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USE OF A HYDROLYSED SOYA ISOLATE-BASED DIET IN THE MANAGEMENT OF CHRONIC IDIOPATHIC INFLAMMATORY BOWEL DISEASE AND DIETARY HYPERSENSITIVITY IN CATS

(With 2 Tables and One Figure)

By

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استخدام غذاء يحتوى على الصويا المهدرجة للسيطرة على الالتهاب المعدى المعوى المزمن وحساسية الغذاء المفرطة فى القطط

نشوى عصمت والى ، فنسنت بيورج ، مايكل داى ، كريستوفر ستوكز ، اندريا هارفى ، ميك بايلى ، تيموثى جريفيث جونز

أجرى البحث لتحديد مدى كفاءة غذاء يحتوى على الصويا المهدرجة للسيطرة على الالتهاب المعدى المعوى المزمن فى القطط. هذا وقد تم مقارنة الغذاء العلاجى بغذاء آخر موجود بالأسواق ، تم إدارج القطط فى التجربة طبقاً لمجموعة من الشروط المتفق عليها ثم تم وضع كل قطة فى واحدة من مجموعتين بصورة عشوائية كالآتى: المجموعة الأولى تم تغذيتها على الغذاء المختبر والمحتوى على الصويا المهدرجة (n=5) والمجموعة الثانية على الغذاء المقارن (n=5). أخذت عينات من الإثنى عشر والقولون باستخدام المنظار من جميع القطط المدرجة فى البحث للتقييم الهستوباثولوجى. الاستجابة الجيدة للغذاء تم تحديدها بناءً على اختفاء الأعراض المرضية. القطط التى لم تستجيب للغذاء الأول تم تحويلها إلى الغذاء المقارن. سبعة من عشر قطط تحسنت بصورة ملحوظة على الغذاء المختبر وثلاثة استجابوا للغذاء المقارن ، علماً بأن هذا الفرق فى النتائج لم يكن معنوياً ($p>0.05$). النتائج أوضحت أن استخدام غذاء يحتوى على الصويا المهدرجة يكون فعالاً فى تحسين حالة القطط المصابة بالالتهابات المعدية المعوية المزمنة.

SUMMARY

A crossover blinded trial was used to assess the efficacy of a hydrolysed soya diet in managing cats with chronic, idiopathic gastrointestinal disorders. A commercial feline prescription diet was used for comparison. Cats that fulfilled the selection criteria were recruited and assigned randomly to one of two groups. Group 1 was fed the hydrolysed soya diet (n=5) and group 2 (n=5) received the comparison diet. Duodenal and colonic endoscopic biopsies were collected from all cats for histopathological assessment. A good response to dietary manipulation was indicated by cessation of presenting signs. Cats failing to improve after feeding the initial diet were switched to the other diet. Seven out of ten cats significantly improved while on the test diet while three cats responded well to the comparison diet but this difference was not statistically significant ($p>0.05$). These results suggest that a hydrolysed soya-based diet can be effective in the management of cats with chronic gastrointestinal signs.

Key words: *Feline IBD, Dietary Trial, hypersensitivity, Immunohistochemistry, Hydrolysed soya diet*

INTRODUCTION

Dietary hypersensitivity and idiopathic inflammatory bowel disease (IBD) are recognised as common and important intestinal disorders of the cat (Sherding, 1982; Tams, 1996; Guilford *et al.*, 2001). An adverse reaction to dietary components can be classified as either food hypersensitivity or food intolerance; food hypersensitivity is an immunological event directed against food components, while food intolerance is an adverse reaction to food with no immunological component (Sampson, 1999).

Diagnosis of IBD requires elimination of all specific causes of gastrointestinal inflammation. Infectious causes as well as tumours and endocrine disorders must be excluded before a diagnosis of IBD is reached. Evaluation of cellular infiltration in haematoxylin and eosin (HE) -stained endoscopic biopsies can be difficult, although since this study was performed the World Small Animal Veterinary Association (WSAVA) gastrointestinal standardization guidelines for mucosal histology have become available in an attempt to improve consistency in interpretation (Day *et al.*, 2008).

Although the underlying aetiopathogenesis of IBD remains unclear, possible contributory factors include a genetic basis, infectious agents and immune dysregulation (Guilford, 1997). Several studies of IBD in the cat

(Denis *et al*., 1992; Hart *et al*., 1994) and dog (Hayden and Van-Cruiningen., 1982; Easley, 1972) have been reported. All of these early reports focused primarily on the clinical features of the disease, and diagnosis was based on routine histopathological assessment of intestinal biopsies. More recently, characterization of mucosal cellular infiltrates using immunohistochemical techniques in canine (Stonehewer *et al*., 1998; German *et al*., 2000 and 2001) and feline IBD (Roccabianca *et al*., 2000; Waly *et al*., 2004) as well as molecular characterization of cytokine expression in intestinal tissues have been reported (Van Nguyen *et al*., 2006, Janeczko *et al.*, 2008).

There is clear evidence that dietary management plays an important role in treating cats suffering from idiopathic chronic vomiting, diarrhoea and weight loss even though specific dietary hypersensitivity or IBD may not always be demonstrated (Guilford *et al*., 2001). The process of hydrolysis breaks down the soya antigen into small fragments that reduces the likelihood of an intestinal immune response (Puigdmont *et al.,* 2006). Therefore, the aim of the present study was to assess the value of a commercially-produced hydrolysed soya diet in the management of cats with chronic, idiopathic gastrointestinal signs consistent with IBD.

MATERIALS and METHODS

Recruitment of cases

Veterinary practices were invited to refer cases that might be suitable for inclusion in the trial to the Feline Centre, University of Bristol, Langford. The inclusion criteria for the trial were set as follows,

1 - Chronic signs suggestive of gastrointestinal disease present for at least two weeks (vomiting, diarrhoea and weight loss).

2 - No less than 2.5 kg in body weight for ease of endoscopy.

3 - No specific cause of the chronic gastrointestinal signs (other than IBD) identified, negative for feline immunodeficiency virus (FIV) and feline leukaemia virus (FeLV) infections on ELISA testing.

4 - An appropriate course of Fenbendazole[a] treatment to have been given at a dose rate of 50mg/kg/day for five days.

5 - Cats must not have access to food other than trial diets, and be confined indoors or strictly supervised by the owner to prevent access to other

foods while on trial.

Clinical investigations

For each case, a clinical and dietary history was taken and a physical examination was performed. All cats entering the trial had routine laboratory investigations (Haematology, biochemistry, FIV and FeLV screening and serum T4 concentration for cats over 7 years of age) including faecal parasitological examination (Particularly for protozoa, giardia and tritrichomonas) and bacterial culture. Thoracic and abdominal radiography were performed. Endoscopic investigation was carried out for evaluation of the gastric, duodenal and colonic mucosa and collection of duodenal and colonic biopsies for histopathology and immunohistochemistry.

Study Protocol

Cases entering the trial were randomly assigned to one of two groups. Group 1 cats were fed the test hydrolysed soya protein diet [b] (RCH) and group 2 cats received a commercial prescription intestinal diet[c] (CON). All cases were discharged after clinical investigation with a supply of the appropriate diet. Owners were asked to provide a daily log of the cat's progress and complete follow-up sheets. Owners were also telephoned after 7-10 days to check on the progress of the cat. Faecal consistency was evaluated by the owners using a photographic scale ranking the different forms of faecal consistency from score 1 showing watery diarrhoea, to a score of 5 defined by hard and dry faeces. A score of 4 is considered optimal on this scale.

Cats that showed marked improvement after the initial 10-14 day period continued to receive the same diet for a further two weeks. Cats were re-weighed, and information was gathered by telephone questionnaire and where necessary a visit for re-evaluation. Cats were then challenged with their original diet for a minimum period of two weeks. Feeding the test diet was then resumed for a further two month period, at the end of which the owners completed a final questionnaire.

Cats failing to show significant improvement after the initial period of feeding of either diet, were reassigned to receive the other diet. When this change led to a marked improvement, the protocol was followed as for those cats showing resolution of clinical signs. The cats did not receive any medical therapy during the first phase of the trial. In cases where the second trial diet did not result in significant improvement, medical treatment with orally administered prednisolone was given at a reducing

dosage over a 6-8 week period starting at 2 mg/kg daily.

A significant or a marked improvement was defined as improvement of faecal quality and significant reduction of the presenting clinical signs, whereas complete resolution of presenting clinical signs, weight gain and optimal faecal consistency indicated resolution of the disease. A cat was considered to have relapsed when and if the challenge with the original diet resulted in return of the presenting clinical signs.

Collection of endoscopic biopsies

Samples of duodenal and colonic mucosa were collected by endoscopy as previously described (Waly *et al.*, 2004). Biopsies were fixed in 10% neutral buffered formalin, and then embedded in paraffin wax, cut at 5μm and stained with HE.

Histopathology and Immunohistochemistry

Biopsies from all cats were scored for pathological changes based on the architecture of the section and cellular infiltration of lamina propria in both villous and crypt areas. All slides were examined by a certified pathologist (MJD) who was unaware of the animal's identity.

Immunohistochemical labelling for MHC class II was performed using formalin-fixed tissues and tissues were examined as previously described (Waly *et al*., 2001). For scoring epithelial expression of MHC class II, areas of both crypt and villous epithelium were scored according to the intensity of MHC class II labelling of the epithelial cells (grades 0-4) as previously described (Waly *et al*., 2001). The density of class II^+ cells in the lamina propria was also scored from 1-4 where 1 indicated normal density, 2 a mild increase, 3 an increase in density of cells and 4 indicated that a densely populated lamina propria with differentiated dendritic cells.

Statistics

To test the null hypothesis that there was no difference between effects of the two diets, a Chi-Square Test procedure was performed using SPSS software package[d].

RESULTS

Signalment

Six cats were less than one-year-old and four cats were over one year of age. There were six male cats (Three neutered) and four female cats (Two neutered). A number of breeds was represented including Ragdoll (n=2) and one each of Burmese, Siamese, Ocicat, Norwegian forest cat and Bengal; the remaining three were domestic shorthair (DSH). The majority

of cats (n=6) had been fed a single source food or a sensitivity control diet two weeks prior to the referral.

All cats were reported to have lost weight prior to referral. Six out of ten cats had signs of large bowel diarrhoea, three cats had signs of small bowel diarrhoea. Two cats showed vomiting as well as diarrhoea and one cat showed only vomiting (Table 1).

No consistent blood abnormalities were detected; two cats showed an increase in the total white blood cell count (Cases 1 and 6) of 25.6 and 23.8 $\times$ 10^9/l respectively. One cat had a mild eosinophilia reaching 2.8 $\times 10^9$/l (Case 1). Five out of ten cats (50%) showed neutrophilia ranging from 13.5-23.9 $\times$ 10^9/l. Serum biochemistry was unremarkable apart from a low total protein but normal levels of albumin in six cats ranging from 58.1-74.2 g/l (mean = 65.98, median = 68.35).

Histopathology and Immunohistochemistry

Routine histopathological examination (HE) of endoscopic biopsies showed colitis (n=6), duodenitis (n=1), or duodenocolitis (n=3). Duodenal biopsies from the majority of cases (n=8) showed mild epithelial expression of MHC class II by enterocytes (Table 1); only two cases had no such epithelial expression. The lamina propria of these biopsies was densely populated with class II^+ cells many of which had dendritic morphology (Fig. 1).

Response to dietary management

A total of four cases responded to the first diet they were assigned (Three to RCH, one to CON); one of these cases did not relapse when re-challenged with the original home diet (The cat that was on CON diet). Six cases responded to the second diet they were assigned (Four RCH, two CON). Of these, one did not relapse when challenged (The cat that was on CON diet). Two clients declined to re-challenge their cats (Both were on RCH). These two unchallenged cases were both started on CON diet and did not respond and were subsequently changed to the RCH diet. A summary of these results is presented in Tables 1 and 2. The difference between the number of cases completing the trial on either of the tested diets (RCH and CON) was not statistically significant (p=0.206).

Table 1: Clinical and histological features of cats in the trial.

Cats	Histopathology	MHC-II epithelial score	MHC-II LP score	Main presenting clinical sign	Weight gain/loss (kg)
1	Colitis	1	2	LI diarrhea	*Yes
2	Colitis	1	2	LI diarrhea	0.12
3	Duodeno-colitis	1	3	LI&SI diarrhea	0
4	Colitis	1	2	LI diarrhea	*Yes
5	Duodeno-colitis	1	2	Vomiting	ND
6	Colitis	0	4	LI&SI diarrhea	0.38
7	Duodeno-colitis	0	1	LI diarrhea	0.46
8	Colitis	1	2	LIdiarrhoea & vomiting	0.4
9	Colitis	1	2	LIdiarrhoea & vomiting	0
10	Duodenitis	1	2	LI diarrhea	*Yes

ND, not done, LI (Large intestinal), SI (Small intestinal).
* Owner did not weigh cats but reported weight gain during the trial.

Table 2: Summary of responses of cats in the trial.

Cats	Diet 1	Response to Diet 1	Diet 2	Response to Diet 2	Medication	Challenge	End of Trial Diet
1	RCH	Good	RCH	NA	No	Relapse	RCH
2	RCH	Good	RCH	NA	No	Relapse	RCH
3	RCH	Resolved	RCH	NA	No	Relapse	RCH
4	RCH	No response	CON	Resolved	No	Relapse	CON
5	RCH	No response	CON	No significant response	No	Relapse	Euthanased
6	CON	No response	RCH	Good	No	Relapse	RCH
7	CON	No response	RCH	Good	Yes	No challenge	Medications and RCH
8	CON	No response	RCH	Good	Yes	No challenge	Medications and RCH
9	CON	No response	RCH	Resolved	No	No relapse	RCH
10	CON	Good	CON	Good	No	No relapse	CON

Diet 1, the first diet cat was assigned on, diet 2; the second diet the cat was assigned on when not responding to the first diet.
RCH (Royal canin's hydrolysed soya diet); CON (Control diet, Hill's I/D);
NA, not applicable.
Medication consists of prednisone at a dose rate of 2mg/kg until resolution of signs then gradual tapering of the dose.

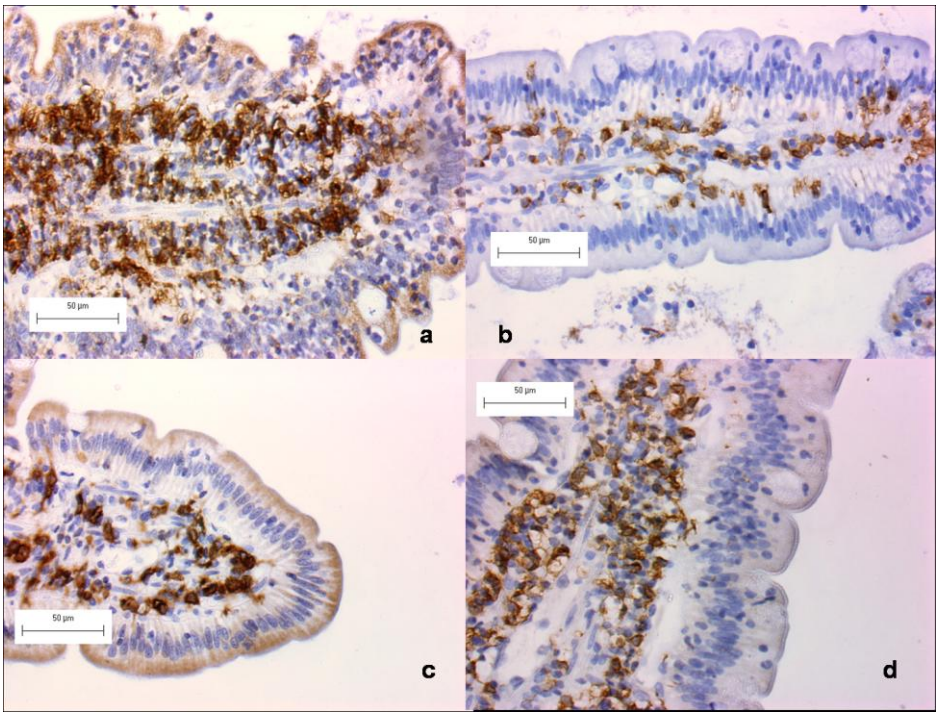

Fig. 1: MHC class II expression in duodenal biopsies from cats with dietary hypersensitivity and IBD. An intestinal biopsy showing (a) MHC class II expression by enterocytes (Score of 3) and the lamina propria densely populated with antigen presenting cells (Grade 4). (b) Absence of MHC class II expression by enterocytes (Score of 0) and a normal population of lamina propria antigen presenting cells (Grade 1). (c) MHC class II expression by enterocytes (Score of 2) with a less dense population of lamina propria antigen presenting cells (Grade 2). (d) Absence of class II expression by enterocytes (Score of 0) but a dense population of lamina propria antigen presenting cells (Grade 3).

DISCUSSION

The present study investigated the potential value of a hydrolysed soya -based diet in the management of cats suffering from IBD and food related chronic GI signs. A larger number of cases would have been preferable but recruitment of cases to a trial of this nature is challenging. A number of cases were offered to the trial but did not fulfil the criteria, the owners subsequently declined to participate or the owners subsequently failed to comply or complete the trial. Nevertheless clear benefits were

evident in the use of such diet for management of chronic GI signs and the response compared favourably to the established diet.

The majority of recruited cases were presented with large bowel diarrhoea and fewer cats suffered from small bowel diarrhoea and signs of vomiting. This is unusual compared with other reports (Guilford *et al.,* 2001) where diarrhoea (25% of the study group), vomiting (56%) or both vomiting and diarrhoea (19%) were the main clinical signs recorded. However, in other studies the breakdown of cases with respect to large versus small bowel diarrhoea is similar to that reported here (Nelson *et al.,* 1984; Peterson, 1995).

The results of routine histopathological examination in 60% of cases showed inflammation of the large bowel and 30% showed inflammation of both the small and large intestine, whereas one case had duodenitis only. In other studies, lesions of the colon have been more significant (Guilford *et al.,* 2001; Leistra and Willemse, 2002) and support a recommendation to that colonic biopsies be examined even in cases presented with signs of small bowel diarrhoea.

The immunohistochemical evaluation of MHC class II expression in these cats was consistent with previous findings where cats with IBD were reported to have up-regulation of both epithelial expression and number of class II $^{+}$ cells in their duodenum (Waly *et al.,* 2004, 2007).

Seven out of the ten cats reported here responded more favourably to the hydrolysed soy-based diet (70%) and only three cats (30%) responded better to the control diet, the difference did not reach statistical significance which my reflect the limited number of cases that completed the trial. A total of six cases relapsed when challenged with their original home diet (75%), whereas two cats (20%) did not relapse after challenge. Others have shown that some cats will respond to dietary challenge but do not fulfil the criteria for food hypersensitivity in that re-challenge does not induce relapse and describe these cases as food responsive (Guilford *et al.,* 2001; Leistra and Willemse, 2002). A relapse suggests that the cat is sensitive to one of its regular dietary components. Precise diagnosis of the inciting factor is achieved by dietary elimination-challenge studies, which was beyond the scope of this trial.

The process of hydrolysis breaks down the soya antigen into small fragments that reduces the likelihood of an intestinal immune response (Puigdmont *et al.,* 2006). It is suggested that these fragments are too small to cross-link IgE molecules on the surface of sensitized mast cells, thereby inhibiting mast cell de-granulation and perpetuation of the intestinal inflammatory response. Such a mechanism may explain the favourable response of 70% of the recruited cases to the test diet.

In conclusion, the present study suggests that a hydrolysed soy-based diet can be effective in controlling the gastrointestinal disturbances associated with IBD and dietary hypersensitivity in cats. A further study on a larger number of cases is still required to confirm these results.

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The work described was undertaken at the Feline Centre, University of Bristol, Langford.

Footnotes

[a] (Granofen, Panacur, Zerofen,)

[b] (Veterinary Diet Feline Hypoallergenic, Royal Canin, Aimargues, France, RCH)

[c] (Prescription Diet Feline I/D, Hill's Topeka, Kansas, CON)

[d] (SPSS 12.0 for windows)

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