Vet Clin Small Anim 36 (2006) 1251–1268
VETERINARY CLINICS
SMALL ANIMAL PRACTICE

Hydrolyzed Protein Diets for Dogs and Cats

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Hydrolyzed proteins have been used as the source of essential and dispensable amino acids in breast-milk replacer formulae for more than 50 years, mainly for the management of infant cow's milk allergy [1–3]. In contrast, diets formulated for dogs and cats that use hydrolysates as the amino acid source have been available for less than a decade, and experience and knowledge in veterinary medicine are still rudimentary.

The primary aim of hydrolyzing proteins for specialized diets is to sufficiently disrupt the protein structure within the diet to remove any existing allergens and allergenic epitopes and thereby prevent immune recognition by patients already sensitized to the intact protein. A secondary aim might be to disrupt the proteins to such an extent that there are no antigens capable of eliciting an immune response and leading to sensitization in a naive individual. An "antigen" is defined as a substance capable of stimulating antibody production. Antigens are usually, although not always, proteins. An "allergen" is an antigen that is capable of eliciting and binding to specific immunoglobulin E (IgE) antibodies and inducing mast cell degranulation after binding to IgE on the cell surface [4]. Ideally, protein hydrolysis prevents mast cell degranulation that would occur in response to the intact protein and enables a patient hypersensitive to the protein to ingest the hydrolysate without clinical signs.

The term *hypoallergenic diet* is, at best, an ambiguous one and has been widely misused. It should be reserved for diets that have at least been demonstrated to possess a substantial reduction in antigenicity and have preferably been shown to be tolerated by the most patients known to be hypersensitive to the intact source protein [5–7]. Any reduction in antigenicity or clinical reactivity at which point a diet could be considered "hypoallergenic" is arbitrary, however, unless it is absolute. Therefore, the use of the term is discouraged, and it is not used further in this review.

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0195-5616/06/$ – see front matter
doi:10.1016/j.cvsm.2006.08.008

FOOD ALLERGENS

Foods contain an enormous variety of proteins, most of which are potentially antigenic, and yet only a few have been shown to be allergenic. It is generally thought that the biochemical properties that make a particular substance an allergen are not species specific and that, in general, significant homology might be preserved in the recognition of allergens. Indeed, that seems to hold true for the allergenic potential of immunoglobulins [8]. Nonetheless, there are species differences in the relative importance of most allergens. For instance, although beef is the most common allergen in dogs and cats, it is not a common cause of allergy among people living in North America, despite its being a significant source of protein in their diets [9–12].

Adverse food reactions in dogs and cats include both non-immunologic (food intolerance) and immunologic (food hypersensitivity) mechanisms. It is probably the case that the majority of true food hypersensitivities are a type-I hypersensitivity that involves mast cell degranulation for the development of clinical signs. Mast cell degranulation requires cross-linking of two or more IgE molecules bound by high-affinity IgE receptors ($F_c\varepsilon R1$s) on the mast cell membrane (Fig. 1A). This requirement for divalency places a minimum size limit on molecules that can stimulate IgE-mediated reactions. Most publications refer to this lower limit as being 10 kd, although smaller peptides could act as haptens [12–14]. Work by van Beresteijn and colleagues [15] and, more recently, by Van Hoeyveld and coworkers [16] suggests that the limit may be much smaller, possibly between 3 and 5 kd. The minimum molecular mass for simple IgE binding seems to be somewhere between 0.97 kDa and 1.4 kDa (see Fig. 1B).

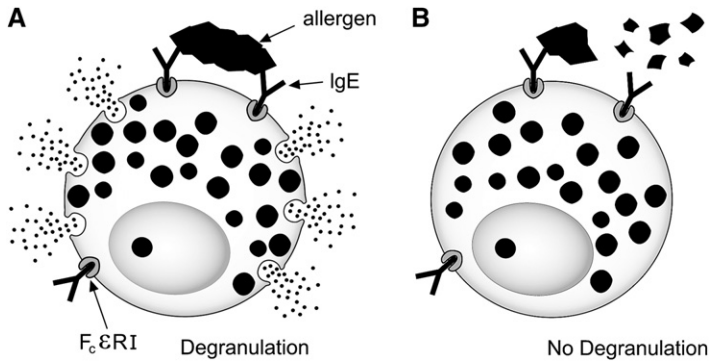

Fig. 1. Requirements for mast cell activation. (*A*) Mast cells are sensitized by IgE binding to the high affinity IgE receptor, to $F_c\varepsilon RI$. Allergen binding to IgE cross-links the $F_c\varepsilon RI$ molecules and induces the release of preformed and de novo synthesized mediators, such as histamine, prostaglandins, enzymes, and cytokines. (*B*) If the allergenic protein is sufficiently hydrolyzed, cross-linking does not occur and the mast cell does not degranulate. This is the case even if some of the fragments retain the ability to bind IgE.

In human beings, peptides as small as 4.5 kd have been shown to retain allergenicity [17]. In contrast, proteins of greater than 70 kd are unlikely to be efficiently absorbed intact through the enteric mucosa, and few food allergens are of that size. However, the size of the smallest fragments that retain allergenicity varies greatly between food protein sources.

Although most of the known food allergens are naturally occurring food proteins or glycoproteins, there is evidence that nonprotein molecules can function as allergens. Certain carbohydrates free of proteins, such as pneumococcal polysaccharides and highly cross-linked dextran, have been demonstrated to induce allergic reactions in human beings [12,18]. Carbohydrate determinants have been implicated as protein-binding haptens (eg, inulin) and as parts of antigenic glycoproteins (eg, β-fructofuranosidase) [19–21]. They are also claimed to be responsible for cross-reactivity between plant allergies and are incriminated in false-positive IgE-binding assays, such as those used in serum ELISA allergy tests [11]. The role of true carbohydrate antigens in human allergology is still controversial and poorly defined, however, and nothing is yet known about their existence in canine and feline patients.

In cases in which a dietary carbohydrate is implicated as a source of allergen (eg, corn [maize]), it is more likely that there is a protein allergen within the carbohydrate source than the existence of a true hypersensitivity to the carbohydrate molecules. Maize zeins, which are 20- to 23-kDa prolamine proteins, have been detected in hydrolyzed casein formulae when corn starch is used as the carbohydrate source [22]. Similarly, lipophilic protein allergens have been isolated in refined vegetable oils [23]. Thus, the carbohydrate and lipid sources chosen for incorporation into hydrolyzed protein diets may be important sources of conventional protein allergens because they are not commonly subjected to enzymatic hydrolysis and should be considered when evaluating commercial diets.

It is important to recognize the limitations of our understanding of canine and feline adverse reactions to food. The precise nature of the immunologic responses in most cases has not been defined. Thus, although type 1 IgE-mediated hypersensitivities are thought to be present in some cases, it is likely that other mechanisms exist in a subset of cases. This is especially true for cases in which only gastrointestinal signs are present. The degree of hydrolysis needed to prevent an adverse reaction may be different when non–IgE-mediated immune responses are present.

REDUCING THE ANTIGENICITY OF FOOD PROTEINS

The antigenicity of a protein is determined by its primary structure (ie, its amino acid sequence), its secondary structure (folding of the amino acid chain into helices or sheets), and its tertiary structure (further folding of the helices and sheets). Reducing the antigenicity can be achieved by (1) disrupting the three-dimensional structure of the protein (secondary and tertiary structures), (2) altering the structure of amino acid side chains (eg, conjugation of amino acids with sugars, oxidation of amino acids), or (3) cleaving peptide bonds (hydrolysis).

The specific methods by which antigenicity can be reduced include heat treatment, pH manipulation, enzymatic hydrolysis, and filtration [24]. The effectiveness of heat treatment depends on the inherent lability of the protein to increased temperatures [25]. Among milk proteins, for example, casein is relatively heat stable and can withstand temperatures of 130°C for longer than 1 hour without significant denaturation [26]. In contrast, whey proteins are much more heat labile, denaturing at around 80°C [27].

Accordingly, in human beings, it has been shown that although heat treatment may reduce the number of whey protein antigens, it has little effect on the casein antigens, despite heating at 121°C for 15 minutes [25]. Thus, heat treatment used alone to reduce the allergenicity of a milk product is of no use in those individuals sensitized to the casein component [28].

It can be assumed that there are a significant number of heat-stable allergens, because many of the food allergies identified in domestic animals include reactions to protein components in commercial diets (dry or canned) that are subject to heat treatment during manufacturing. Alternatively, it may be that some proteins increase in allergenicity with heat treatment. The effect of heat treatment is mostly to change the three-dimensional conformation of the protein [29]. Although this may disrupt some allergens, it may equally uncover previously hidden allergenic determinants. Other reactions occurring at high temperatures include the Maillard reactions, which involve the reactions between certain amino acids and reducing sugars to produce compounds called melanoidins, which give a characteristic brown color. Melanoidins can be more or less allergenic than the original protein by acting as haptens or by reducing peptide absorption, respectively [30,31]. These findings may explain some of the observations pertaining to differences between home-prepared elimination diets and commercial diets. It has been demonstrated recently that heat treatment during canning of a purified diet containing casein, starch, sucrose, and corn oil can create new antigens that are more immunogenic than the uncooked diet [32].

Alterations in the pH of the solution can be used to reduce the antigenicity of a protein further in addition to the conformational changes that occur at high temperatures. Most food allergens are usually quite resistant to moderate acid treatments, however, particularly those acid concentrations simulating stomach acid conditions [13]. For example, the peanut allergen "Ara h 2," soy allergen "Gly m 1," and milk β-lactoglobulin are resistant to acid digestion at pH 2.8 in contrast to the nonallergenic peptides [33,34].

Therefore, heat treatment and pH adjustments alone cannot be relied on to reduce the allergenicity of parent compounds significantly, and, as discussed, some reactions may actually increase allergenicity.

Enzymatic Hydrolysis

Cleavage of a protein molecule by enzymatic hydrolysis into small fragments is the most reliable way of reducing antigenicity. If the cleavage occurs within an antigenic peptide sequence, it is immunologically inactive. Additionally,

because many antigenic determinants rely to some degree on the three-dimensional structure of the peptide, disruption of the surrounding amino acid sequence may lead to a sufficient change in three-dimensional conformation so that loss of antigenicity occurs.

Hydrolysis of proteins is achieved by using food-grade proteolytic enzymes. The resultant hydrolysate varies in composition according to the composition of the parent compound, the specificity of the proteolytic enzymes chosen, the method by which the hydrolysis is conducted, and any further processing of the resultant product.

The selection of enzymes is important, because the specific site at which the particular enzymes act determines the likelihood of degradation of the particular epitopes responsible for the hypersensitivity reactions. Because the amino acid sequence and three-dimensional structure of the individual epitopes are rarely known, trial and error with in vitro evaluation is usually the method by which a particular hydrolytic enzyme is selected. A variety of proteases have been used from various sources, including mammalian pancreas, porcine stomach, bacteria, fungi, and some fruits [25].

Ultrafiltration

Hydrolysates usually contain residual amino acid sequences that were resistant to the hydrolysis plus traces of the enzymes used in the hydrolysis process. The hydrolysates therefore contain a variety of fragments, which may range from single amino acids to large-molecular-weight polypeptides depending on the degree of hydrolysis. Removal of the larger fragments via physical separation or molecular filtration can have a significant influence on the "quality" of the finished product. Currently, ultrafiltration of the hydrolysate is the most widely used method to remove the large-molecular-weight fragments. The size of the filter and the efficiency of the filtration process determine the success of ultrafiltration. Understandably, ultrafiltration of a protein hydrolysate would add a considerable cost to the final product if used.

EVALUATING AND COMPARING HYDROLYZED PROTEIN DIETS

Ingredients

Initial selection of a commercial hydrolyzed protein diet for a particular patient should probably be based on the protein source. None of the currently available commercial diets are sufficiently hydrolyzed to guarantee the complete absence of all allergens. Therefore, it is prudent to select a diet that does not contain a protein source that the patient is known or suspected to be sensitized to. Secondary consideration should be given to the sources of carbohydrate and lipid as sources of potential protein allergens and (unproven) as sources of carbohydrate or lipid antigens. The hydrolyzed protein diets currently widely available are presented in Table 1.

Table 1
Complete and balanced hydrolyzed protein diets available for dogs

Diet[a]	Protein source	Carbohydrate source	Lipid source
Hill's z/d Ultra Allergen Free	Chicken	Corn starch, cellulose	Soybean oil
Hill's z/d Low Allergen	Chicken, potato	Potato, potato starch, cellulose	Soybean oil
Nestle-Purina HA	Soy	Corn starch, cellulose, vegetable gums (gum arabic and guar gum)	Coconut oil, canola oil, corn oil
Royal Canin Hypoallergenic	Soy, poultry liver	Rice, beet pulp, fructo-oligosaccharides	Poultry fat, soybean oil, borage oil, fish oil

Ingredients taken from manufacturers' product guides (January 2006).
[a]Hill's Pet Nutrition, Inc. Topeka, Kansas, USA; Nestlé Purina PetCare Company, St. Louis, Missouri, USA; Royal Canin, Aimargues, France.

Nutritional Evaluation

The digestibility of a protein hydrolysate is predicted to be superior to that of the intact protein source. In fact, numerous studies have shown that small peptides are even better absorbed from the intestine than free amino acids [35,36]. Thus, extensively hydrolyzed proteins seem to be the ideal source of amino acids for maximal digestibility. The digestibility of the protein fraction of a soy-hydrolysate–based diet is reported by the manufacturer to be 90.7% [37]. By comparison, intact soy protein isolates have been shown to have apparent total tract digestibilities of 84.7% to 89.3% [38]. In addition, the apparent ileal protein digestibility of a chicken-based hydrolysate diet has been found to be 82.4% [39]. Thus, although the digestibility may be higher, it is not dramatically so compared with some forms of the intact protein source.

Despite the higher digestibility and absorption of protein hydrolysates, use of the amino acids seems to be different in extensively hydrolyzed formulae compared with the intact protein. Nitrogen use seems to be reduced when compared with conventional formulae [40]. Reduced growth, decreased serum albumin, and increased serum urea have been observed in newborn human infants fed from birth to 1 month with various hydrolysates when compared with feeding intact protein or breast milk [40,41]. Alterations in calcium and phosphorus absorption and differences in the serum amino acid profiles can occur between infants fed whey-protein and those fed a whey hydrolysate [40,41]. These reduced nutritional aspects of protein hydrolysates are seen with extensively hydrolyzed formulae and not with moderately hydrolyzed formulae, such as those currently available in commercial veterinary diets.

Therefore, although the digestibility and nutritional value of hydrolysates cannot be assumed to be the same as those of the parent protein, it is unlikely that the degree of hydrolysis used by the currently available hydrolyzed

protein diets is associated with a detrimental effect on nitrogen balance. In addition, products that have been subjected to suitable feeding trials, such as the protocols recommended by the American Association of Feed Control Officials (AAFCO), have been demonstrated to be suitable for long-term feeding.

Physicochemical and Immunologic Evaluation

Laboratory-based testing provides manufacturers with the opportunity to characterize various molecular and immunologic properties of hydrolysates before their incorporation into complete feeds. The antigenicity and allergenicity of a hydrolysate are partly but not wholly dependent on the molecular weight of the remaining peptides. As stated previously, the smaller the resulting fragments are, the less likely it is that residual epitopes remain. Physicochemical analyses of hydrolysates describe the extent of the hydrolysis and the distribution of the molecular weights of the remaining peptide fragments. This is often the starting point in the selection of a candidate hydrolysate [42]. High-performance size-exclusion chromatography has been commonly used to describe the molecular weight profile of infant formulae and has been applied to the peptide component of one veterinary hydrolysate diet [6]. The molecular weight profile and the accumulative percentage curve of the hydrolysate are presented in Fig. 2, and the molecular weight distribution and peptide concentrations are presented in Table 2. As can be seen from Fig. 2, molecular weight profiles are complex data sets that cannot, and should not, be reduced to simple

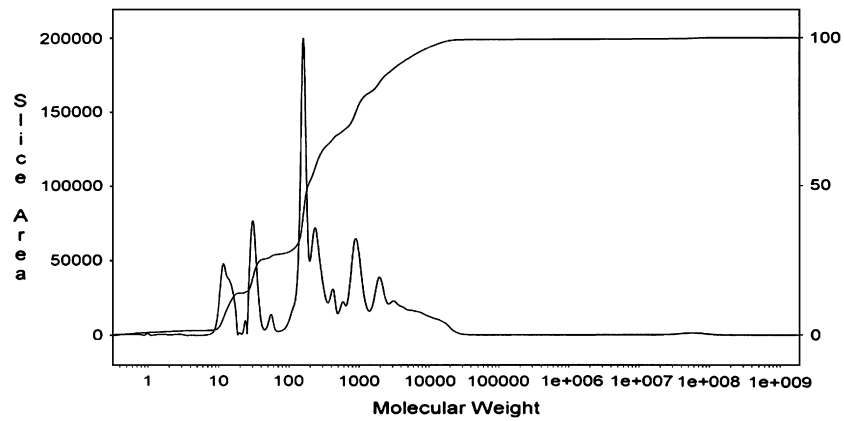

Fig. 2. Chromatographic separation of peptides in a hydrolysate derived from chicken liver and heart. High-performance size-exclusion chromatography separation was performed using a gel filtration column. Absorbance was measured at 205 nm. The molecular weight (x-axis) in Daltons is plotted against absorbance at 205 nm (y-axis). The cumulative percentage (%) curve (z-axis) is plotted after completion of sample elution. (*From* Cave NJ, Guilford WG. A method for in vitro evaluation of protein hydrolysates for potential inclusion in veterinary diets. Res Vet Sci 2004;*77*(3):235; with permission.)

Table 2
Molecular weight distribution of a chicken-protein hydrolysate

Molecular weight range (kd)	Percentage of total sample (wt/wt)
< 0.5	67.6
0.5–1	10.8
1–3	10.8
3–5	3.7
5–10	4.0
> 10	3.1

From Cave NJ, Guilford WG. A method for in vitro evaluation of protein hydrolysates for potential inclusion in veterinary diets. Res Vet Sci, 2004; 77(3):232.

molecular weight averages. Clearly, the expression of a hydrolysate in terms of its average molecular weight provides limited information on the potential for and significance of intact antigens within the formulation.

It is difficult to determine the limit under which the remaining peptide fragments are small enough to prevent clinical signs in sensitized patients, however. Indeed, although there is an absolute limit for all proteins, the actual limit for individual proteins varies, as mentioned, according to the antigenic epitopes within that protein.

Ensuring that a hydrolysate has no peptides greater than 3 kd, or even 1 kd, would ensure the greatest chance of eliminating any residual allergens. The expense involved in extensive hydrolysis and ultrafiltration makes this, at least for pet food manufacturers, an unrealistic objective, however. Additionally, hypersensitivity reactions in children have been identified in even the most hydrolyzed formulae [43]. Suggested explanations include the presence of an allergen within the carbohydrate component, a hapten effect, or reassembly of old or new epitopes in vivo or in vitro during or subsequent to formulation. Importantly, it should also be realized that the presence of fragments of greater than 5 kd, or even greater than 10 kd, does not guarantee allergenicity. As detailed previously, reduction of allergenic epitopes is dependent on the specificity for the proteolytic enzymes as to whether any given epitope is cleaved or disrupted and rendered nonallergenic.

Immunochemical analyses can semiquantitatively estimate hydrolysate reactivity with preformed antibody. The use of ELISAs and RAST assays to assess residual antibody binding is widespread in human medicine. The ability of hydrolysate-based products to induce an immune response can be evaluated by using animal models, such as the passive cutaneous sensitivity test or laboratory animal hyperimmunization [44,45]. It is the responsibility of the manufacturer to choose the appropriate combination of laboratory-based tests and to use them to document product consistency, thus helping to ensure consistent clinical performance.

At present, detailed in vitro analysis is only available for one of the commercially available hydrolysate diets, which limits product comparisons [6].

Ultimately, however, controlled clinical studies are necessary to demonstrate conclusively the biologic efficacy of these formulations in their target species.

PROBLEMS WITH HYDROLYSATES

The most significant problem that manufacturers of hydrolysate formulae face is persistent immunogenicity. Although a particular process may significantly reduce the allergenicity of the product, it does not abolish the risk of producing an immune-mediated reaction. In the initial stages of an enzymatic hydrolysis, it is common for previously hidden antigenic sites to become exposed and for the product to increase in allergenicity, which is only reduced with further hydrolysis. In extremely hypersensitive children, the reactions to hydrolysate formulae can be life threatening. As the number of hydrolyzed protein formulae appearing on the market for use in allergic human patients increases, so do the number and range of reported hypersensitivity reactions, even anaphylaxis [1,46–48]. It has been shown that only small amounts of intact allergenic epitopes are required to elicit significant and even fatal IgE-mediated responses in sensitized individuals [49]. The best guarantee of producing a truly nonallergenic diet resides in the production of purified amino acids and small peptides. Unfortunately, the widespread use of these elemental products is cost-prohibitive, they have poor acceptance by patients, and they are not easily fed enterally because of their exceptionally high osmolarity.

Preserving palatability for human beings is difficult with the more extensively hydrolyzed products [50]. Peptides and amino acids produce a variety of flavors. The sweet taste of some amino acids and peptides has long been known [51]. Bitterness offers the greatest hurdle to palatability, however. The bitter taste sensation of peptides is, to some degree, related to their hydrophobicity, which, in turn, is a product of their amino acid composition [52]. When a protein is hydrolyzed, the peptide fragments that contain hydrophobic side chains are exposed and can be tasted. Thus, as hydrolysis proceeds, bitterness tends to increase. The most bitter tasting peptides in soy hydrolysates occur between 4 and 2 kDa [53]. As the peptide fragments decrease in size to less than 1 kDa, or even free amino acids, bitterness declines [52]. Hydrolysates produced from more hydrophobic proteins, such as casein, are more likely to be bitter tasting than heterogeneous protein sources, such as meat proteins.

It should be noted that a variety of flavors of peptides are described by human subjects, including bitter, sweet, meaty, and yeasty [52,54]. Also, although individual amino acids and peptides may have a bitter taste, the taste of a hydrolysate is dependent on the mixtures of peptides and cannot be assumed to be any one flavor or easily predicted from the protein source of known hydrophobicity [52,53]. Finally, taste preferences among mammals vary and are not identical to human taste preferences [54–57]. For instance, leucine is a bitter tasting amino acid to human beings, but is a positive flavor enhancer to cats [54,58]. Indeed, protein hydrolysates have long been used to enhance the palatability of commercial dog and cat foods.

In a study of 63 dogs fed a commercial chicken-based hydrolysate diet, palatability was reported to be good or excellent in 48 (76%) and was described by owners of 10 dogs (16%) as being poor but was only refused by 4 dogs (6%) [59]. In another study, although palatability was not reported, 58 (97%) of 60 dogs successfully completed a 2-month feeding trial when a soy-based hydrolysate diet was prescribed, which is consistent with adequate palatability of that diet [60]. Based on data published to date, the rate of acceptance by dogs fed hydrolyzed protein diets as elimination diets is similar to that of those fed conventional select protein diets.

In addition to changes in taste and digestibility, osmolarity increases significantly with increasing hydrolysis and has been blamed for a high incidence of diarrhea in infants fed extensively hydrolyzed formulae [61]. Although the osmolarity of jejunal contents after a normal meal is mildly hyperosmolar (300–350 mOsm/L), feeding high-osmolarity enteral solutions (up to 800 mOsm/L) has been associated with diarrhea in human beings [62,63]. Even higher osmolarities can cause sloughing of enterocytes [64]. In studies of acute diarrhea in children, an osmolarity of 250 mOsm/L or less is associated with improved rehydration, lower stool volume, and less vomiting compared with a solution of 311 mOsm/L, indicating an increased sensitivity to osmolarity in enteritis [65]. However, the osmolarity of the jejunal contents following a feed of a complete hydrolyzed diet is not easily predicted, as it is affected by other ingredients and by the rate of gastric emptying. Thus a protein hydrolysate will produce a different intestinal luminal osmolarity when it is administered as a solution compared with when it is incorporated within a complex diet.

The osmolarity of one chicken-based hydrolysate diet has been determined to be 682 mOsm/L when mixed 1:1 wt/wt with water compared with 293 mOsmol/L for a standard intact protein maintenance diet [39]. Therefore, it is conceivable that hydrolyzed proteins and high food osmolarity could be detrimental in some dogs. Nevertheless, in 46 dogs fed the diet for 6 to 8 weeks as part of an evaluation for suspected food hypersensitivity, only 4 dogs developed soft feces that had been normal on their original diets [59]. Also, of the 46 dogs, 21 had gastrointestinal signs as part of their original presentation, and the feces of all 21 improved on the hydrolysate diet. These findings combine to suggest that hyperosmolar diarrhea is not a significant problem with that diet.

Finally, the use of enzymatic hydrolysis, with or without ultrafiltration, and the selection of purified carbohydrate sources, such as starch, incurs considerable cost to the manufacturer. Consequently, the protein hydrolysate diets available are at least 50% more expensive on a per-calorie basis than normal premium maintenance diets.

USE AND EVIDENCE OF EFFICACY OF HYDROLYZED PROTEIN DIETS

When considering reports of the efficacy of hydrolysate diets, it should remembered that nutritional factors other than the hydrolysis of the protein component may be responsible for reported clinical improvements. Nutritional

variables that could affect clinical responses include dietary digestibility, correction of vitamin or mineral deficiencies, a lowered ω (n)-6/n-3 fatty acid ratio, and the potential for an immunomodulatory effect of soy isoflavones (eg, genistein) within the diet, especially in cases of intestinal disease. A study that would definitively demonstrate the efficacy of protein hydrolysis alone would compare two diets in which the only difference is that one of the diets has the protein component hydrolyzed.

Elimination Diets

The primary role for the use of hydrolyzed protein diets is for the diagnosis or management of food hypersensitivity in all its manifestations. Whenever the feeding of a novel protein diet is recommended, a hydrolyzed protein diet could be considered. Increasingly, feline and canine patients are being exposed to a wide variety of protein sources as the range of commercial diets increases. The identification of a truly novel protein in patients presented for evaluation of dietary hypersensitivity can be difficult. Hydrolyzed protein diets allow greater confidence in the instigation of an elimination trial when a dietary history is uncertain or reveals prior exposure to multiple proteins.

Protein hydrolysate diets have been reported to be effective and well tolerated when used as elimination diets for the diagnosis of adverse food reactions in dogs [59,60]. In those studies, owner compliance was excellent, whereby 73% and 97% of dogs completed the 6- to 8-week trial periods. The high completion rates are similar or superior to those reported by authors using home-cooked or commercial novel protein diets (64%–80%) for elimination diet trials [66–68].

The protein sources incriminated in the adverse reactions were not reported in the study by Loeffler and colleagues [59]. Therefore, it is impossible to comment on the efficacy of that diet in cases in which the patient is sensitized to the intact source protein. In the study by Biourge and coworkers [60], however, two dogs that did not improve when fed the soy- and chicken-based hydrolysate did improve when fed a home-prepared soy-based diet or a commercial rabbit and rice diet. Those findings suggest sensitization to the chicken or other protein fractions within the hydrolysate diet or the creation of novel dietary antigens as the result of the food processing, as has been demonstrated to occur [32].

Finally, Jackson and colleagues [7] evaluated the efficacy of a soy-based hydrolysate diet when fed for 2 weeks to 14 cross-breed dogs that were known to be allergic to soy or corn. Of the 14 dogs, 3 reacted adversely to the hydrolyzed soy diet, all 3 of which were hypersensitive to soy and corn; thus, it is uncertain to what fraction the dogs were reacting. This study demonstrated for the first time that a commercially available hydrolysate diet can be fed to most dogs sensitized to the intact source protein without eliciting clinical signs. It also indicated that a significant proportion (21%) of dogs sensitized to the intact compounds still react adversely to the hydrolyzed diet, however. This re-emphasizes the limitations of the currently available hydrolyzed protein diets.

For maximum confidence in performing an elimination diet trial, it is still important, even when using a hydrolyzed protein diet, to obtain an accurate dietary history and to choose a diet that contains ingredients the patient is unlikely to be sensitized to.

Inflammatory Bowel Disease

Novel protein diets have been proven effective in dogs and cats with a range of small and large intestinal inflammatory bowel disease (IBD) [69–72]. Guilford and coworkers [72] reported that in 16 cats with chronic gastrointestinal signs in which elimination challenge trials had proven dietary hypersensitivity, all 16 had mild to severe inflammatory infiltrates in at least one region of the bowel. The infiltrates were lymphocytic, lymphocytic-plasmacytic (most cases), or eosinophilic (two cases). All cats responded completely to the elimination diet alone without the need for immunosuppression. In a report of 13 dogs with lymphocytic-plasmacytic colitis (LPC), clinical signs resolved in all dogs with the introduction of a novel protein diet, and 9 of 11 dogs rechallenged with their original diet relapsed [70]. In a further report of 6 cats with LPC, all responded completely to an elimination diet [69]. A complete clinical response to an elimination diet has been reported in a cat with duodenal and ileal lymphocytic infiltrates so marked that a histopathologic diagnosis of intestinal lymphosarcoma was made [73]. These reports emphasize the importance of the diet as a source of provocative antigens in a subset of cases of IBD.

Hydrolyzed protein diets have the advantage over an intact novel protein diet in the management of IBD because there is less concern about sensitization to the new diet during the initial treatment phase. The concern over newly acquired dietary hypersensitivity has led to the concept of using "sacrificial proteins" when treating intestinal disorders with loss of oral tolerance [74]. Theoretically, hydrolyzed protein diets might lead to a more rapid improvement if inappropriate immune responses directed against novel dietary antigens are contributing to ongoing enteritis. Anecdotally, hydrolyzed protein diets seem to be effective adjuncts to pharmacologic therapy, and even as the sole therapy in IBD. Clinical resolution with histopathologic improvement has been reported in four of six dogs with refractory IBD when treated with a soy-based hydrolysate diet alone [75]. Although small and uncontrolled, these results are supportive of the role of the hydrolysate, because five cases had previously failed suitably conducted elimination diet trials using intact novel proteins.

Acute Enteritis

Acute enteritis from any cause can conceivably lead to temporary sensitization to food antigens, which would be expected to prolong clinical signs. Bacterial adjuvants, such as fimbriae from enterotoxigenic *Escherichia coli*, lipo-oligosaccharide from *Campylobacter*, and cholera toxin, can induce sensitization to ingested proteins if administered concurrently [76,77]. If sensitization does occur during acute enteritis, feeding a hydrolysate diet during recovery from intestinal disease would be expected to abrogate such an effect.

The effect of feeding a hydrolyzed diet during intestinal recovery from an acute mucosal insult or intestinal resection has not been evaluated in dogs or cats. Nevertheless, it is becoming increasingly clear that the early introduction of food after severe mucosal injury can more rapidly restore normal permeability; decrease bacterial translocation; decrease time to normalization of demeanor, appetite, vomiting, and diarrhea; and decrease mortality [78,79]. It has also been shown that the form of the diet is important during recovery. Marks and colleagues [80] reported that intestinal recovery in cats after treatment with a toxic dose of methotrexate was maximized when a complex diet was fed and was impaired when a purified amino acid diet was fed.

These findings are consistent with the knowledge that intestinal recovery is dependent on the production of trophic factors, such as glucagon-like peptide-2 (GLP-2) and insulin-like growth factor-1 (IGF-1) [81]. The ileum and colon are the primary intestinal sites of synthesis and secretion of GLP-2 and IGF-1, which are released in response to the presence of nutrients, especially peptides, in the intestinal lumen. Thus, luminal nutrients are essential for maximal and rapid mucosal recovery, which is stimulated largely by enterically derived GLP-2 and IGF-1. Therefore, concerns when feeding semielemental diets are whether there is, by virtue of the hydrolysis procedure, less stimulation for intestinal recovery and whether the speculated benefit of avoiding transient or persistent food hypersensitivity outweighs a risk of impairing intestinal adaptation. Some studies have shown improved villous recovery after starvation when hydrolysates are fed compared with intact proteins or free amino acids [82,83]. In other studies, however, extensively hydrolyzed proteins have been shown to impair intestinal recovery [84].

It is likely that the degree of hydrolysis currently incorporated for the production of hydrolysate diets in veterinary medicine does not have any detrimental effect on intestinal recovery after a mucosal insult. In fact, it may be that the oligopeptide component is ideal for feeding in acute and chronic inflammatory enteropathies. This is an area that warrants further study.

Prevention of Food Hypersensitivity

Perhaps some of the most interesting work on protein hydrolysates has been the recent discovery of so-called "tolerogenic" peptides in partially hydrolyzed formulae. Fritsche and coworkers [85] investigated whether oral tolerance can be induced with protein hydrolysates. The authors investigated a partially hydrolyzed formula and an extensively hydrolyzed whey-protein formula and found that the partially hydrolyzed formula was able to induce immunologic tolerance to the intact protein when administered before and during experimental sensitization, whereas the extensively hydrolyzed formula was not. The significance of these findings is that they introduce the possibility of inducing tolerance in a sensitized patient even when the patient is sensitized to the parent protein. As long as there are no peptides large enough to induce an IgE-mediated response but there are sufficiently large fragments to be antigenic in some way, the establishment of tolerance to the parent protein may be hastened. In

addition, if the feeding of a hydrolysate were to be considered as a prophylactic measure in patients "at risk" of developing a food hypersensitivity, the inclusion of some low-molecular-weight antigens might be advantageous. These findings raise the intriguing possibility that hydrolysates may have a role in preventing hypersensitivity in individuals at risk as well as for managing already sensitized individuals. In high-risk infants who are unable to be completely breast fed, there is evidence that prolonged feeding with a hydrolyzed formula compared with a cow's milk formula reduces infant and childhood allergy to cow's milk [86].

Exocrine Pancreatic Insufficiency

The benefit of hydrolyzed protein diets for the management of exocrine pancreatic insufficiency (EPI) could be argued on the basis of increased digestibility and reduced antigenicity. Predigestion of the protein seems intuitively beneficial for cases of EPI. However, there is no evidence that protein malnutrition occurs following successful treatment of EPI. It is likely that intestinal brush border endopeptidases compensate for the loss of pancreatic proteases and enable adequate protein and peptide digestion. Regardless, adverse reactions to food have been reported in up to 10% of dogs with EPI [87,88]. Biourge and Fontaine [89] reported the efficacy of a soy- and chicken-based hydrolysate diet in three German Shepherds with EPI and adverse food reactions that had been inadequately managed for a prolonged period. In all three dogs, fecal quality, dermatologic signs, and body weight improved within 3 weeks of commencing feeding. Although this was an uncontrolled study with inadequate numbers to draw firm conclusions, the results are sufficiently provocative to suggest that the hydrolyzed protein diets may be beneficial in the management of refractory cases of EPI.

SUMMARY

Although true food hypersensitivity is relatively uncommon in dogs and cats, it is an important differential diagnosis for chronic pruritic skin disease and gastrointestinal disease alike. Given the ever-increasing range of dietary proteins that our patients are exposed to, hydrolyzed protein diets offer a convenient and proven option for the diagnosis and management of food hypersensitivity. As experience with hydrolyzed protein diets in veterinary medicine increases, so should our appreciation for the range of their benefits in diseases, such as IBD, acute enteritis, and EPI. Comparing the currently available hydrolysate diets beyond basic ingredients is difficult because of the absence of standard evaluations. Determining the optimal degree of hydrolysis is even more difficult and likely differs according to protein, patient, and disease process. The degree of hydrolysis currently used in veterinary diets may be ideal from nutritional and palatability perspectives but cannot guarantee an absence of intact allergens. As such, the use of hydrolyzed protein diets does not expunge the need for a detailed dietary history when dietary hypersensitivity is suspected.

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