HYDROLYSED PROTEIN DIETS
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Introduction
Hydrolyzed proteins have been used as the source of essential and dispensible amino acids in breast-milk replacer formulae for more than 50 years, mainly for the management of infant cow’s milk allergy.\(^1\) In contrast, diets formulated for dogs and cats that utilize hydrolysates as the amino acid source have been available for less than a decade, and experience and knowledge in veterinary medicine is still rudimentary.

The primary aim of a hydrolyzed protein diet is to sufficiently disrupt the proteins within the diet as to remove any existing allergens and prevent recognition by a patient sensitized to the intact protein. A secondary aim might be to disrupt the proteins to such an extent that there are no longer any antigens capable of eliciting an immune response and leading to sensitization in a naïve individual. An “antigen” is defined as a substance capable of stimulating antibody production. Antigens are usually, though not always, proteins. An “allergen” is an antigen that is capable of eliciting and binding to specific IgE antibodies, and inducing mast cell degranulation following binding to IgE on the cell surface. Ideally, hydrolysis will prevent mast cell degranulation that would occur in response to the intact protein, and will enable 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 the very least, been demonstrated to possess a substantial reduction in antigenicity, and preferably been shown to be tolerated by the vast majority patients known to be hypersensitive to the intact source protein.\(^4\) However, defining at which point in the reduction in antigenicity or clinical reactivity a diet could be considered “hypoallergenic” is arbitrary, unless it is absolute and the use of the term is discouraged.

Although the majority of the known food allergens are naturally occurring food proteins or glycoproteins, there is evidence that non-protein molecules can function as allergens. Certain carbohydrates, free of proteins, such as pneumococcal polysacharides and highly cross-linked dextran, have been demonstrated to induce allergic reactions in man.\(^7\) Carbohydrate determinants have been implicated as protein-binding haptons (e.g. inulin), and as parts of antigenic glycoproteins (e.g. β-fructofuranosidase).\(^9\) They are also claimed to be responsible for cross-reactivity between plant allergies, and are incriminated in false-positive IgE-binding assays, such as used in serum ELISA allergy tests.\(^11\) However, the role of true carbohydrate antigens in human allergology is still controversial and poorly defined, and nothing is yet known about their existence in canine and feline patients. In cases where a dietary carbohydrate is implicated as a source of allergen, e.g. maize, it is more likely that there is a protein allergen within the carbohydrate source, than the existence of a trye hypersensitivity to the carbohydrate molecules. Maize zeins, which are 20 to 23 kDa proteins, have been detected in hydrolyzed casein formulae when corn starch is used as the carbohydrate source.\(^12\) Similarly, lipophilic protein allergens have been isolated in refined vegetable oils.\(^13\) Thus the carbohydrate and lipid sources chosen for incorporation into hydrolysed protein diets may be an important source of conventional protein allergens since they are not subjected to enzymic 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 immunological responses in the vast majority of cases has not been defined. Thus although type 1, IgE-mediated hypersensitivities are thought to be present in some, it is likely that other mechanisms exist in a subset of cases. This is especially true for cases where only gastrointestinal signs are present. The degree of hydrolysis needed to prevent an adverse reaction may be different, when adverse, non-IgE-mediated immune responses are present.

Commercial hydrolysed protein diets
Initial selection of a commercial hydrolyzed protein diet for a particular patient, should probably be based upon the protein source. None of the currently available diets are sufficiently hydrolyzed to guarantee the complete absence of any 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.
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 enzymic 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 appear on the market for the use in allergic human patients, so the number and range of reported hypersensitivity reactions, even anaphylaxis, increases. 1,14-16 It has been show that only very small amounts of intact allergenic epitopes are required to elicit significant and even fatal IgE mediated responses in sensitized individuals. 17 The best guarantee of producing a truly non-allergic diet resides in the production of purified amino acids and small peptides. Unfortunately, the widespread use of elemental products is cost prohibitive and such products cannot be easily fed enterally due to their exceptionally high osmolarity.

Preserving palatability for humans is difficult with the more extensively hydrolysed products. 18 Peptides and amino acids produce a variety of flavours. The sweet taste of some amino acids and peptides has long been known. 19 However, bitterness offers the greatest hurdle to palatability. The bitter taste sensation of peptides is to some degree, related to their hydrophobicity, which is in turn a product of their amino acid composition. 20 When a protein is hydrolysed, the peptide fragments that contain hydrophobic side chains are exposed and can be tasted. Thus, as hydrolysis proceeds, bitterness tends to increase, with the most bitter tasting peptides in soy hydrolysates between 4 to 2 kDa. 21 As the peptide fragments decrease in size to less than 1kDa or even free amino acids, bitterness declines. 22 Hydrolysates produced from more hydrophobic proteins such as casein, are more likely to be bitter tasting that heterogenous protein sources such as meat proteins.

However, it should be noted that a variety of flavours of peptides are described by human subjects including bitter, sweet, meaty, and yeasty. 20,22 Also, although individual amino acids and peptides may have a bitter taste, the taste of a hydrolysate is dependant on the mixtures of peptides and cannot be assumed to be any one flavour, or easily predicted from the protein source of known hydrophobicity. 20,21 Finally, taste preferences amongst mammals vary and are not identical to human taste preferences. 22,26 For instance leucine is a bitter tasting amino acid to humans, but is a positive flavour enhancer to cats. 27 Indeed protein hydrolysates have long been used to enhance the palatability of commercial dog and cats 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%), was described by owners of 10 dogs (16%) as being poor, but was only refused by 4 dogs (6%). 27 In another study, although palatability was not reported, 58 of 60 (97%) 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. 28 Based on data published to date then, the rate of acceptance by dogs fed hydrolyzed protein diets as elimination diets, is similar to 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 diarrhoea in infants fed extensively hydrolysed formulae. 29 Although the osmolarity of jejunal contents following a normal meal is mildly hyperosmolar (300 – 350 mOsm/L), feeding high osmolarity eneteral solutions (up to 800 mOsm/L) has been associated with diarrhoea in humans. 30,31 Even higher osmolalities can cause sloughing of enterocytes. 32 In studies of acute diarrhoea in children, an osmolarity of ≤ 250 mOsm/L 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. 33 However, the osmolarity of the jejunal contents following a feed of a complete hydrolysed diet is not easily predicted, as it is affected by other ingredients, and by the rate of gastric emptying. Thus a low fat hyperosmolar solution will produce a different intestinal luminal osmolarity to a complex extuded dry 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 mOsm/L for a standard intact protein maintenance diet. 34 Therefore it is conceivable that the high osmolarity could be detrimental in some dogs. However, 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. 27 Also, of the 46 dogs, 21 dogs 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 diarrhoea 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 a considerable cost to the manufacturer, and 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 in food hypersensitivity**

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 n-6:n-3 fatty acid ratio, and the potential for an immunomodulatory effect of soy isoflavones (e.g. 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 hydrolysate.

The primary role for the use of hydrolysed 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 hydrolysed 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 where a dietary history is either 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. In those studies, owner compliance was excellent, whereby 73% to 97% of dogs completed the 6 to 8 week trial periods. The high completion rates are similar or superior to those reported by authors utilizing home-cooked or commercial novel-protein diets (64 – 80%) for elimination diet trials.

The protein sources incriminated in the adverse reactions were not reported in either study by Loeffler et al (2004, 2006), therefore it is impossible to comment on the efficacy of that diet in cases where the patient is sensitized to the intact source protein. However, in a study using the Royal Canin soy hydrolysate diet, 2 dogs that did not improve when fed the soy and chicken-based hydrolysate, did improve when fed either a home-prepared soy-based diet, or a commercial rabbit and rice diet. Those findings suggest either sensitization to the chicken or other protein fraction within the hydrolysate diet, or the creation of novel dietary antigens as the result of the food processing as has been demonstrated to occur.

The Nestle-Purina HA soy-based hydrolysate diet was fed for 2 weeks to 14 cross-breed dogs that were known to be allergic to soy and/or corn. Of the 14 dogs, 3 reacted adversely to the hydrolysed soy diet, all of which three were hypersensitive to both soy and corn, so it is uncertain to what fraction the dogs were reacting to. This study demonstrated for the first time, that a commercially available hydrolysate diet can be fed to the majority of dogs sensitized to the intact source protein without eliciting clinical signs. However, it also indicated that a significant proportion (21%) of dogs sensitized to the intact compounds will still react adversely to the hydrolyzed diet. This re-emphasizes the limitations of the currently available hydrolysed protein diets. For maximum confidence in performing an elimination diet trial, it is still important, even when using a hydrolysed protein diet, to obtain an accurate dietary history, and to choose a diet that contains ingredients the patient is unlikely to be sensitized to.

**Conclusion**

Although true food hypersensitivity is relatively uncommon in both dogs and cats, it is an important differential for chronic pruritic skin disease and gastrointestinal disease alike. Given the every increasing range of dietary proteins that our patients are exposed to, hydrolysed 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 will our appreciation for the range of their benefits in diseases such as IBD and acute enteritis. 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 will likely differ according to protein, patient, and disease process. The degree of hydrolysis currently employed in veterinary diets may be ideal from nutritional and palatability perspectives, but cannot guarantee an absence of intact allergens. As such, the use of hydrolysed protein diets does not expunge the need for a detailed dietary history when dietary hypersensitivity is suspected.
Table 1. Complete and balanced hydrolyzed protein diets available for dogs and cats

<table>
<thead>
<tr>
<th>Diet</th>
<th>Protein source</th>
<th>Carbohydrate source</th>
<th>Lipid source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hill’s z/d Ultra Allergen Free</td>
<td>Chicken</td>
<td>Maize starch, cellulose</td>
<td>Soybean oil</td>
</tr>
<tr>
<td>Hill’s z/d Low Allergen</td>
<td>Chicken, potato</td>
<td>Potato, potato starch, cellulose</td>
<td>Soybean oil</td>
</tr>
<tr>
<td>Hill’s Feline z/d Ultra canned</td>
<td>Chicken</td>
<td>Corn starch</td>
<td>Soybean oil</td>
</tr>
<tr>
<td>Hill’s Feline z/d Low Allergen dry</td>
<td>Chicken, Brewers rice</td>
<td>Brewers rice</td>
<td>Soybean oil</td>
</tr>
<tr>
<td>Nestle-Purina HA</td>
<td>Soy</td>
<td>Corn starch, cellulose, vegetable gums (gum arabic and guar gum)</td>
<td>Coconut oil, canola oil, corn oil</td>
</tr>
<tr>
<td>Royal Canin Hypoallergenic</td>
<td>Soy and poultry liver</td>
<td>Rice, beet pulp, fructo-oligosaccharides</td>
<td>Poultry fat, soybean oil, borage oil, fish oil</td>
</tr>
</tbody>
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Ingredients taken from product guides

References
9. van Ree R. Carbohydrate epitopes and their relevance for the diagnosis and treatment of allergic diseases. International Archives of Allergy and Immunology 2002; 129:189-197.
Small Animal Medicine Chapter