THE NUTRITIONAL MANAGEMENT OF FOOD HYPERSENSITIVITY IN DOGS AND CATS: AN ASSESSMENT OF A PROTEIN HYDROLYSATE

by

NICHOLAS J. CAVE

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ABSTRACT

Adverse reactions to food are exceedingly common reasons for the presentation of cats and dogs to veterinarians. Of those cases, a relatively small number involve a truly immune-mediated reaction to the food substance. However, differentiating those that are from the more common food intolerances is usually difficult and often impossible. In addition, certain individuals with a true food hypersensitivity are difficult to manage with conventional diets. The identification and availability of nutritionally complete commercially prepared diets with a protein component that is truly novel to the patient under investigation is often the stumbling block to successful diagnosis and management of food hypersensitivity. The recent development of protein hydrolysate based pet foods for dogs and cats provides an exciting tool for more reliable diagnosis and management or food hypersensitivity in those species. Chapter 1 of this thesis describes the immunological and pathophysiological basis of food hypersensitivity and presents some of the key areas of recent research that have lead to a deeper, if still incomplete understanding of the aetiological mechanisms responsible. The development and maintenance of oral tolerance is discussed including the key roles that the resident antigen presenting cells in the mucosa play. From that description follows a presentation of some of the current hypotheses regarding mechanisms by which oral tolerance is lost or not established. These include the action of mucosal adjuvants, parasitism, IgA deficiency and alterations in mucosal permeability. Building on this discussion is an examination of the methods currently available to veterinarians for the diagnosis of food hypersensitivity, their clinical usefulness and limitations.

The importance of obtaining a complete and accurate dietary history is emphasized. The difficulties in doing so and the consequences of not doing so are discussed. As stated, the recent development of diets in which the protein content has been hydrolysed provides a new tool for the veterinarian. Some of the practical aspects behind producing hydrolysate diets are presented and the theoretical basis, especially the importance of the molecular weight of remaining polypeptide fragments, is emphasized. Finally recommendations as to their use and the role that they may play in the future are discussed.

Chapter 2 describes the initial assessment of 2 candidate hydrolysates, one made from fish and the other from chicken proteins. The method used for this initial experiment was high-performance size-exclusion liquid chromatography. The investigation revealed the chicken hydrolysate to have the more favourable molecular weight profile of the two. The finding that 92.9% of the hydrolysate was of a molecular weight less than 5kDa is supportive of its potential value in a hypoallergenic diet. The molecular weight profile was then compared with a selection of those published in the human medical literature. Cow's milk hydrolysates have been widely available and used for the past 2-3 decades. The chicken hydrolysate appeared to compare very favourably to a number of extensively hydrolysed human infant formulae that have been demonstrated experimentally and clinically to be truly hypoallergenic. Despite the extensive use of molecular weight profiles to compare hydrolysates, they remain unreliable as predictors of allergenicity.

Chapter 3 describes the experimental evaluation of the *antigenicity* of the chicken hydrolysate. Following successful immunization of dogs to the intact parent protein, an IgG inhibition ELISA was developed using sera from the immunized dogs. It was demonstrated that the hydrolysate retained some ability to bind IgG but that at equal

levels of antibody binding, the concentration of the hydrolysate solution required was 66 times greater than that of the intact parent protein. It is likely that this represents a clinically highly significant reduction in antigenicity.

Of the limitations of the inhibition ELISA study, perhaps the greatest from the perspective of hydrolysate diet analysis is its inability to differentiate the molecular weight of the IgG-binding fragments. This is important since if they are less than 6-10kDa, they are unlikely to participate in IgE-mediated allergic reactions. Chapter 4 describes the experiment chosen to determine the size of the remaining IgG-binding fragments, namely Western blotting. It was established that the major antigenic fraction remaining in the parent protein following SDS-PAGE separation was a c.69kDa protein consistent with chicken serum albumin. It was demonstrated by both the Western blotting and the HP-SEC that this antigen was absent from the hydrolysate. The actual size of the few remaining binding fragments in the hydrolysate was not, however, clearly elucidated.

It was concluded that the chicken hydrolysate assessed during this thesis is a promising candidate for inclusion as the peptide component of a diet for the diagnosis and management of food hypersensitivity in dogs and cats. In addition, the diet has theoretical promise for the prevention of food hypersensitivity during periods of mucosal inflammation such as idiopathic inflammatory bowel disease and acute gastroenteritis. Ultimately, clinical trials are required to conclusively demonstrate the value of the hydrolysate in the diagnosis and management of these disorders.

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