective dogs. Dogs were referred to the Veterinary Specialist Centre "The Wagenrenk" between December 2001 and January 2003 for investigation and management of signs of gastrointestinal disease of at least 2 months' duration. The terminology adopted in the current study was similar to that used in some recent studies[4,5] in that the aim was to recruit dogs suffering from the syndrome of CE, of suspected small intestinal origin. Accordingly, dogs were included if clinical signs of small intestinal disease were present, signs suggestive of a large intestinal disorder were absent, and a complete diagnostic investigation (including endoscopy) had been performed to eliminate other causes (eg, systemic disorders, infectious diarrhea, and architectural gastrointestinal diseases such as partial obstructions of the intestinal tract). Dogs were excluded if they had received corticosteroid therapy in the 3 weeks before initial enrollment, or when there was evidence of hypoproteinemia (low total protein concentration, serum albumin concentration <20 g/L).

A total of 340 dogs were referred with gastrointestinal signs during the time frame of the study, 194 of which underwent endoscopy (Fig 1). After exclusion of other causes and elimination of dogs that had recently received corticosteroid therapy, 65 dogs fitted the study inclusion criteria. Of these, the owners of 26 dogs ultimately agreed to participate, whereas the owners of the remaining 39 dogs declined the invitation to participate.

Diagnostic Investigations

In order to minimize study variation, the primary study investigator (P.J.J.M.) assessed all dogs at all visits. A complete history was taken and a physical examination performed. Subsequently, detailed laboratory investigations were performed, including a complete blood count, a serum biochemistry profile (eg, urea, creatinine, alkaline phosphatase, alanine aminotransferase, total bilirubin, bile acids, calcium, phosphate, total protein, albumin, globulins, and protein electrophoresis), serum trypsin-like immunoreactivity, fecal parasitology (2 separate samples tested for Giardia by zinc sulfate centrifugal flotation and nematode parasites by flotation with sugar solution), and fecal bacteriological culture (for *Salmonella* spp. and *Campylobacter* spp.). Thereafter, survey abdominal radiography and abdominal ultrasonography were performed. Finally, the primary study investigator performed upper gastrointestinal endoscopy in all individuals. During this procedure, multiple mucosal biopsies were collected from the stomach (body and fundus) and upper small intestine (duodenum and, where feasible, jejunum) for histopathologic analysis. Trained animal technicians cared for all dogs during their hospitalization for clinical procedures.

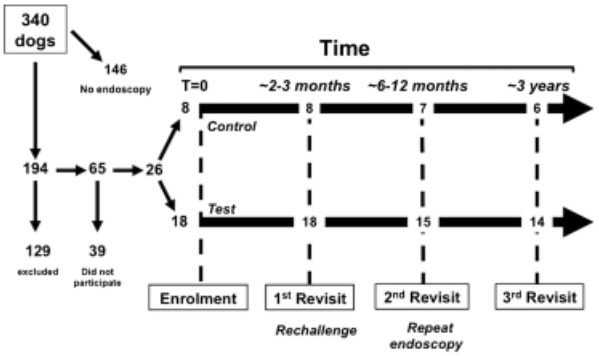

Fig 1. Summary of the trial design and inclusion of dogs. Other than the timeline, all numbers refer to dogs recruited and remaining within the trial at the respective points.

Randomization Procedure and Allocation of Study Diets

The study was designed as a prospective, open-label, randomized, positively controlled trial. After obtaining informed consent, the dogs were numbered sequentially and then randomly allocated, either to the test or control diet, in a 2:1 ratio. The primary study investigator performed the randomization and allocated dogs to the different diet groups. The random number function of computer software[a] was used to generate the case sequence. The study was open-label in that both the primary observer and participant were aware of which diet the study subjects received. Given that the study used diets which were commercially available in their normal packaging (rather than plain packaging), it was not feasible to blind participants. However, when discussing the trial and during all follow-up appointments, the investigators avoided inadvertently biasing participants, by not expressing opinions as to which diet was expected to be superior.

During the study period, participants maintained a diary, in which they recorded any gastrointestinal signs and listed any changes from the normal routine of the dog. This diary was reviewed by the primary study observer at each re-evaluation, and also was used to assign a severity score to the clinical signs.

Diets

The test diet was a commercially available diet containing hydrolyzed soy protein (Table 1),[b] whereas the control food also was commercially available, was highly digestible, and contained proteins from a variety of sources (Table 1).[c] As well as the difference in the type of protein incorporated, the test diet had a lower protein and higher carbohydrate content relative to the control; but, macronutrient and energy contents were otherwise broadly similar. Diets were fed as per the manufacturers's recommendations. For dogs in ideal body condition, the normal weight guide on the packet was used to determine the initial daily allocation, and this level of energy intake approximated to a maintenance energy requirement (MER) of $132 \times (\text{body weight in kg})^{0.73}$ kilocalories (Kcal)/d. For dogs that were underweight, the underweight feeding scale was used, approximating $1.25 \times$ MER, whereas in dogs deemed to be overweight, the overweight guide was used which approximated $0.75 \times$ MER. The amount fed was adjusted at each visit, based upon response (ie, ration was increased or decreased by 10–20% if there had been an increase or decrease in body weight, respectively). However, the exact amounts of food given at all stages in the study were not recorded as part of the study. Participants were instructed to switch their dog to the new diet progressively, over 7 days, by gradually increasing the proportion of new diet fed. Instructions also were given about ensuring that the chosen diet was fed exclusively (ie,

Table 1. Composition of the diets used in the study.

	Test Diet (4,182 kcal/kg)[a,c]		Control Diet (4,268 kcal/kg)[b,c]	
Nutrient	g/100 g[d]	g/1,000 kcal[e]	g/100 g[d]	g/1,000 kcal[e]
Moisture	9.0	21	9.0	21
Protein	21.0	50	30.0	70
Fat	19.0	45	20.0	47
Carbohydrate	37.6	90	27.4	64
Total dietary fiber	5.4	13	6.3	15
Minerals	6.5	19	7.3	17
Omega 6	4.4	10.5	3.9	9.1
Omega 3	0.8	1.9	0.7	1.6
EPA + DHA	0.3	0.8	0.4	0.9
List of ingredients	Rice, soy protein isolate digest, animal fat, mineral salts, beet pulp, hydrolyzed poultry liver, vegetable oils (including borage oil), zeolite, fructo-oligosaccharides, fish oil, L-tyrosine, chelated trace elements, taurine, DL-methionine, marigold flower extract (with high lutein content), vitamins.		Poultry meal, rice (extruded), corn meal, animal fat, poultry liver, vegetable oil (including copra oil), egg powder, brewer's yeast, mineral salts, soy protein isolate, beet pulp, purified cellulose, fructo-oligosaccharides, fish oil, zeolite, trace elements (including chelated trace elements), yeast extracts (with high mannan-oligosaccharide content), taurine, marigold flower extract (with high lutein content), vitamins.	

[a]Hypoallergenic diet, Royal Canin, Aimargues, France.
[b]Intestinal diet, Royal Canin, Aimargues, France.
[c]Metabolizable energy (ME) content of the diet, measured by bomb calorimetry.
[d]Nutrient content expressed in g/100 g of product as fed.
[e]Nutrient content expressed in g/1000 kcal ME.
EPA, eicosapentanoic acid; DHA, docosahexanoic acid.

avoiding consumption of treats or table scraps and avoiding access to food of other dogs in the household by feeding individual dogs separately and lifting up food bowls when not in use). No other medication was given throughout the trial, and dogs were only allowed to drink fresh water.

Study Follow-Up

First Re-Evaluation. Participants and their dogs returned to the clinic for an initial reassessment, approximately 3 months after the start of the study. Any dog that had not responded by this time was considered to be a nonresponder, and other treatments were added, as deemed necessary by the attending clinician. Participants of dogs that responded to the diet were instructed to challenge their dogs with the original diet, for a period of 7 days, and an ARF was confirmed when clinical signs recurred.[11]

Second Re-Evaluation. A second detailed re-evaluation was performed approximately 6–12 months after the 1st visit. Dogs were re-evaluated by the primary study investigator, and subjective response again was determined. Additionally, if the participant gave consent, gastrointestinal endoscopy was repeated. If dogs had relapsed at this stage, other therapies were offered, based upon the clinical opinion of the primary study observer.

Third Re-Evaluation. A final re-evaluation was performed approximately 3 years after the beginning of the trial to determine long-term efficacy of dietary management. As with the previous assessments, clinical response was determined subjectively. If dogs had relapsed at this stage, other therapies again were offered, and the primary study investigator determined the type of therapy used.

Other Client Communication. In addition to the official re-evaluation visits, contact was maintained with clients using e-mail updates and, if necessary, follow-up telephone calls. The amount of contact required varied depending upon the individual case.

Outcome Measures

Primary Outcome Measure. The primary outcome measure of interest was subjective response to therapy, and this was scored as complete, partial, or no response. Assessment of this criterion was made at all 3 re-evaluations by the primary study observer, with reference to the information provided by the owner at the time of re-evaluation. The term complete response was assigned to cases in which clinical signs had abated completely (total absence) or had returned to what the owner believed to be normal for the animal before the onset of the problem. The term no response was used either when there had been no noticeable change in the frequency and severity of clinical signs, or when any improvement was not convincingly greater than the natural variation in frequency of clinical signs for that dog (ie, a change to 3 times weekly signs from 4 times weekly). Finally, the term partial response was used when noticeable improvement had occurred, but clinical signs remained and their frequency was greater than what the owner believed to be the animal's normal situation. In all such cases, frequency of signs had decreased by at least 50%.

Secondary Outcome Measures. The 2 main secondary outcome measures were change in body weight and change in severity of clinical signs, as determined retrospectively with reference to clinical data recorded at the time of assessment. In addition, any additional therapies that were required in any dogs were reported, although group differences in frequency of use of such therapies were not assessed statistically.

Retrospective Grading of Clinical Signs. In order for results to be comparable with recent studies, clinical signs were retrospectively reviewed and scored according to a validated scheme for recording severity of gastrointestinal signs (canine inflammatory bowel disease activity index [CIBDAI]).[12] Briefly, 6 gastrointestinal signs (ie, attitude and activity, appetite, vomiting, stool consistency, stool frequency, and weight loss) were scored in terms of severity (from

0 to 3); the scores then were summed to produce a composite score (0–3, not clinically relevant; 4–5, mild; 6–8, moderate; ≥9, severe). All clinical records, taken at the time of evaluation, and the owner diary records were used to assign CIBDAI at the initial visit, as well as the first and second re-evaluations. Insufficient details were available from the notes taken at the final follow-up to estimate CIBDAI at this stage. The records first were made anonymous (such that it was not possible to determine the case and which visit), and then reviewed by the primary study investigator on a single occasion.

Histopathologic Assessment

Tissue samples collected during endoscopy were fixed in 10% neutral buffered formalin, routinely dehydrated, and embedded in paraffin. Slides (4 μm) were stained with hematoxylin and eosin. The results of the histopathologic assessment performed at the time (by a number of pathologists within the department) were used in case management (ie, by excluding cases that were not consistent with CE such as lymphoma or lymphangiectasia). However, in order for the results of the current study to be compared with more recent research, as many slides as possible were reviewed on a single occasion, by an European board-certified pathologist (T.S.G.A.M.I.) in conjunction with a pathology resident in training (not available), and graded using recent internationally accepted criteria.[13] For this assessment, gastric biopsy slides were available from 12/18 test diet dogs and 4/8 control diet dogs, whereas intestinal biopsy slides were available from 17/18 test diet dogs and 8/8 control diet dogs.

Data Handling and Statistics

Statistical analyses were performed with a computer software program,[d] and descriptive statistics were used to report baseline data (either median and range or mean ± standard deviation). Before the study, the sample size calculation function of the statistical software package was used to estimate the required number of subjects. For the calculation, we assumed a 2:1 case:control recruitment rate, an expected response rate of 49%,[11] and assumed that the response rate in control dogs would be half the response rate in cases. Based upon an α value of 0.05, 18 cases and 9 controls would be required to produce an 80% power for detecting such a difference.

Given the fact that study numbers were small, and the outcome measures were either proportions (eg, response to therapy, type of histopathologic inflammation) or categorical data (eg, CIBDAI, severity of inflammation), nonparametric statistical tests were used throughout. Initially, baseline characteristics were compared to determine the appropriateness of the randomization process and comparability of the groups. These included age, sex, weight, CIBDAI, and severity of histopathologic changes. Tests used included the Mann-Whitney test or Fisher's exact test and, given that multiple comparisons were performed, a modified Bonferroni's correction was applied.[14] To confirm further that groups were similar, the proportion of cases that relapsed after challenge with the original diet (at the 1st revisit) also was assessed, again by Fisher's exact test.

For comparisons of the primary outcome measure (subjective response to therapy), the proportion of cases responding at each visit was assessed (within each group) by the χ^2 test for trend. Comparisons between groups were made at each visit by Fisher's exact test, with a modified Bonferroni correction for multiple comparisons. For assessment of the secondary outcome measure (CIBDAI scores), changes in scores between each visit were made within groups by Friedman's test (nonparametric 2-way analysis of variance), with Conover posthoc comparisons. Comparisons between diet groups were made at each visit with the Mann-Whitney test, with a modified Bonferroni correction for multiple comparisons. The level of statistical significance was set at $P < .05$ for 2-sided analyses.

Results

Dogs

Twenty-six dogs were finally included in the study, 8 of which were assigned to the control diet and 18 to the test diet. Before referral, a variety of medications had been given by the referring veterinarians, including antiemetics (6 dogs), acid-blocking drugs (5 dogs), sucralfate (8 dogs), antibacterials (7 dogs), and sulfasalazine (3 dogs), all without effect. A range of diets also had been administered before referral, including highly digestible low residue diets (7 dogs), standard and premium grocery foods (8 dogs), novel protein diets (9 dogs), and homemade diets (3 dogs). However, none of these diets had been used strictly or given for a period of >3 weeks, and none of the dogs had responded to any of these diets (data not shown). There was no significant difference in frequency of administration of any therapy or diet type between groups (data not shown).

There were no reported palatability issues with either diet, and none of the dogs needed to be withdrawn as a consequence. A range of ages, sexes, breeds, and body weights were included, with no specific differences between groups (Table 2). There also was no significant difference between groups for duration of clinical signs before presentation (test group: median, 211 days [range, 67–2,931 days]; control group: median, 165 days [range, 90–1,080 days]; Table 2, $P = .71$), and starting body weight (test group: median, 29.6 kg [range, 6.3–39.5 kg]; control group: median, 10.3 kg [range, 4.7–40.0 kg]; Table 2, $P = .126$).

Despite randomization, the severity of clinical signs, as retrospectively judged by the CIBDAI, was significantly worse in dogs on the test diet than the control diet dogs (test group: median, 5 [range, 2–11]; control group: median, 3 [range, 2–7]; Table 3, $P = .013$). This was, in part, related to the fact that the 3 dogs classified as having severe disease (CIBDAI > 9) all were allocated to the test group during randomization.

Endoscopy and Histopathology

Endoscopic and histopathologic assessments of both gastric and duodenal biopsies were performed in all dogs before starting dietary management. Gross findings were not evident in 15 dogs, an increased tendency to hemorrhage after biopsy was seen in 11 dogs (usually in both stomach and duodenum), and the duodenal mucosal surface was irregular and hyperemic in 5 dogs. There were no differences in the presence of such lesions between the 2 diet groups ($P = .96$). When the available histopathology specimens were independently reviewed in accordance with recently published guidelines, gastric changes were described as lymphoplasmacytic inflammation (test 4, control 1), mixed inflammation (test 3, control 1), or normal (test 4, control 2); intestinal changes were classified as lymphoplasmacytic inflammation (test 2, control 2), eosinophilic

Table 2. Starting characteristics of dogs on the 2 different diets.

Criterion	Test Diet	Control Diet	*P* Value
Breed	Berner Sennenhond	Boxer	—
	Border Collie, Boxer	Huskie	
	CKCS (3), Crossbred	Jack Russell Terrier	
	Duitse Herder, Groenendaeier	Kooikerhondje	
	Golden Retriever (2)	Lakeland Terrier	
	Hovawart, Labrador Retriever	Rhodesian Ridgeback	
	Newfoundland, Niesenschnauzer	Shetland Sheepdog	
	Rhodesian Ridgeback	Shih Tzu	
	Rottweiler, Tibetan Terrier		
Sex	Male (5)	Male (3)	.569
	Neutered male (4)	Neutered male (0)	
	Female (2)	Female (2)	
	Neutered female (7)	Neutered female (3)	
Age (years)	3.3 (0.6–11.0)	3.5 (0.5–10.8)	.605
Duration of signs (days)	211 (67–2931)	286 (90–1386)	.714
Body weight	29.6 (6.3–39.5)	10.3 (4.7–40.0)	.126

Numerical data are expressed as median (range).

inflammation (test 7, control 2), mixed inflammation (test 4, control 2), or normal (test 4, control 2). The severity of gastric inflammation was scored as mild (test 4, control 2), moderate (test 2, control 0), severe (test 1, control 0), or normal (test 3, control 2); the severity of intestinal inflammation was scored as mild (test 8, control 3), moderate (test 4, control 3), or normal (test 4, control 2). None of the histopathologic specimens from either stomach or duodenum had changes consistent with severe inflammation. There were no significant differences in either the type (gastric, $P = .99$; small intestinal, $P = .93$), or severity (gastric, $P = .61$; small intestinal, $P = .92$) of inflammation between the test and control dogs.

Table 3. Outcome variables in the dogs on the 2 different diets.

Criterion	Test Diet	Control Diet	*P* Value
Dogs remaining in trial			
First recheck	18 (100)	8 (100)	>.999[a]
Second recheck	15 (83)	7 (88)	.846[a]
Final recheck	14 (78)	6 (75)	.871[a]
Response rate[b]			
First recheck	12, 4, 2 (89)	6, 1, 1 (88)	.871[a]
Second recheck	13, 2, 0 (87, 13, 0)	2, 0, 5 (28, 0, 72)	.0012[a]
Final recheck	11, 3, 0 (79, 11, 0)	1, 0, 5 (12, 0, 88)	<.001[a]
Body weight (kg)			
First recheck	29.3 (6.3–42.0)	10.4 (5.4–36.4)	.063[c]
Second recheck	30.0 (6.5–47.0)	10.3 (5.0–44.0)	.089[c]
Final recheck	NA	NA	NA
CIBDAI			
Initial visit	5 (2–11)	3 (2–7)	.013[c]
First recheck	0 (0–6)	0 (0–5)	.825[c]
Second recheck	0 (0–3)	1 (0–4)	.010[c]
Final recheck	NA	NA	NA

CIBDAI, chronic inflammatory bowel disease activity index; NA, not available.

CIBDAI, where 6 gastrointestinal signs (eg, attitude and activity, appetite, vomiting, stool consistency, stool frequency, and weight loss) are severity scored (from 0 to 3); the scores are summed and a composite score produced and categorized as follows: 0–3 = clinically insignificant; 4–5 = mild; 6–8 = moderate; ≥ 9 = severe.

[a]*P* values quoted are for Fisher's exact test, with modified Bonferroni's correction, comparing the test and control diets.

[b]For response rate, the numbers represent complete responders, partial responders and nonresponders, respectively, while the number in parentheses represents the percentage of responders (complete and partial combined). Body weight and CIBDAI data are expressed as median (range).

[c]*P* values for body weight and CIBDAI are for the Mann-Whitney test, with modified Bonferroni's correction, comparing the test and control diets.

First Re-Evaluation

Initial re-evaluation was conducted after a median of 90 days (range, 43–223 days) when 16/18 (89%) of dogs on the test diet had responded, 12 completely and 4 partially (eg, occasional vomiting and diarrhea). For the control diet, 7/8 dogs had responded, 6 completely and 1 partially. There was no significant difference in the initial response rate between diets ($P = .87$). CIBDAI decreased significantly in both groups (Table 3), and there was no significant difference in CIBDAI between groups at the initial re-evaluation ($P = .82$). However, given that starting CIBDAI was higher in the test group, the magnitude of decrease was significantly greater in this group ($P < .001$).

Body weight increased significantly in dogs fed the test diet (median, 4%; range, −3–22%; $P = .012$) with increases noted in 13 dogs, a decrease in 1 dog, and stable weight in the other 4 dogs. In contrast, weight did not change significantly in the control diet group (median, 0%; range, −9–17%; $P = .16$), with increases noted in 4 dogs, decreases in 2 dogs, and stable weight in 2 dogs. There was no difference in body weight, however, between groups ($P = .063$) at the time of this first re-evaluation.

Twenty-two of the 23 dogs that responded subsequently were rechallenged with their original diet; the other dog (on the test diet) was not challenged because of the wishes of the participant. Eleven of 16 (69%) of the dogs on the test diet relapsed within a week of rechallenge, whereas signs did not relapse in the remaining 5

dogs (31%). Similarly, 4/6 (67%) of the dogs on the control diet relapsed on rechallenge. There was no significant difference in the rate of relapse upon rechallenge in either group ($P = .92$).

Second Re-Evaluation

A second re-evaluation was conducted on 22 of the 23 responding dogs after a median of 232 days (range, 100–476 days). The other dog (on the test diet) had died before re-evaluation from an unrelated nongastrointestinal disease. Of the 15 dogs assigned to the test diet that were re-evaluated, 13 (87%) had remained asymptomatic provided that the test diet was fed, and the remaining 2 were partial responders in that they had improved but had occasional gastrointestinal signs. In contrast, significantly fewer dogs (2/7, 28%, $P = .0012$) assigned to the control diet had remained asymptomatic and, as a consequence, CIBDAI was significantly higher in these dogs than in those on the test diet (Table 3, $P = .010$). For the test diet dogs, median (range) body weight had increased significantly compared with initial enrollment (median, 5%; range, −7–28%; $P < .001$), but there was no significant difference between the 1st and 2nd re-evaluation ($P = .323$). For the control diet dogs, there was no significant difference in body weight between the second re-evaluation and either initial enrollment ($P = .17$) or the first re-evaluation ($P = .17$). Again, there was no difference in body weight between groups ($P = .089$) at this evaluation.

As a result of the relapsing signs, additional therapy was administered to 5 (71%) of the dogs on the control diet, including prednisolone (n = 2), hyoscine butylbromide (n = 1), and switching to the test diet (n = 1). Repeat endoscopy was performed in 5 dogs on the test diet (all free from clinical signs) and in 3 dogs originally on the control diet (all relapsed, and currently on prednisolone [n = 2] or the test diet [n = 1]). There was no significant change in either the type or severity of histopathologic changes in any of the dogs ($P > .90$ for all).

Third Re-Evaluation

A final follow-up was performed a median of 1,284 days (range, 619–1,562 days) after the start of the study. Twenty of the 23 dogs had remained in the trial, 14 (78%) of which had started (and remained) on the test diet and 6 (75%) that had started with the control diet. Occasional gastrointestinal signs were noted in 1/14 test diet dogs (7%), whereas an additional 2 (14%) were asymptomatic on the test diet, but developed clinical signs if additional food materials were consumed. In contrast, significantly fewer dogs (1/6, 17%, $P < .001$) originally given the control diet were still in remission. The test diet was subsequently given to 3 of these dogs, and clinical signs resolved. One of the remaining 2 dogs responded to additional therapy (prednisolone and azathioprine), whereas the remaining dog continued to show signs despite the use of a variety of therapies.

Discussion

The current study assessed the efficacy of a hydrolyzed protein diet for the management of CE in dogs. The study has been reported according to the recently published "Reporting Guidelines for Randomized Control Trials" (REFLECT statement).[10] Twenty-six dogs were selected from a group of 340 dogs referred with chronic gastrointestinal signs, and dogs were randomized to either a test (hydrolyzed protein) or control diet. The latter diet differed from the control diet, in that it was highly digestible and contained a mix of proteins predominantly from poultry sources. Therefore, it was not a single-source protein (exclusion) diet and, as a result, no comment can be made on the comparative efficacy of hydrolyzed protein diets and conventional exclusion diets in management of CE. Additional studies would be needed to answer this question. In addition, the protein content of this diet was higher than the test diet. However, it is not currently known whether differences in protein content (specifically a lower protein content) can provide a clinical benefit (ie, by decreasing antigenic load). Again, additional studies could be warranted.

Regardless of the diet that the dogs of the current study received, a high initial response rate was noted with 23 of 26 (88%) dogs responding, with no significant difference between the control and test groups. The high response rate to dietary management is better than reported in previous studies in companion animals,[4,11] but similar to the response rate when another hydrolyzed protein diet was used in a trial of dogs with pruritus, almost half of which had concurrent gastrointestinal signs.[15] Possibilities for discrepancies among studies include differences in methodology, the exact diets used, and variability in the recruited study population. For instance, in 1 study of dogs, diets were only fed for a period of 10 days before they were deemed to have failed.[4] This might suggest that delayed responses to dietary therapy may occur in dogs, and that longer diet trial periods should be recommended to make certain that ARF is not the cause of clinical signs. Additional possibilities relate to the fact that only dogs with small intestinal signs were included, that no dogs had histopathologic evidence of severe intestinal inflammation, that cases with current hypoproteinemia were excluded (often the more severe cases) and, based upon the retrospective CIBDAI data and histopathologic assessment, the severity of cases may have been lower in the current study, compared with previous work.[4]

Although initial response rate did not differ between groups, the long-term remission rate was significantly better on the test diet. Given that hydrolyzed proteins are reportedly less immunogenic,[8,9] this improvement may be because of the dogs on the control diet subsequently becoming sensitized to ingredients in their diet. This hypothesis is supported by the fact that 4 of the dogs that relapsed while on the control diet subsequently achieved clinical remission on the test diet and without resorting to any other therapy. An alternative explanation for improved long-term remission is the improved

digestibility of the hydrolyzed protein diet. A final possibility would be the fact that the study was an open-label trial, which may have meant that biases were unknowingly introduced. This is a key study limitation and, ideally, the 2 diets should have been presented in identical plain bags, and a crossover design used. Even though the attending clinician attempted to avoid unduly biasing the client when discussing the diets, the client still could have developed his or her own opinion on efficacy, for instance using an internet-based search. Furthermore, the primary study investigator could have subconsciously biased the outcome when grading response to therapy. However, the pattern of group differences (ie, no initial difference in response, but improved long-term remission) would perhaps be unusual for bias introduced from the owner and attending veterinarian. Instead, if 1 treatment were perceived to be superior, differences in initial response would be more likely, with subsequent regression to the mean in the absence of any genuine benefit. Nonetheless, additional trials are recommended, trials that are not only randomized and controlled, but larger and subject to double blinding. Increasing the population size also would insure that the full extent of any treatment benefit of a hydrolyzed diet was determined.

The majority of dogs were rechallenged with their original diet, which led to recurrence of signs in two thirds of the dogs, with the speed of relapse equivalent to that reported previously for ARF.[11,16] Given the similar proportion of dogs relapsing in both groups, it is unlikely that a difference in the number of ARF cases accounts for the different response rates noted between groups. Interestingly, many other dogs responded well to dietary management, and then did not relapse upon rechallenge, as with a previous study of dietary management for feline CE.[11] The reason for this phenomenon is not known.

Despite the fact that cases were randomized to their respective groups and most baseline characteristics were similar, a difference was noted in median starting CIBDAI, with higher scores noted in the test diet group, and suggesting that cases in this group were more severe. Again, this is a limitation of the study and may have resulted in variable responses between the groups. However, such a difference is unlikely to account for the difference in response rate noted because, if anything, the expected response would have been less favorable in the test diet group. In fact, this finding may imply that the beneficial effect of the test diet was more marked than was shown. Nonetheless, caution should be exercised in interpreting these results given that CIBDAI scores were applied retrospectively, based upon a review of the original written records and study data. It may, therefore, have been that scores were assigned inaccurately and, as a result, this was not used as our primary outcome measure. Furthermore, it is noteworthy that a number of starting CIBDAI scores were low (ie, 0–3) in both groups, and would be defined as clinically insignificant disease.[12] This may have resulted from scores being assigned retrospectively, so that the full extent of the severity of signs before referral was underestimated. Moreover, such scores sometimes can be spuriously low in cases in which clinical signs wax and wane, and if the dog is examined during a period when severity is low (A.J. German, personal observations). Interestingly, low CIBDAI scores before therapy also have been reported in previous trials, perhaps for similar reasons.[4,5] Therefore, a prospective study, which scores severity at the time of assessment, is recommended to confirm the validity of the results of the current trial.

A final key limitation of the study is the low recruitment rate, with only 23 of 65 (35%) dogs that fulfilled the inclusion criteria actually being enrolled, and this might have introduced some bias. In this respect, it is possible that the dogs not enrolled were more severely affected and hence less likely to respond. As a result, the response rate may have been overestimated. Against this, however, cases were randomized to the different diets after enrollment, and no differences in the majority of baseline variables were noted, except for CIBDAI as discussed above. This suggests that groups were similar at the outset and that the findings were valid. The reasons why the recruitment rate was so low are not clear, but possibilities include unwillingness to commit to return for multiple follow-up examinations and the fact that no specific incentive was offered.

In keeping with previous studies, there was no obvious improvement in histopathologic findings despite the excellent clinical response rate.[4,5] The reasons for such findings are not clear but could suggest that outward clinical signs can be adequately controlled with diet, yet underlying inflammation persists. Alternatively, it may relate to variability in the reliability of histopathologic interpretation of gastrointestinal biopsy specimens, as previously documented.[17] A final possibility is that more substantial histopathologic changes existed in other regions of the small intestine, given the recent demonstration that severity of duodenal pathology is not reflective of ileal pathology.[18] Indeed, many gastroenterologists now recommend that the ileum routinely be assessed when investigating dogs presenting with small intestinal diarrhea. However, when the current study was conducted (2001–2003), this was not common practice. Nonetheless, this should be a consideration for similar projects in the future.

In conclusion, the current study examined the efficacy of a hydrolyzed protein diet for the management of chronic gastrointestinal disease in dogs. Whereas no difference in the initial response rate was noted when compared with the highly digestible control diet, long-term remission was better. This confirms the efficacy of such diets for the management of canine chronic small bowel enteropathies. Given that the hydrolyzed protein diet used in the study is commercially available, and the cases enrolled had naturally occurring disease, it is reasonable to expect that the findings of the study will translate into benefits in clinical practice. It is less clear, however, whether the results can be extrapolated to other diets that incorporate hydrolyzed proteins, because the native protein used can be different, as can formulation of such diets.

Footnotes

[a] Excel, Microsoft Corporation, Redmond, WA
[b] Hypoallergenic diet, Royal Canin, Aimargues, France
[c] Intestinal diet, Royal Canin
[d] Stats Direct, version 2.6.2, Stats Direct Ltd, Altrincham, UK

Acknowledgments

The authors thank Mrs Tanja Senders and Mrs Agnes Felder for their assistance during the study. Diagnostic imaging studies were kindly performed by Mrs Melinda Schmidt and Mariette Vink-Nooteboom. AJG's senior lectureship is funded by Royal Canin. The study was not supported by a research grant.

References

1. Hall EJ, German AJ. Diseases of the small intestine. In: Ettinger S.J., Feldman E.C., eds. Textbook of Veterinary Internal Medicine, 7th ed. Philadelphia, PA: WB Saunders; 2009:1333–1378.

2. Kennis Food allergies: Update of pathogenesis, diagnoses, and management. Vet Clin North Am: Small Anim Pract 2006; 361:175–184.

3. German AJ, Hall EJ, Day MJ. Chronic intestinal inflammation and intestinal disease in dogs. J Vet Intern Med 2003;17:8–20.

4. Allenspach K, Wieland B, Grone A, Gaschen F. Chronic enteropathies in dogs: Evaluation of risk factors for a negative outcome. J Vet Intern 2007;21:700–708.

5. Burgener IA, Konig A, Allenspach K, et al. Upregulation of toll-like receptors in chronic enteropathies in dogs. J Vet Intern Med 2008;22:553–560.

6. von Berg A, Koletzko S, Grubl A, et al. The effect of hydrolyzed cow's milk formula for allergy prevention in the first year of life: The German Infant Nutritional Intervention Study, a randomized double-blind trial. J Allerg Clin Immunol 2003;111:533–540.

7. Olivry T, Bizikova P. A systematic review of the evidence of reduced allergenicity and clinical benefit of food hydrolysates in dogs with cutaneous adverse food reactions. Vet Dermatol 2010;21:31–40.

8. Puigdemont A, Brazis P, Serra M, et al. Immunologic responses against hydrolyzed soy protein in dogs with experimentally induced soy hypersensitivity. Am J Vet Res 2006;67: 484–488.

9. Serra M, Brazis P, Fondati A, et al. Assessment of I9E binding to native and hydrolyzed soy protein in serum obtained from dogs with experimentally induced soy protein hypersensitivity. Am J Vet Res 2006;67:1895–1900.

10. O'Connor AM, Sargeant JM, Gardner IA, et al. The REFLECT statement: Methods and processes of creating reporting guidelines for randomized controlled trials for livestock and food safety. J Vet Intern Med 2010;24:57–64.

11. Guilford WG, Jones BR, Markwell PJ, et al. Food sensitivity in cats with chronic idiopathic gastrointestinal problems. J Vet Intern Med 2001;15:7–13.

12. Jergens AE, Schreiner CA, Frank DE, et al. A scoring index for disease activity in canine inflammatory bowel disease. J Vet Intern Med 2003;17:291–297.

13. Day MJ, Bilzert T, Mansell J, et al. Histopathological standards for the diagnosis of gastrointestinal inflammation in endoscopic biopsy samples from the dog and cat: A report from the world small animal veterinary association gastrointestinal standardization group. J Comp Pathol 2008;138:S1–S40.

14. Holm S. A simple sequentially rejective multiple test procedure. Scand J Stat 1979;6:65–70.

15. Loeffler A, Lloyd DH, Bond R, et al. Dietary trials with a commercial chicken hydrolysate diet in 63 pruritic dogs. Vet Rec 2004;154:519–522.

16. White SD. Food hypersensitivity in 30 dogs. J Am Vet Med Assoc 1989;188:695–698.

17. Willard MD, Jergens AE, Duncan RB, et al. Interobserver variation among histopathologic evaluations of intestinal tissues from dogs and cats. J Am Vet Med Assoc 2002;220:1177–1182.

18. Casamian-Sorrosal D, Willard MD, Murray JK, et al. Comparison of histoptahologic findings in biopsiefrom the duodenum and ileum in dogs with enteropathy. J Vet Intern Med 2010;24: 80–83.